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TITLE: Vaccination of High-Risk Breast Cancer Patients with Carbohydrate Mimicking Peptides

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14. ABSTRACT
The expression of the Tumor Associated Carbohydrate Antigens such as the neolactoseries antigen Lewis Y (LeY) and gangliosides such as GM2 and GD2/GD3 are amplified on breast cancer cells and is linked to poor prognosis and high risk of disease relapse. Immunotherapy to direct responses to TACA is, therefore, perceived to be of clinical benefit. To overcome this deficiency, we developed mimotopes of TACA to induce more robust cross-reactive and tumor-specific responses. In preclinical studies, immunization with these mimotopes reduce tumor burden and inhibited metastatic outgrowth of murine tumor cells expressing TACA structural homologues. Thus, peptide mimotopes of TACA represent a new and very promising tool to induce a strong immune response to TACA expressed on Breast Cancer cells. Based on encouraging preclinical results, our objectives are for the current funding period (years 1 and 2) are to develop the necessary preclinical data required by the Food and Drug Administration (FDA) for filing an Investigational New Drug (IND). In this context we: 1.) Developed the necessary procedures for the required Good Laboratory Practice (GLP) studies; 2.) Defined problems in scale up of the manufactured mimotope vaccines; 3.) Identified alternative mimotopes of TACA that circumvent the scale up problems.

15. SUBJECT TERMS
1.) Developed the necessary procedures for the required Good Laboratory Practice (GLP) studies; 2.) Defined problems in scale up of the manufactured mimotope vaccines; 3.) Identified alternative mimotopes of TACA that circumvent the scale up problems.

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INTRODUCTION

Carbohydrates are the most abundantly expressed self-antigens on tumor cells and consequently they are perceived as viable targets for immunotherapy. Examples of tumor-associated carbohydrate antigens include the gangliosides GM2, GD2, GD3, and fucosyl GM1, Globo H, polysialic acid, STn and the neolactoseries antigens sialyl-Lewis x (sLex), sialyl-Lewis a (sLea) and Lewis Y (LeY). A major approach to induce responses to these tumor associated carbohydrate antigens (TACA) is carbohydrate-conjugate vaccines. Representative examples of these vaccines in clinical development include those directed toward gangliosides, polysialic acid, Globo, Lewis Y (LeY), and the STn antigen. Because TACA are T-cell–independent antigens and self-antigens, conjugation to immunologic carrier protein is perceived essential to recruit T cell help for antibody generation. Conjugation of TACA does not, however, ensure an increase in immunogenicity because conjugation strategies do not uniformly enhance carbohydrate immunogenicity.

Carbohydrate Mimetic Peptides (CMPs) functioning as T cell dependent surrogates of TACA are proposed to augment responses to TACA. Our rational is based upon our preclinical studies that these surrogate antigens, also referred to as mimotopes, induce immune responses that inhibit tumor growth in animal models. Our purpose is to induce TACA reactive antibodies and cellular responses in breast cancer patients by using a mimotope. We expect to observe after vaccination a robust anti-TACA response in individuals that should positively impact on tumor recurrence.

BODY

The major goals of this application are to determine the safety and tolerability of immunization with a CMP imunogen; and to determine whether immunization with the CMP generates an immune response against TACAs and TACA expressing breast cancer cell lines.

We have defined aims one through three for period three of years three through five. This progress report reflects the revised statement of work (SOW), submitted in the 2008 Annual Report submitted in May of 2008. Below is the revised SOW:

Revised SOWs for years 3-5

YEAR 3

Preclinical [Aim 1]	
1. Complete preclinical studies	Q2
2. Finalize in-house reports.	Q1-Q2
3. Request pre-IND meeting, Prepare pre-IND meeting package, Meet with FDA.	Q3
4. File IND	Q4
Clinical [Aim 2 and 3]	
1. Finalize Investigator Brochure, protocols (Phase 1A and 1B), informed consent, etc	Q1-Q2
2. Obtain IRB and IBC approval from both UAMS and ARMY	Q2
3. Obtain FDA approval for IND	Q3
4. Initiate Phase IA trial	Q3
5. Develop validation assays for Immuno and Functional assays:	Q3-Q4

ELISA	
6. Calculate Coefficient Variance and modify procedures for validation	Q4
7. Complete Subject Enrollment, Clinical evaluations, Immunoassays, Biostatistical analyses	Q3-Q4
8. Implement data management system and clinical database	Q1-Q4
9. Complete data analyses for Phase IA study – if 9 patients required	Q4
10. Prepare Clinical Study Report for Phase IA	Q4

YEAR 4

CMC - clinical supplies [Aim 3]	
1. Manufacture, Release more clinical supplies (if needed) for Phase 1B	Q1-Q2
Clinical – Phase IA [Aim 2]	
1. Complete data analyses for Phase IA study – latest if 18 patients required	Q2
2. Determine if SID has been met for repeat dose-finding for OID –latest if 18 patients required	Q2
3. Prepare Clinical Study Report for Phase IA – latest if 18 patients required	Q2
Clinical - Phase IB [Aim 3]	
1. Obtain FDA approval to initiate Phase IB	Q2
2. Initiate Phase IB trial	Q2
3. Enrollment of Study Subjects	Q2-Q3
4. Clinical Evaluation of Study Subjects while undergoing immunization	Q2-Q4
5. Standard clinical evaluation of Study Subjects after completion of immunization protocol	Q4
6. Perform antibody immunoassays	Q4
7. Perform laboratory studies of T cell responses	Q4

YEAR 5

Clinical - Phase IB [Aim 3]	
1. Standard clinical evaluation of Study Subjects following immunization	Q1-Q2
2. Lock data base for Phase 1B clinical	Q2
3. Complete antibody data analyses	Q3
4. Complete analyses of T cell responses	Q3
5. Complete biostatistical analyses and prepare Summary Report	Q4

Based of the revised stated in the above SOW we emphasize our progress of Aim1 subtasks 1-4 and Aim 2 – 3 subtask 1 -10. All other aims will be listed in future reports.

A. Aim 1. Preclinical

Subtask 1: Complete preclinical studies

Subtask 2: Finalize in-house reports.

Subtask 3: Request pre-IND meeting, Prepare pre-IND meeting package, Meet with FDA

Subtask 4: File IND

B. Aim 2 and 3. Clinical

Subtask 1: Finalize Investigator Brochure, protocols (Phase 1A and 1B), informed consent, etc

Subtask 2: Obtain IRB and IBC approval from both UAMS and ARMY

Subtask 3: Obtain FDA approval for IND

Subtask 4: Initiate Phase IA trial

Subtask 5: Develop validation assays for Immuno and Functional assays: ELISA

Subtask 6: Calculate Coefficient Variance and modify procedures for validation

Subtask 7: Complete Subject Enrollment, Clinical evaluations, Immunoassays, Biostatistical analyses

Subtask 8: Implement data management system and clinical database

Subtask 9: Complete data analyses for Phase IA study – if 9 patients required

Subtask 10: Prepare Clinical Study Report for Phase IA

A. Aim 1 Preclinical (Year 3)

Subtask 1: Complete preclinical studies

The *"Vaccination of High-Risk Breast Cancer patients with Carbohydrate Mimicking Peptide"* grant's third year ended as of April 23, 2009. The third moved the study into its GLP preclinical phase. All administrative required approvals and study staff security clearances have been met as of study initiation date of May 9, 2008. The Study completion date and Final report dates are ending on the final report from the UAMS Quality Control and Assurance Unit. The preclinical study was conducted at the GLP facilities in the Central Arkansas Veterans Healthcare System. The pre-clinical portion of the grant is held under Code of Federal Regulation Title 21 Part 58 (21CFR58) Good Laboratory Practice (GLP) for Non-clinical Laboratory Studies. All standard operating procedures (SOPs) are in place and are in accordance with the Code of Federal Regulation Title 21 Part 58 (21CFR58). All SOPs were reviewed for their Annual Review as of April 30, 2008. The staff members' training is in compliance with 21CFR 58. All staff member training is current and is in accordance with 21CFR58 and research administration by-laws of the University of Arkansas for Medical Sciences (UAMS). The Draft of the final report, and its associated appendix is found in **Appendix I** of this report.

Subtask 3 and 4: Request pre-IND meeting, Prepare pre-IND meeting package, Meet with FDA

After sign off of the GLP report by the UAMS Quality Control and Assurance Unit and Completion of the IND application we will submit to the FDA. Expected submission is June 30 2009. This strategy necessitates the FDA to respond in thirty days after the IND submission.

B. Aim 2 and 3. Clinical (Year 3)

Subtask 1: Finalize Investigator Brochure, Human protocols (Phase 1), and informed consent.

The Human protocol and consent forms are complete. The Investigative Brochure is near completion as we await final GLP sign off. The draft of Investigational Brochure, and final versions of the human protocol and consent form are found in **Appendix II of this progress**

reprot.

Subtask 2: Obtain IRB and IBC approval from both UAMS and ARMY

We have submitted the Phase I protocol and consent forms to the UAMS Clinical Research and Data Management (CRDM) office. Data coordination and management for clinical trials conducted at the Winthrop P. Rockefeller Cancer Institute is provided by the 12-person Cancer Institute CRDM office. The primary mission of the CRDM office is to provide all Cancer Institute clinicians with easy access to complete copies of active clinical protocols, assist with selecting protocols for patients, check patient eligibility for trials, collect protocol-required data, and submit that data to the appropriate sponsor. The CRDM office also ensures that all clinical trials are conducted according to the UAMS institutional review board and Food and Drug Administration Good Clinical Practice guidelines. After the GLP report is finalized and signed off the Investigator Brochure will be finalized and sent along with the Phase I protocol and consent form to the UAMS IRB. It is expected that IRB submission will occur on June 1, 2009. The trial will not begin until FDA approval of the IND.

Subtask 3: Obtain FDA approval for IND

The IND will be submitted at the end of June 2009 if not earlier. FDA approval should take 90 days – 30 days approval and potentially 60 day clinical hold.

Subtask 4: Initiate Phase IA trial

Not started and expect to do so in Sept/Oct 2009.

Subtask 5: Develop validation assays for Immuno and Functional assays: *ELISA*
P10s induces immune responses in experimental animals: Immunization with P10s-PADRE induces an immune response to itself (**Figure 1**). Plates were coated with a multivalent form (Multivalent Antigen Peptide –MAP devoid of the PADRE component) of P10s (P10s-MAP) and tested against serum raised in animals during the GLP study. Figure 1 validates that the P10s-PADRE induces P10s reactive antibodies at high titer (1:100000) antibodies in the mice in the GLP studies. Both test doses – 300 ug and 500 ug- induced similar anti-P10s responses with the higher dose resulting in slightly higher reactivity to P10s.

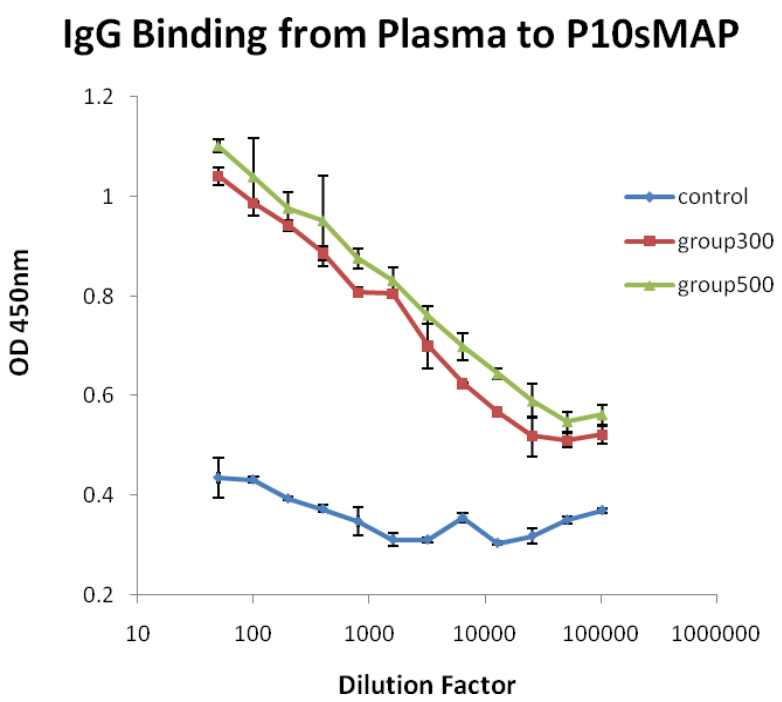

Figure 1. ***IgG binding to P10s***. *Plates were coated with P10s-MAP (1μg/well). Plasma samples were collected from 3 groups of mice (4 mice per group) after 5th immunization with P10s-PADRE. Samples of plasma pooled from each group were added to the peptide coated plates in serial dilution ranging from 1:50 to 1:100000 and binding was visualized with anti-mouse IgG peroxidase (Sigma, St Louis,MO). Absorbance was measured at 450 nm using a Bio-Tek ELIZA reader (Bio-Tek instrument, Inc, Highland Park,Vermont). Mean absorbance and standard deviations are presented for each plasma dilution.*

P10s-PADRE induced responses cross-react with GD2.

Analyzing ganglioside antibodies by ELISA has always been plagued with difficulties and inconsistence forcing the development of carefully designed methodology optimized at each step of the assay. Even with highly standardized ELISA the unnatural presentation of the ganglioside epitopes on a solid phase introduces noise and uncertainty of the results. For instance, we have found that mimotope reactive antibody in the preimmune IgG repertoire in humans show consistently very high reactivity to hydrophobic structures. On solid phase the hydrocarbon chains of the fatty acid components of the gangliosides are exposed and are targets for at least some of the binding antibodies observed. This binding most probably is unrelated to the biological effect of the vaccine since in its natural presentation the ganglioside molecule is inserted in the cellular membrane and its hydrophobic part is hidden in the external lipid layer of the membrane. To test the antibody response in a more natural presentation while preserving the highly defined nature of the binding assay (having minimum diversity of potentially cross-reactive structures) we introduced a quantitative assay of anti-ganglioside antibodies based on binding to ganglioside containing liposomes and flow cytometry (**Figure 3**). To avoid storing ganglioside containing liposomes which seem to lose some of the ganglioside contents after thawing (may be due to the amphipathic nature of the molecule) it was considered more reproducible to load ex tempore phosphatidylcholine liposomes from a frozen stock with GD2 by

coincubating a defined volume and concentration of liposomes with 0.1 mg/ml of GD2 added as 10 times lower volume of 1mg/ml methanol solution.

To facilitate the inclusion of the ganglioside in the membrane the mixture is subjected to 1h sonication at 40^{o}C. The loading is followed by washing away the GD2 remaining in the solution before staining with the tested sera. We have an SOP prepared based on this protocol. The quantitative standardization of the assay will be ensured by: 1) Standardizing the PMT voltage setting and linearity of the flow cytometer using LinearFlow™ Green Flow Cytometry Intensity Calibration Kits for 488 nm Excitation and 515 nm Emission (Molecular probes, Invitrogen detection technologies) and a target intensity for the 100% and the 0.02% emitting beads of respectively 9000 and 1.8 fluorescence channels; 2) Using a defined lot of secondary antibody for the mouse immunoglobulins and 3) Inclusion of samples of liposomes stained with a standard solution of anti-ganglioside antibody (a GMP grade solution of ME36.1 antibody) followed by a defined lot of secondary antibody. The ME36.1 staining will be performed at 0.01, 0.03 and 0.05 mg/ml of the antibody and the highest value will be considered level at saturation. This is done to avoid lower binding levels of higher concentrations due to crowding phenomena. The binding of the sera at different dilutions will be presented as relative to the saturation level of ME36.1 binding.

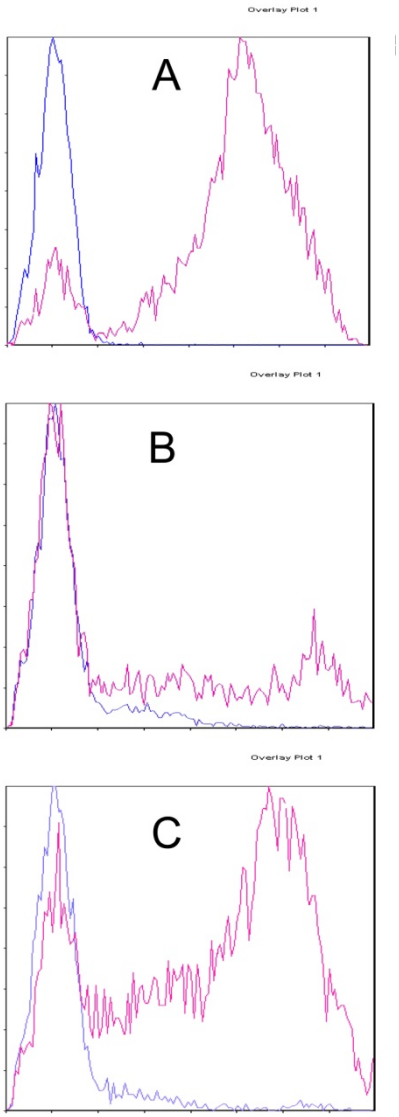

Figure 2. Flow cytometry data of staining GD2 liposomes with mouse serum from p10s immunized mice. *Stock of DMPC liposomes was pelleted and 0.15 ml (200mM) of this preparation was incubated with GD2 at 0.1 mg/ml in PBS 10% methanol for 1h at 40°C in a sonicator bath (the suspension was sonicated all through the incubation). The GD2 loaded liposomes were brought to 5ml with PBS and centrifuged for 20min at 80 000xg. The pellet was resuspended in 5 ml PBS and this suspension was used further for staining and FACS. The different antibody solutions were incubated for 30 min at RT followed by washing by centrifugation at 20000xg for 30 min. In all experiments GD2 loaded liposomes (red line) were compared to non-loaded liposomes (blue line). A – staining with 0.01 mg/ml anti-GD2 antibody ME36.1 followed by FITC conjugated secondary antibody; B – Binding of naïve serum at 1:100 dilution and C- binding of P10s immunized mice sera at 1:100 dilution. In the mice serum binding experiments anti-mouse IgM FITC conjugate was used as a secondary antibody. This and all following flow cytometry data is acquired and analyzed on Beckmann COULTER® EPICS® XL™ and XL-MCL™ flow cytometer.*

Figure 3. Binding of total immunoglobulin from GLP study mice plasma to GD2 liposomes using the method described above. *The reactivity (presented as mean fluorescent intensity - MFI) was higher in the group immunized with 0.3 mg P10s than in the 0.5 mg immunized group, which may be due to a high dose tolerance.*

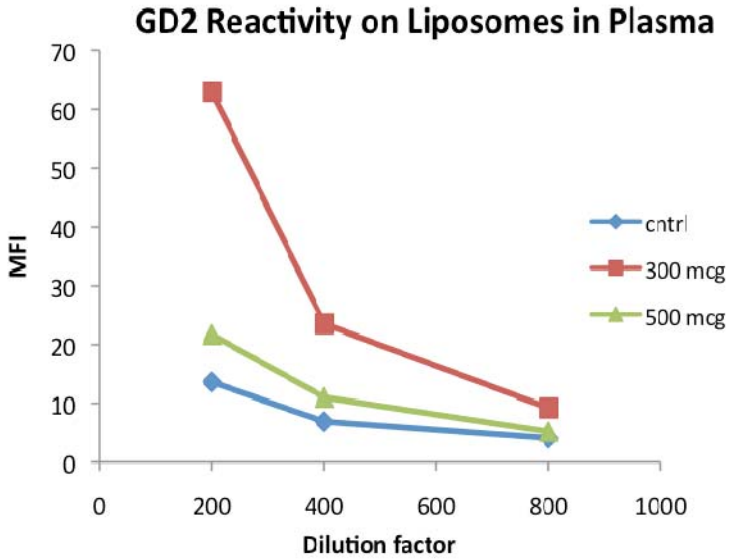

Figure 3

Plasma from P10s-PADRE immunized (300μg dose) was shown to be reactive with the GD2-lipisome complex (**Figure 3**) suggesting that perhaps high tolerance might be operative in the immunization. As an additional prove of consistency of the mimotope property of the P10s peptide and also an indirect confirmation of the flow cytometry based quantitation of mimotope induced reactivities a correlation between data generated from studying active immunization induced antibodies in mice and preimmune cross-reactive human IgG is shown on **Figure 4**.

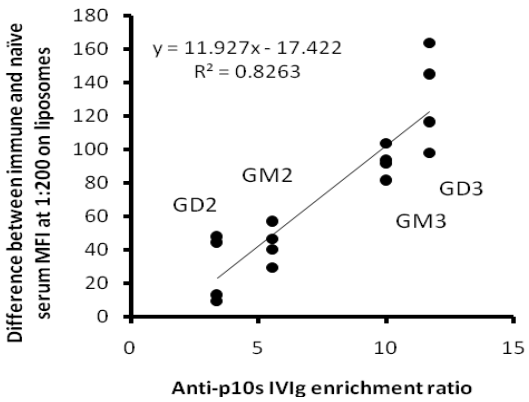

Figure 4*. Correlation between the data obtained from liposome loaded ganglioside assay and binding profile of anti-p10s preimmune antibodies isolated by immuno-affinity chromatography from normal human IgG (IVIg). Human IgG was isolated by passing dialyzed IVIg at 50 mg/ml through a p10s affinity column and eluting the bound antibodies by acid and alkaline elution. The antibodies eluted were dialyzed against PBS and concentrated. On the abscissa are shown the values of relative enrichment of anti-ganglioside reactivity in this affinity prepared fraction as compared to the starting IgG solution. These values are derived from the binding data from glycan array experiment carried out at the Consortium for Functional Glycomics (The Scripps Research Institute, La Jolla, CA, http://www.functionalglycomics.org/fg/site_guide/about.shtml). On the ordinate are shown reactivities of mouse sera with liposomes loaded with the same gangliosides presented as MFI. The sera are from BALB/c mice immunized with p10s 4 times with two weeks intervals. The MFI of naïve animals' sera is subtracted from the MFI of sera from individual immunized mice.*

P10s-PADRE reactive human antibodies might be catalytic

A key feature of the carbohydrate mimotope P10s is the polyspecific recognition both by the antibody repertoire in general but also in terms of the peptide's properties as a mimic. This means that the peptide presents a number of different conformations and even more different epitopes associated with TACA determinants. Proofs of this concept exist in our previous publications and this finding led us to propose the term "multiple antigen mimotopes". The mimotope polyspecificity prompted us to hypothesize that a diverse epitope space might contain also footprints of transition state analogues that could recruit catalytic antibodies capable of transforming or unmasking new carbohydrate antigens. To probe this possibility, we compared the lectin recognition profiles of a breast cancer cell line MDA-231 after treatment with P10s cross-reactive antibodies or no treatment. The anti-P10s IgG was isolated from pooled human immunoglobulin G preparation for intravenous use (IVIg) by affinity chromatography. Since these antibodies also contain species that bind with sufficient avidity to the cellular surface and may also affect the physiology of the cells and the turnover of glycans, we used cells fixed with 1% formaldehyde to avoid signaling events and eluted the bound antibodies by 2 cycles of acid (pH 2.7) and alkaline (pH 11) elution. The results of this experiment are presented in **Figure 5**.

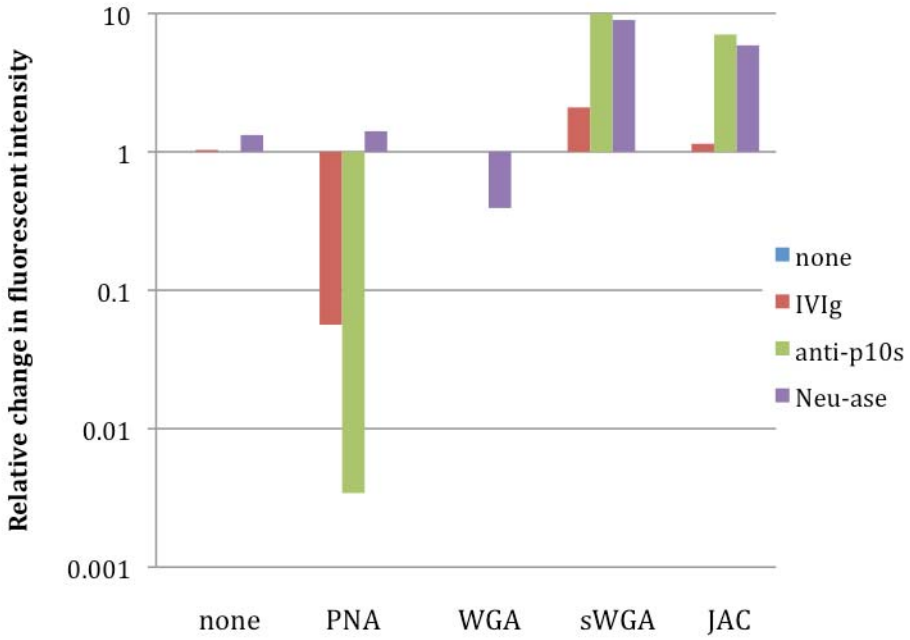

Figure 5. Relative change in the median fluorescence intensity of lectin stained MDA 231 cells after treatment with antibodies or neuraminidase measured by flow cytometry. *MDA-231 cells were fixed with 1% formaldehyde for 10 min, treated with non-fractionated IVIg (0.1mg/ml) or anti-p10s fraction of IVIg (0.1 mg/ml) as well as neuraminidase and washed with 2 cycles of acid (0.05M glycine/HCl, 0.15 M NaCl pH2.7) and alkaline(0.1 M Diethanolamine, 0.15M NaCl pH11) buffer, followed by PBS. The cells showed high human IgG binding before and no residual binding after the elution step (data not shown). At the end the different samples were stained with 0.02 mg/ml of different biotinylated lectins followed by streptavidin-phycoerythrin. The WGA stained, IVIg treated sample is missing. PNA – peanut agglutinin, WGA – wheat germ agglutining, sWGA – succinylated WGA, JAC – jacalin, Neu-ase – neuraminidase type II from V. cholerae.*

Neuraminidase (type II from *Vibrio cholerae*) reduced the WGA binding although the effect was less than expected since this neuraminidase is known to catalytically remove α2-3, α2-4, α2-6 and α2-8 linked sialic acid. This may be due to a steric hindrance for some of the sialic acid residues. Interestingly, both IVIg and the anti-P10s fraction affected the expression of the different lectin binding reactivities but in all cases anti-P10s antibodies had a stronger effect. In the case of PNA this loss of binding was almost complete for anti-P10s. The antibody fractions were prepared from clinical grade sample of IVIg under sterile conditions sterile filtered and kept at 4^{o}C. This reduced maximally the probability that the effect is due to a bacterial glycosidase contamination.

It seems unlikely that anti-P10s antibodies possess neuraminidase activity. The pattern of change observed, i.e. – decrease of PNA activity and increase of both sWGA and JAC activities was analyzed on the basis of the reactivity patterns for each one of these lectins as demonstrated in experimental data made public by the Consortium for Functional Glycomics. **Table 1** shows the high binding glycans for each of the lectins using a 264 glycan array. The changes are consistent

with a galactosidase activity, which cleaves terminal Galβ1-3 sugars and unmasks underlying JAC and sWGA binding sites. **Table 1** also presents a number of candidate glycans that fit this hypothetical model. Anti-P10s fraction of IVIg was found to bind to these glycans with moderate to high avidity in a previous experiment. A hypothetical galactosidase activity of this type in anti-P10s antibodies may lead to conversion of T to Tn antigens and increase the expression of the latter. Such an antigen modification alone may lead to an increased immunogenicity of the cancer cells after P10s immunization. The tumor specificity in this case may come from the higher density of some of the carbohydrate epitopes on the tumor cells and shifts in the glycan profile mostly due to incomplete synthesis of glycans.

Table 1. ***Binding data (in relative units) from the Consortium for Functional Glycomics for the lectins used in this study as well as for anti-p10s fraction of IVIg.*** *The set marked in green represents glycans that have high binding to PNA but low to the other two lectins. The cleavage of the terminal Galβ1-3 sugar would destroy the PNA binding site but unmask a WGA binding site. JAC has almost overlapping specificity with PNA so the results are somewhat surprising (e.g. – the blue set) yet there are lectins with high JAC and low PNA binding (some of the red set), which could also be products of cleavage of PNA binding sites. All of the glycans carrying PNA sites that can be cleaved to sWGA/JAC binding sites (green set) are also recognized by the anti-p10s antibodies with reactivity varying from marginal (3200 units) to very high (25000 units) . The lower reactivity in this case may be an indicator of intrinsic enzymatic activity in a subset of the antibodies.*

Glycan Formula	Binding in relative units			
	PNA	JAC	WGA	Anti-P10s IgG
Galβ1-3(Neu5Acβ2-6)GalNAcα-Sp8	39480.80	302	220.29	3213
Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ-Sp0 GM1	47172.40	73	107.79	5222
Galβ1-3GalNAcβ1-4Galβ1-4Glcβ–Sp8	48788.52	52	375.01	25328
Neu5Acα2-6(Galβ1-3)GalNAcα–Sp8	50444.81	31	440.68	7347
Neu5Acβ2-6(Galβ1-3)GalNAcα–Sp8	31622.02	52	235.96	9690
Galβ1-3GalNAcα-Sp8 T antigen	51903.83	16827	522.04	
Galβ1-3GalNAcβ–Sp8	51480.65	11518	223.36	
Galβ1-3(GlcNAcβ1-6)GalNAcα-Sp8	46426.22	1296	54848.48	
Galβ1-3(Galβ1-4GlcNAcβ1-6)GalNAcα-Sp8	49367.98	0	45237.19	
GlcNAcβ1-6(Galβ1-3)GalNAcα–Sp8	43653.36	5325	42688.87	
Galβ1-4GlcNAcβ1-6(Galβ1-3)GalNAcα–Sp8	41615.79	45	42411.68	
GalNAcβ1-3GalNAcα–Sp8	233.88	18882	269.31	
GalNAcβ1-3Galα1-4Galβ1-4GlcNAcβ-Sp0	-2.62	11977	51174.56	
GalNAcβ1-3(Fucα1-2)Galβ-Sp8	-26.57	11366	341.62	
β-GalNAc–Sp8 β-Tn antigen	267.33	3276	19015.94	
β-GlcNAc–Sp8	354.06	1458	44705.27	

An alternative explanation would be removal of the whole antigen/antibody complex from the surface of the cell during the elution procedure. Since the non-IgG treated samples were subjected to the same elution procedure this hypothesis requires a preferential effect on the complex as opposed to the antigen alone, which would be even less likely.

T cell reactivity with P10s and a hetroclitic analogue: The identification of specific tumor antigens has significantly advanced the field of tumor immunology, in particular, the development of cancer vaccines. Improved understanding of the molecular basis of antigen

recognition has resulted in the development of rationally designed tumor antigen specific vaccines based on motifs predicted to bind to human class I or class II major histocompatibility molecules (MHC). Peptide-based vaccines have advanced from pre-clinical to human clinical studies over the last decade and several important issues have been elucidated during this clinical progression. First, investigators have developed powerful paradigms to choose peptide(s) for immunization and established the pre-clinical evaluation needed to develop a peptide-based tumor vaccine. Secondly, the importance of class II peptides in active immunization is being increasingly defined. The generation of an endogenous $CD4^+$ T cell response will improve the magnitude and maintenance of $CD8^+$ immunity generated with class I binding epitopes. Finally, methods have been developed to modify peptides to improve the immunogenicity of epitope specific vaccines.

In our studies, the P10s peptide (WRYTAPVHLGDG) was modified being three residues shorter than the parent peptide P10 (GVVWRYTAPVHLGDG) peptide, optimized for antibody binding. Both P10 and P10s are predicted binders for class II and class I HLA, and thus potential Th and CTL epitopes, respectively. The P10s peptide was optimized with regard to antibody binding, but P10 proves to carry more and more diverse HLA binding motives. The predicted HLA class I and class II binding motifs are summarized in the **Table 2** below. Out of 41 studied class I alleles 16 are predicted to bind 10 motifs common for the P10s and P10 sequences while 6 other alleles bound motifs that are found only in P10. Out of 51 studied class II alleles 22 are predicted to bind common motifs, while P10 had specific motifs that were predicted to bind to 14 more alleles. Thus, it could be expected that P10 is a more promiscuous T cell epitope both for Th and TCL lymphocytes and could induce T cells in different genetic background. The software used (PROPRED1 - http://www.imtech.res.in/raghava/propred1/index.html for class I and HLApred for class II http://www.imtech.res.in/raghava/hlapred/index.html by Dr. G.P.S. Raghava's group) provided an option for BLAST search of similar sequences in the human genome but none were found.

Epitopes recognized by $CD8^+$ T cells are, in general, peptides of 8-10 amino acids in length anchored at each end within the major groove of MHC class I molecules. As a consequence, class I molecules exhibit more amino acid sequence specificity based on these anchoring residues, than class II molecules which may be more promiscuous in the binding of peptides. Most investigators have focused on defining class I epitopes for HLA-A2 molecules. Most likely the interest in HLA-A2 is fueled by the increased prevalence of this allele in many human populations. However, as early studies of peptide-based vaccines have shown that cancer patients can be immunized against a variety of tumor antigens more studies are focusing on HLA-A alleles other than A2 as well as HLA-B motifs. A potential problem in the development of CTL epitope-based vaccines is the large degree of MHC polymorphism and the need for knowledge of HLA restrictions in the population to be vaccinated. However, it is now known that that HLA class I molecules can be divided into several families or supertypes based on similar peptide-binding repertoires. For example, the A2 supertype consists of at least eight related molecules and of these, the most frequently observed are HLA-A*0201, A*0202, A*0203, A*0206, and A*6802. In addition, the A2 supertype is expressed in all major ethnicities; 39-46% range of most common populations. Many peptides that bind A*0201 also exhibit degenerate binding, i.e. binding to multiple alleles, thus, an A2 supertype multi-epitope vaccine could be designed to provide broad population coverage. Recent investigations have demonstrated peptides that bind

HLA-A0201 with high affinity will cross-react with other A2 family alleles. Studies of in vitro binding of peptides to other HLA-A molecules demonstrated over 70% of peptides that bound HLA-A0201 with high affinity bound at least two other members of the A2 superfamily. Although class I motifs have been assumed to be very specific and restrictive, studies such as those described above suggest large overlaps in specificity can be found. As newer clinical trials translate these in vitro observations of the broadening of class I activity in vivo, it may be that class I peptide vaccines will be less restrictive in use than what had been previously assumed.

Table 2. HLA class I and II binder motifs within the P10/P10s sequence were predicted using PROPRED1 (for classs I) and HLApred (for class II) software. *The residues in red mark the beginning of a binding motif while the rest of the residues of motif are colored in blue. Only the "binders" are shown as determined by the algorithm using the default settings.*

HLA Class I		HLA Class II	
Allele	**Binding motif**	**Allele**	**Binding motif**
HLA-A2	GVVWRYTAPVHL	HLA-DRB1*0101	GVVWRYTAPVHLGDG
HLA-A*0201	GVVWRYTAPVHL	HLA-DRB1*0102	GVVWRYTAPVHLGDG
HLA-A*0205	GVVWRYTAPVHL	HLA-DRB1*0305	GVVWRYTAPVHLGDG
HLA-A2.1	GVVWRYTAPVHL	HLA-DRB1*0309	GVVWRYTAPVHLGDG
HLA-B14	GVVWRYTAPVHL	HLA-DRB1*0401	GVVWRYTAPVHLGDG
HLA-B*2702	GVVWRYTAPVHL	HLA-DRB1*0402	GVVWRYTAPVHLGDG
HLA-B*2705	GVVWRYTAPVHL	HLA-DRB1*0405	GVVWRYTAPVHLGDG
HLA-B*3901	GVVWRYTAPVHL	HLA-DRB1*0421	GVVWRYTAPVHLGDG
HLA-B*3902	GVVWRYTAPVHL	HLA-DRB1*0426	GVVWRYTAPVHLGDG
HLA-B*5102	GVVWRYTAPVHL	HLA-DRB1*0701	GVVWRYTAPVHLGDG
HLA-B*5103	GVVWRYTAPVHL	HLA-DRB1*0703	GVVWRYTAPVHLGDG
HLA-B*5201	GVVWRYTAPVHL	HLA-DRB1*0801	GVVWRYTAPVHLGDG
HLA-B*5301	GVVWRYTAPVHL	HLA-DRB1*0802	GVVWRYTAPVHLGDG
HLA-B*5401	GVVWRYTAPVHL	HLA-DRB1*0804	GVVWRYTAPVHLGDG
HLA-B*51	GVVWRYTAPVHL	HLA-DRB1*0806	GVVWRYTAPVHLGDG
HLA-Cw*0301	GVVWRYTAPVHL	HLA-DRB1*0813	GVVWRYTAPVHLGDG
		HLA-DRB1*0817	GVVWRYTAPVHLGDG
		HLA-DRB1*1101	GVVWRYTAPVHLGDG
		HLA-DRB1*1102	GVVWRYTAPVHLGDG
		HLA-DRB1*1114	GVVWRYTAPVHLGDG
		HLA-DRB1*1120	GVVWRYTAPVHLGDG
		HLA-DRB1*1121	GVVWRYTAPVHLGDG
		HLA-DRB1*1128	GVVWRYTAPVHLGDG
		HLA-DRB1*1301	GVVWRYTAPVHLGDG
		HLA-DRB1*1302	GVVWRYTAPVHLGDG
		HLA-DRB1*1304	GVVWRYTAPVHLGDG
		HLA-DRB1*1305	GVVWRYTAPVHLGDG
		HLA-DRB1*1307	GVVWRYTAPVHLGDG
		HLA-DRB1*1321	GVVWRYTAPVHLGDG
		HLA-DRB1*1322	GVVWRYTAPVHLGDG
		HLA-DRB1*1323	GVVWRYTAPVHLGDG
		HLA-DRB1*1327	GVVWRYTAPVHLGDG
		HLA-DRB1*1328	GVVWRYTAPVHLGDG
		HLA-DRB1*1502	GVVWRYTAPVHLGDG
		HLA-DRB5*0101	GVVWRYTAPVHLGDG
		HLA-DRB5*0105	GVVWRYTAPVHLGDG

Similar promiscuous binding was predicted also to the mouse I-A^b and I-A^d alleles. We checked the proliferative response to P10s (WRYTAPVHLGDG) and to the parent peptide P10 (GVVWRYTAPVHLGDG) in P10s immunized mice. The splenocyte cultures were stimulated

for 10 days with bone marrow derived mouse dendritic cells loaded with the appropriate peptides and driven to maturation by LPS containing monocyte conditioned medium. After one week of stimulation the cells were labeled with [^{3}H] thymidine for 16h, harvested on filters and the incorporated radioactivity measured by beta-counter (Wallac 1450 MicroBeta TriLux). Splenocytes from the P10s immunized animals showed higher proliferative response (**Figure 6**).

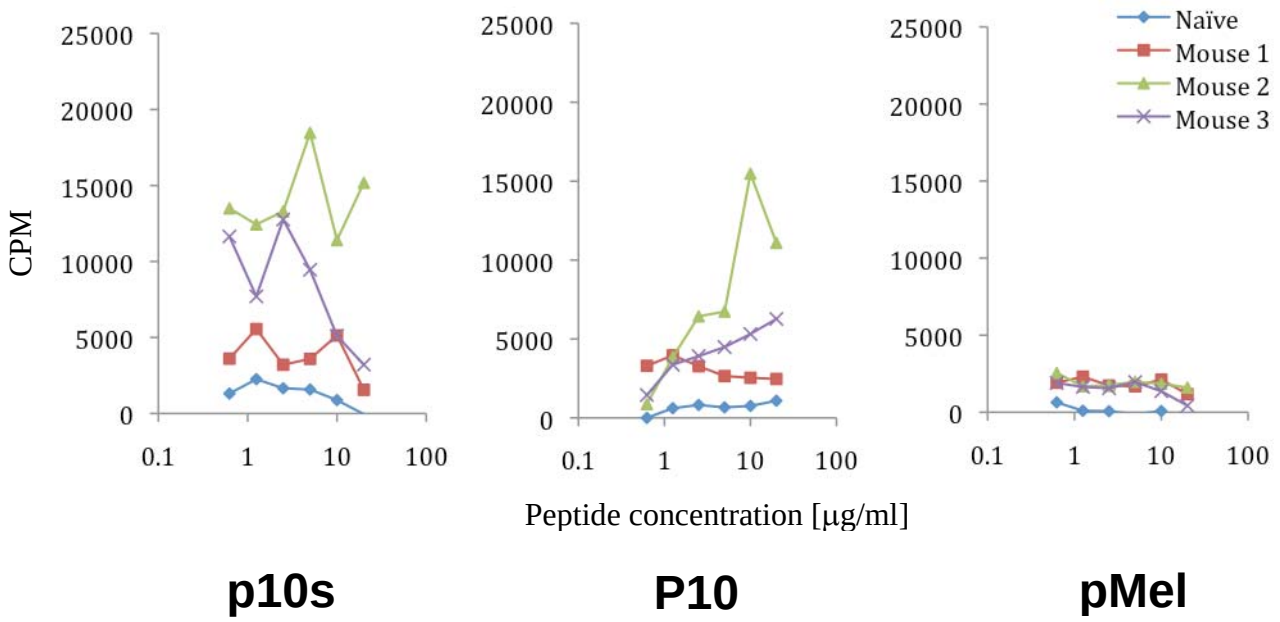

Figure 6. ***P10s activates T cells.*** *Proliferative response of splenocytes from BALB/c mice immunized s.c. 5 times at two weak intervals with P10s/QS21 after stimulation with P10s, P10 and another peptide mimotope with overlapping T cell epitope containing an YRY motive. Splenocytes were stimulated in vitro for 10 days with bone marrow derived dendritic cells, loaded with the peptides and driven to maturation with monocyte conditioned medium and LPS. During the last 16h the cultures were labeled with [^{3}H] thymidine, the cells harvested onto filters and the radioactivity was read using beta counter.*

The T cell involvement in the antitumor responses, induced by P10s, is probably mostly indirect by recruiting the help of Th cells to carbohydrate reactive B cells that recognize the mimotope. At the same time the presence promiscuous CTL epitopes prompted us to check also the possibility that anti-P10s CTL are directly involved in tumor cell killing by cross-reactivity with a putative tumor antigen. To this end, we had the VVWRYTAPVHLGDG version of the peptide synthesized and coupled to HLA-A*0201 pentamers labeled with phycoerythrin (Proimmune, Oxforde, UK). We checked the frequency of the preexisting CTL clones in the peripheral blood of normal (non-tumor bearing) donors and they were barely detectable. On the other hand we could detect P10s/A*0201 specific CD8 T cells in other donors, which may be due to allorectivity. Nevertheless, the reactivity seems to be specific as the frequency of the positive cells indicates (**Figure 7**). This approach however provides us with a reliable method to identify and validate the frequency of induced CTLs as a surrogate in the vaccination protocol.

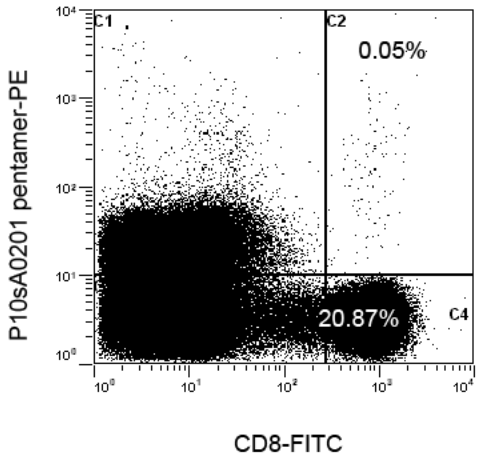

Figure 7. P10s can isolate T cells. *Staining with P10s/A*0201 pentamers of CD8 positive peripheral mononuclear cells from a healthy non-immunized donor of unknown HLA haplotype formula. The frequency of the pentamer positive cells is typical for antigen specific lymphocyte clones.*

These pentamer positive $CD8^+$ cells were sorted by flow cytometry and stimulated with allogeneic peripheral mononuclear cells in the presence of IL-2 and IL-15 as well as with A*0201 monocyte derived dendritic cells loaded with P10s and in the presence IL-4. The CTL line is being established at the moment and we shall test the specific cytotoxicity of these cells for breast cancer tumor cell lines MDA-231 and MCF7. If this proves true, allogeneic, P10s specific T cells may be used by themselves as an antitumor agent in the context of mismatched CTL treatment of A*0201 patients. We are acquiring a MHC Class II pentamer coupled with P10s and P10 to be used in monitoring the frequency of T cells in P10s-PADRE immunized subjects in our Phase I studies.

Subtasks 6-10 will commence once we have FDA approval of the IND.

KEY RESEARCH ACCOMPLISHMENTS

• P10s appears to enrich for antibodies with catalytic properties exposing the Tn antigen for interaction with NK cells.
• P10s immunization displays a safety profile sufficient for clinical studies.

REPORTABLE OUTCOMES

None

CONCLUSION

In year three, we had experienced delays that followed from year 2. The biggest issue is that we are bringing forward a new peptide analog. This peptide scales up to manufacture clinical grade material and displays anti-tumor efficacy but is mechanistically different from our lead mimotope. Within this year, we have finalized the GLP studies, developing the infrastructure required to conduct the preclinical studies. In the GLP studies, the peptide displays a safety profile that should not preclude it from clinical study. Sign off on the GLP final report by the UAMS Quality Control and Assurance group is expected at the end of May 2009. The IND application is nearing final draft status and it is expected that submission to the FDA will occur at the end of June 2009. We anticipate we shall be able to start our Phase IA studies in Sept-Oct 2009. We have identified an unexpected and novel property of this carbohydrate mimetic peptide analog. It seems that the antibodies induced by the mimotope enhances for catalytic antibodies that unmask carbohydrate epitopes that allows for NK cells to infiltrate into tumor sites. A patent disclosure is being filed on this concept. This finding indicates that P10s will be a medical product with unique properties.

References:

None

Appendices I Preclinical

UNIVERSITY OF ARKANSAS
FOR MEDICAL SCIENCES

Final Report

Study Title:

Vaccination of High-Risk Cancer Patients with Carbohydrate-Mimicking Peptides

Protocol Number:

2935

Sponsor Name and Address:
U.S. Army Medical Research and Material Command
Fort Detrick, Maryland 21702-5012

Testing Facility Name and Address:
Central Arkansas Veterans Healthcare System John L. McClellan Memorial Veterans Hospital
4300 W 7th St
Little Rock, AR 72205

Test Site Name and Address:
Central Arkansas Veterans Healthcare System John L. McClellan Memorial Veterans Hospital
4300 W 7th St
Little Rock, AR 72205

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2 **GLP Compliance Statement:**

3 **Quality Assurance Statement:**

UAMS **Office of Breast Cancer Research**

Quality Assurance Statement

The Quality Assurance Unit of the UAMS Research Support Center (RSC), formerly the office of Research Support and Regulatory Affairs, was designated by study management to perform the duties of the quality assurance unit for the non-clinical laboratory study entitled "*Determination of the Safety and Tolerability of Immunization with a LeY Peptide Mimotope Vaccine in Mice.*"

In-Phase inspections were conducted on seven separate occasions throughout the study to assure the integrity of the data collected and to confirm that the study protocol and standard operating procedures were followed as written. Written reports of each inspection were forwarded to the study director and study management indicating any findings resulting from the inspection. No deviations from approved protocols or standard operating procedures were made without proper authorization and documentation.

The inspection in-phase inspection calendar is shown below:

UAMS QAU In-Phase Inspection Calendar

Date of Inspection	Phase of Study Inspected	Person Performing Inspection	Date of Inspection Report
5/23/08	Week -1: Quarantine	R.C. Anderson	5/29/08
5/27/08	Week 1: Immunization I	R.C. Anderson	5/30/08
6/09/08	Week 3: Necropsy I	R.C. Anderson	6/09/08
7/8-9/08	Week 7: Immunization IV	Tracy L. Gatlin	7/10/08
7/21/08	Week 9: Necropsy II	Tracy L. Gatlin	7/21/08
9/30/08	Week 19: Immunization V	Tracy L. Gatlin/ R.C. Anderson	10/01/08
10/13/08	Week 21: Necropsy III	Tracy L. Gatlin	10/15/08

R.C. Anderson 5/19/09

R.C. Anderson
Research Support Center
Quality Assurance Manager

Date

Tracy L. Gatlin 5/19/08

Tracy L. Gatlin
Research Support Center
Good Tissue Practice Specialist

Date

Original document is located in the Appendix

4. Study Dates and Responsible Personnel:

Key GLP Study Dates

Monday May 5, 2008	Mice ordering
Friday May 9, 2008	Study Initiation Date
Monday May 12, 2008	Mice arrival
From Monday May 12- 26, 2008	Quarantine
Monday May 26, 2008	Group assignment
Monday May 27, 2008	First Immunization
Tuesday June 3, 2008	Second Immunization
Monday June 9, 2008	First sacrifice
Tuesday June 10, 2008	Third Immunization
Tuesday July 1, 2008	Tail bleeding
Tuesday July 8, 2008	Fourth Immunization
Monday July 21, 2008	Second Sacrifice
Tuesday Sept 23, 2008	Tail bleeding
Tuesday Sept 30, 2008	Fifth Immunization
Monday October 13, 2008	Third Sacrifice
Wednesday May 20, 2009	Study Completion Date
Draft	Final Report

Key Personnel

Artaud, Cecile	Study Coordinator
Gomes, Tina	Research Technologist
Hennings, Leah	Protocol Director
Hutchins, Laura	Clinical Director
Jousheghany, Fariba	Research Assistant
Karbassi , Behjatolah	Research Associate
Kieber-Emmons, Thomas	Study Director
Milton, Lateefah	Project Manager
Siegel, Eric	Biostatistician
Anderson, Raymond C	Quality Assurance Manager
Gatlin, Tracy	GTP/ GLP Specialist
Henning, Kimberly	Research Technologist
James, Jennifer	Research Assistant
Mullin, Shirley A	Research Assistant
Woods, Howard B	Research Technologist

5 **Summary**

The current study was performed to determine the safety and tolerability of the administration of immunogenic mimetic peptides, which were designed to induce immune responses specific for tumor associated carbohydrate antigens (mimotopes), in mice. Cancer vaccines are argued to facilitate tissue damage in a manner akin to the induction of autoimmunity. Carbohydrate targeting tissue damage is best typified by the natural antibody response directed against the alpha-Gal epitope, a major barrier in porcine-to-human xenotransplantation. The peptide P10s, that mimics a broad spectrum of tumor associated carbohydrate antigens (TACAs), is potent enough to induce an anti-tumor response. Therefore, a preclinical safety study was performed to provide a gross characterization of the nature, frequency and severity of adverse responses following P10s-based vaccine administration in a tolerant mouse setting. The preclinical study provides an initial basis for determining whether the vaccine exhibits a safety profile appropriate for further study. A dose escalation study was performed in groups of 8 mice using QS-21 as adjuvant, with an adjuvant control group. Each treatment was administered at weeks 1, 2, 3, 7, and 19 for a total of five treatments at each dose, to closely mimic the proposed Phase 1 study design. Treatment and control animals were monitored twice weekly for injection-site reactions and changes in weight or general health status. Measurements included urinalysis and histological assessment of tissues from control and treatment mice after the 5 immunizations. There was no evidence of pathological tissue damage in any of the immunized mice. These results demonstrate that vaccination with P10s-based vaccine can enhance responses to TACAs without inducing immune pathology.

6 **Introduction**

We had defined a series of carbohydrate mimetic peptides (mimotopes) that induced tumor-directed, carbohydrate-reactive humoral and cellular responses in experimental animals[1-3]. These studies demonstrated that immunization with mimotopes reduced tumor burden and prolonged survival in mice. Paramount to these studies is the identification of mimotopes that contain the amino acid sequence tract Trp-Arg-Tyr which are mimics of each other in that they induce similar sets of TACA reactive antibodies and also have overlapping T cell specificities. In the current study we determined the safety and tolerability of the administration of a carbohydrate mimetic peptide, defined as P10s with the amino acid sequence WRYTAPVHLGDG which was designed to enhance TACA specific immune responses against cancer breast cancer cells[4]. P10s is a higher fidelity mimic of TACA compared to the homologue peptide P10 with the amino acid sequence GVVWRYTAPVHLGDG which also induces anti-tumor responses [5]. Since P10s enhances responses to TACA, which are essentially self-antigens, safety profile studies with a P10s-based vaccine provides the worst-case scenario concerning the potential of antibody-mediated pathology. This safety and tolerability study in mice was a required step towards developing these immunogens for clinical use as an immunotherapy to prevent recurrence of breast cancer in high-risk breast cancer patients, with the ultimate goal of impacting relapse and prolonging survival. The preclinical safety study followed a master list of Standard Operating Procedures (SOPs)(Appendix).

7 Materials and Methods

7.1 Test Material:

Peptide: P10s-PADRE (Vaccine)

Peptide Seq: WRYTAPVHLGDG-aK-Cha-VAAWTLKAAa
Capital letters – one letter code for L-amino acids
Small letters – one letter code for D-amino acids
Cha - Cyclohexylalanine

This peptide, P10s mimotope covalently linked with PADRE, was synthesized by NeoMPS Inc (San Diego, CA). PADRE is a synthetic, non-natural Pan HLA-DR binding peptide that binds with high or intermediate affinity to 15 of 16 of the most common HLA-DR types tested to date. Because of its binding promiscuity, PADRE should overcome the problems posed by the extreme polymorphism of HLA-DR molecules in the human population. Furthermore, the PADRE peptide was specifically engineered to be immunogenic in humans. This property represents another significant feature of PADRE, suggesting its potential utility as a carrier to induce T cell "help" in vaccine constructs designed for human use.

Adjuvant

QS-21 is an immunological adjuvant. Immunological adjuvants can modulate the humoral (i.e., stimulation of antibody quantity, avidity, affinity, persistence, and/or isotype switching) and/or cellular [(i.e., stimulation of delayed-type hypersensitivity and cytotoxic T lymphocytes (CTL)] immune responses to vaccine antigens. QS-21 has been shown to stimulate both humoral and cell-mediated immunity. QS-21 is a naturally occurring saponin molecule purified from the South American tree *Quillaja saponaria* Molina. It is a triterpene glycoside with the general structure of a quillaic acid 3, 28-O-bis glycoside with the formula $C_{92}H_{148}O_{46}$, and a molecular weight of 1990. QS-21 was supplied in solid powder in an amber glass vial containing 10 mg by Antigenics (Framingham, MA).

7.2 Test Article (Vaccine):

- The P10S-PADRE, 200 mg, Lot No. XF207//042-31-12 was received on 4/18/08 from NeoMPS Inc in powder condition and was stored frozen at ≤-20º C+/-5°C for maximum stability until its use. Temperature logs were maintained and recorded on business days (except holidays) from date of receipt.

7.3 **Control Article, buffer and water:**

- QS-21, 10 mg, Lot No. IF-001-198 was received from Antigenics Inc on 12/16/06 in powder condition and was stored at ≤ -20°C +/-5°C. The shelf life at this storage condition is four (4) years. The expiration date for QS-21 was 5/19/2010 and listed on the vial label and on the certificate of analysis. Temperature logs were maintained and recorded on business days (except holidays) from date of stock solution preparation. Logs were treated as raw data.

- DPBS, 1X (Dulbecco's Phosphate Buffer Saline) without calcium and magnesium, volume 1 L, sterile, Cat. No.21-031-CM, Lot No. 21031279 from Mediatech, Inc., Expiration date. 03/2011, stored at room temperature.
- Distilled, Deionized water, volume 1L, sterile, Cat No. 25- 055-CM, Lot No.25055246, from Mediatech, Inc., Expiration date. 08/2009, stored at room temperature.

7.4 **Test and Control Article Characterization:**

- The P10s-PADRE, 200 mg, Lot No. XF207//042-31-12 was received on 4/18/08 from NeoMPS Inc in powder condition. The sequence of the peptide was H-WRYTAPVHLGDG-aK-Cha-VAAWTLKAAa-NH2 with 96.3% purity, 5% acetate, 6% water and endotoxin concentration of less than 0.41 unit (EU)/mg. The peptide was stored at <-20º C+/-5°C upon arrival for maximum stability until its use.
- QS-21, 10 mg, Lot No. IF-001-198 was received from Antigenics Inc on 12/16/06 in the form of a white powder. The purity of QS-21 in this lot was 99% with less than 20μg of protein in 1mg of QS-21, 3% residual moisture and less than 16 ppm residual solvent. Endotoxin level was less than 1.25 EU/mg, with a bioburden of <1 CFU/mg. The QS-21 was stored at <-20°C +/-5°C according to manufacturer's recommendation.The data of manufacture was 05/19/06 and the shelf life at the above storage temperature is four (4) years. The expiration date for QS-21 was 5/19/2010. Temperature logs were maintained and recorded on business days (except holidays) from date of stock solution preparation.

7.5 **Inventory and Disposition**:

In 1st immunization 30mg P10s –PADRE was weighed and used for immunization. In order to have some extra vaccine preparation to compensate for handling loss, we decided to aliquot P10s- PADRE peptide in aliquots of 35mg for the 2nd immunization, 25mg for 3rd and 4th immunization and 15mg for 5th immunization. For the whole experiment, 130 mg P10S-PADRE was used, 70mg of the peptide left that is stored at <-20º C+/-5°C GLP Freezer.

20 aliquots of QS-21 (2mg/ml, 250μl/ aliquot) were stored at <-20º C+/-5°C GLP freezer. After 1st immunization, we realized that for total immunization we needed more QS-21. We added 6 more aliquots of QS-21,

Lot No. IF-00-198, (2mg/ml), 250µl/aliquot. From number 1 to 22 of QS21 aliquots used for all immunization and 4 (from 23 to 26) excess aliquots stored at <-20º C+/-5°C GLP freezer.

7.6 **Preparation of Dose Formulations**

The test material was prepared according to the Standard Operating Procedure IMM002. Briefly, 20µg of QS-21 per mouse admixed with the appropriate quantity of vaccine (100µg, 300µg and 500µg/mouse) in sterile phosphate buffered saline. The syringe was loaded with the vaccine mixture in the animal procedure room just before its application. Lab personnel wore suitable protective clothing such as laboratory coat and gloves according to UAMS policy.

7.7 **Analysis of Dose Formulations**

7.7.1 **Stability:**

Peptide was prepared freshly from powder that was stored at <-20º C+/-5°C, an aliquot of QS-21 was thawed at room temperature and promptly admixed with peptide and the mixture was kept on ice. PBS was stored at room temperature, mixed with thawed QS-21 and kept on ice. Syringes of test material (peptide/QS-21) and control material (PBS/QS-21) were loaded right before injection.

7.8 **Test System:**

- Test system: Mouse
- Number of animals: 106 mice (10 extra mice were ordered)
- Body weight range: 15-25 grams
- Sex: Female
- Strain: BALB/c
- Age of the test system upon receipt: 4 to 6 weeks
- Source of supply. Charles River Laboratoires International, Inc. (Wilmington, MA)

7.9 **Receipt and Description, Housing and Animal Identification:**

Female, 4-6 weeks old BALB/c mice were purchased from Charles River Laboratories Inc (Wilmington, MA). Upon arrival the mice were quarantined for two weeks (Standard Operating Procedure ANCA007). The animals were housed in the animal facility located at the Veterinary Medical Unit of the VA hospital. The animal receiving and care were conducted according to Standard Operating Procedure ANCA003 and ANCA002 respectively.

Animals were housed in group cages holding no more than 4 mice/cage according to Standard Operating Procedure ANCA002. The animals were identified using an ear notching system and according to Standard Operating Procedure ANCA009. A log assigning animal notch number and cage number to their respective group were maintained.

7.10 **Justification of Test System and Number of Animals:**

The use of peptide mimotopes of TACA provides an alternative approach to generating responses against TACA because, unlike TACA, protein surrogates are T-cell-dependent antigens. Short peptides encoding an epitope capable of binding an anti-TACA antibody, which mimic an unrelated structure, are termed mimetics. Mimetics contain key chemical groups spatially arranged in a conformation that allows cross-reactivity with an anti-TACA antibody. Mimetics that induce cross-reactive responses to TACA are called mimotopes. Mimotopes function by selecting *in vivo* for antibodies that have similar binding properties as TACA. Not all peptides capable of binding to the variable region of an antibody are mimotopes. To truly be considered a mimotope, the peptide must be capable of generating antibodies *in vivo* that recognize the original carbohydrate antigen. Peptides that simply bind the antibody, but do not generate an appropriate immune response, are termed mimetics. Importantly, in preclinical prophylactic and therapeutic vaccination studies, peptide mimotopes of TACA were efficacious in eliciting immune responses that reduced tumor burden and inhibited metastatic outgrowth [1-3, 5]. Thus, peptide mimotopes of TACA represent a new and very promising tool to overcome T-cell independence of TACA and to increase the efficiency of the immune response to glycan antigens.

We have shown that rational design principles can be used to optimize mimotopes to induce more robust TACA-reactive antibodies[4]. Among the mimotopes we have developed are a series that contain the amino acids Trp-Arg-Tyr as a centralized motif. Mimotopes with this motif display an ability to induce antibodies cross-reactive with tumor cells [1, 2], induce cellular responses to tumor cells [3, 5, 6]and induce or activate natural killer (NK) cells with anti-tumor activity (unpublished observations). Preclinical efficacy studies in mice (not under GLP conditions) with vaccines containing P10s (with the sequence WRYTAPVHLGDG) or P10 (a mimotope that contains the entire P10s sequence but is three amino acids longer - GVVWRYTAPVHLGDG) have demonstrated these mimotopes induce a robust immunogenic response that includes cross-reactivity with breast cancer cell lines, stimulation of tumor cell reactive cellular responses and/or stimulation of tumor targeting NK cells. Although the mechanism of action appears to vary depending upon the peptide (P10s or P10), coupling agent and adjuvant (KLH vs. PADRE and QS-21) employed, all vaccines tested in mice to date that contain P10s or P10 have consistently inhibited metastatic outgrowth of murine tumor cells expressing TACA structural homologues.

Antibodies raised against our P10s-PADRE vaccine are tumor reactive, and thus contribute, along with NK activation, to immune surveillance reminiscent of anti-pathogen vaccines. NK cells recognize many tumor

cells but not normal self cells, and they are thought to aid in the elimination of nascent tumors. The major function of NK cells in fighting cancer is likely to be in surveillance and elimination of cells that become malignant before they can cause a tumor. Collectively, these data provide the experimental foundation for evaluating peptide mimotopes as potential cancer vaccines in patients with breast cancer.

P10s has been shown to bind to monoclonal antibodies reactive with the LeY antigen, and to monoclonal antibodies reactive with the gangliosides GD2 and GD3. P10s has been shown to compete with the LeY antigen for anti-LeY antibody binding, and has shown to compete with GD2 for binding to anti-GD2 antibody. P10s reacts with antibodies that are also cross-reactive with TF and TN antigens. P10s therefore cross-reacts with several different classes of TACA-reactive monoclonal antibodies and human antibodies, suggesting that P10s is a broad-spectrum mimetic.

The preferred animal model for toxicity testing is an animal expressing the relevant tumor antigen. The neolactoseries antigen LeY is not expressed in mice, but the ganglioside GD2, GM2 and GD3 and GM3, also mimicked by mimotope P10s [4], are endogenously expressed on murine tumors. Therefore, we performed a preclinical safety study to provide a gross characterization of the nature, frequency, and severity of adverse responses observed following P10s-based vaccine administration in this tolerant mouse setting. The preclinical study provides an initial basis upon which to determine the vaccine safety profile in a manner to support Phase 1 clinical testing of a P10s-based vaccine.

Sample-size calculations for the planned study were motivated by the fact that, from each planned necropsy, we were to screen organ weights and a large number of hematologic and serum-chemistry endpoints via Kruskal-Wallis (KW) test (for any dose-group difference) and Spearman correlation analysis (for trend with dose) using a multiple-comparison-adjusted alpha of 0.01. Because one form of the KW test consists of one-way ANOVA conducted on ranks, KW sensitivity calculations were made via power calculations for one-way ANOVA conducted on quantiles (ranks divided by sample size); under the null hypothesis of no difference among groups, each dose group's quantiles will be distributed in discrete approximation to the Uniform [0, 1] distribution, which has standard deviation (SD) equal to 0.2887. For the screening tests to be considered sufficiently sensitive, it was required (a) that an individual KW test should yield a P of 0.01 or less when the dose groups' mean quantiles showed a dispersion of $\geq$0.173 (an effect size of 0.600 SDs or greater), and (b) that an individual Spearman correlation analysis should yield a P of 0.01 or less when the correlation coefficient was greater in magnitude than ±0.45. Minimum significant values of the means dispersions and Spearman correlations (i.e., minimum values that would yield $P \leq 0.01$) were made using PASS 2005 software by setting beta equal to 0.50 and alpha equal to 0.01. At each planned necropsy, there would be data

from four dose groups. With a sample size of 8 mice per group (for a subtotal of 32 mice per necropsy), the dispersion in mean quantiles has a minimum significant value of 0.172 (a 0.597-SD effect size), while the absolute value of the Spearman correlation coefficient has a minimum significant value of 0.443. On the other hand, with a sample size of only 7 mice per group (for a subtotal of only 28 mice per necropsy), the dispersion in mean quantiles has a minimum significant value of 0.187 (a 0.649-SD effect size), while the absolute value of the Spearman correlation coefficient has a minimum significant value of 0.471. These minimum-significant-value calculation show that 8 mice per group provide both the KW tests and the Spearman correlations with sufficient sensitivity for screening purposes, whereas 7 mice per group do not. Eight mice per dose group (for a subtotal of 32 mice per necropsy) is thus the minimum number of animals needed by the study to provide the large number of screening tests with the required level of sensitivity. With three planned necropsies, an overall total of 96 mice is thus the minimum number required to meet the study's screening objectives.

The study groups include: **Adjuvant control:** 20µg QS-21 per mouse in 100 µl of PBS. **Mimotope-PADRE (Vaccine) and adjuvant:** 100 µg, 300 µg, 500 µg of P10s-PADRE vaccine per mouse was admixed with QS-21 adjuvant (20 µg/mouse) in 100 µl of PBS. There were four dosing groups (3 test and 1 control). 24 mice were assigned to each dosing group and subsequently sacrificed over 3 time points at a rate of 8 mice per dosing group per time point. The total number of animals used was 96 mice.

7.11 **Veterinary Care:**

The animals were housed in the animal facility located at the Veterinary Medical Unit (VMU) of the VA hospital. The animals receiving and care were conducted according to Standard Operating Procedure ANCA003 and ANCA002 respectively.

7.12 **Assignment to Study Groups:**

Upon their receipt, the animals were quarantined for two weeks. After the two week period, each animal was assigned randomly to a dose-group cage. Each cage (and thus the block of four animals per cage) was assigned randomly to a sacrifice time, while pre-sacrifice transfer to a urine-collection cage was assigned randomly to the individual mice in each combination of dose group by sacrifice time. The randomization schedule for assignment to peptide treatment, sacrifice time, and urine-collection caging are shown in Tables A, B, and C, respectively, and were generated by a block randomization scheme implemented in Microsoft® Office Excel 2003. Block sizes were 4 for peptide P10s-PADRE, 3 for sacrifice times, and 8 for urine-collection caging. Animals had their ears punched according to the group assignment tables. Animals and individual animal data record where then tracked by the naming convention of dose-cage designation, and ear-mark. For example mouse 100-1A-L1 means a mouse in the 100 ug dose

group, housed in Cage 1A, and identified with one hole in the left ear. Ear punches correspond to no holes “0”, Left ear “L1”, Right ear “R1”, “B1” corresponds to one hole in each ear.

The study groups were as follows: **Adjuvant control:** 20µg QS-21 per mouse. **Mimotope (Test article) with adjuvant:** 100 µg, 300 µg, 500 µg per mouse.

Table 7.12.1 Group Assignment. In the Cage column Control or Dose group is indicated.

Mouse Order	Punch	Cage #	Mouse Order	Punch	Cage #	Mouse Order	Punch	Cage #
1	“0”	300 µg, 1A	**37**	“L1”	500 µg, 2A	**73**	“R1”	500 µg, 3A
2	“0”	Control, 1A	**38**	“L1”	Control, 2A	**74**	“R1”	300 µg, 3A
3	“0”	100 µg, 1A	**39**	“L1”	300 µg, 2A	**75**	“R1”	100 µg, 3A
4	“0”	500 µg, 1A	**40**	“L1”	100 µg, 2A	**76**	“R1”	Control, 3A
5	“L1”	300 µg, 1A	**41**	“R1”	100 µg, 2A	**77**	“B1”	500 µg, 3A
6	“L1”	Control, 1A	**42**	“R1”	Control, 2A	**78**	“B1”	100 µg, 3A
7	“L1”	100 µg, 1A	**43**	“R1”	300 µg, 2A	**79**	“B1”	Control, 3A
8	“L1”	500 µg, 1A	**44**	“R1”	500 µg, 2A	**80**	“B1”	300 µg, 3A
9	“R1”	300 µg, 1A	**45**	“B1”	500 µg, 2A	**81**	“0”	Control, 3B
10	“R1”	500 µg, 1A	**46**	“B1”	100 µg, 2A	**82**	“0”	100 µg, 3B
11	“R1”	Control, 1A	**47**	“B1”	300 µg, 2A	**83**	“0”	300 µg, 3B
12	“R1”	100 µg, 1A	**48**	“B1”	Control, 2A	**84**	“0”	500 µg, 3B
13	“B1”	500 µg, 1A	**49**	“0”	300 µg, 2B	**85**	“L1”	Control l, 3B
14	“B1”	300 µg, 1A	**50**	“0”	500 µg, 2B	**86**	“L1”	100 µg, 3B
15	“B1”	100 µg, 1A	**51**	“0”	100 µg, 2B	**87**	“L1”	500 µg, 3B
16	“B1”	Control, 1A	**52**	“0”	Control, 2B	**88**	“L1”	300 µg, 3B
17	“0”	300 µg, 1B	**53**	“L1”	100 µg, 2B	**89**	“R1”	500 µg, 3B
18	“0”	500 µg, 1B	**54**	“L1”	500 µg, 2B	**90**	“R1”	Control, 3B
19	“0”	Control, 1B	**55**	“L1”	Control, 2B	**91**	“R1”	100 µg, 3B
20	“0”	100 µg, 1B	**56**	“L1”	300 µg, 2B	**92**	“R1”	300 µg, 3B
21	“L1”	Control, 1B	**57**	“R1”	Control, 2B	**93**	“B1”	100 µg, 3B
22	“L1”	100 µg, 1B	**58**	“R1”	100 µg, 2B	**94**	“B1”	300 µg, 3B
23	“L1”	500 µg, 1B	**59**	“R1”	300 µg, 2B	**95**	“B1”	500 µg, 3B
24	“L1”	300 µg, 1B	**60**	“R1”	500 µg, 2B	**96**	“B1”	Control, 3B
25	“R1”	500 µg, 1B	**61**	“B1”	500 µg, 2B			
26	“R1”	100 µg, 1B	**62**	“B1”	Control, 2B			
27	“R1”	Control, 1B	**63**	“B1”	300 µg, 2B			
28	“R1”	300 µg, 1B	**64**	“B1”	100 µg, 2B			
29	“B1”	100 µg, 1B	**65**	“0”	300 µg, 3A			
30	“B1”	Control l, 1B	**66**	“0”	Control, 3A			
31	“B1”	500 µg, 1B	**67**	“0”	100 µg, 3A			
32	“B1”	300 µg, 1B	**68**	“0”	500 µg, 3A			
33	“0”	100 µg, 2A	**69**	“L1”	100 µg, 3A			
34	“0”	500 µg, 2A	**70**	“L1”	300 µg, 3A			
35	“0”	300 µg, 2A	**71**	“L1”	500 µg, 3A			
36	“0”	Control, 2A	**72**	“L1”	Control, 3A			

Table 7.12.2 Sacrifice order

Cage #	Sacrifice order
100-2A	1
100-3B	1
100-3A	2
100-1B	2
100-1A	3
100-2B	3
300-1A	1
300-1B	1
300-3A	2
300-3B	2
300-2A	3
300-2B	3

Cage #	Sacrifice order
500-3A	1
500-3B	1
500-1A	2
500-1B	2
500-2A	3
500-2B	3
Control-2A	1
Control-1B	1
Control-3A	2
Control-3B	2
Control-1A	3
Control-2B	3

Table 7.12.3. Urinalysis assignment

Cage-punch	Assignment	Cage-punch	Assignment	Cage-punch	Assignment
100-3A-O	8	100-2A-O	3	100-1A-O	5
100-3A-L1	1	100-2A-L1	2	100-1A-L1	2
100-3A-R1	4	100-2A-R1	4	100-1A-R1	3
100-3A-B1	2	100-2A-B1	6	100-1A-B1	6
100-1B-O	7	100-3B-O	5	100-2B-O	7
100-1B-L1	3	100-3B-L1	7	100-2B-L1	1
100-1B-R1	5	100-3B-R1	1	100-2B-R1	4
100-1B-B1	6	100-3B-B1	8	100-2B-B1	8
300-1A-O	4	300-2A-O	5	300-3A-O	5
300-1A-L1	3	300-2A-L1	8	300-3A-L1	6
300-1A-R1	5	300-2A-R1	6	300-3A-R1	2
300-1A-B1	6	300-2A-B1	1	300-3A-B1	3
300-1B-O	1	300-2B-O	4	300-3B-O	7
300-1B-L1	2	300-2B-L1	2	300-3B-L1	1
300-1B-R1	8	300-2B-R1	3	300-3B-R1	8
300-1B-B1	7	300-2B-B1	7	300-3B-B1	4
500-1A-O	4	500-2A-O	2	500-3A-O	4
500-1A-L1	1	500-2A-L1	7	500-3A-L1	7
500-1A-R1	3	500-2A-R1	5	500-3A-R1	3
500-1A-B1	5	500-2A-B1	4	500-3A-B1	5
500-1B-O	6	500-2B-O	1	500-3B-O	8
500-1B-L1	7	500-2B-L1	3	500-3B-L1	2
500-1B-R1	8	500-2B-R1	6	500-3B-R1	1
500-1B-B1	2	500-2B-B1	8	500-3B-B1	6
Control-1A-O	4	Control-2A-O	2	Control-3A-O	7
Control-1A-L1	7	Control-2A-L1	7	Control-3A-L1	6
Control-1A-R1	3	Control-2A-R1	8	Control-3A-R1	8
Control-1A-B1	5	Control-2A-B1	3	Control-3A-B1	1
Control-2B-O	8	Control-1B-O	5	Control-3B-O	5
Control-2B-L1	6	Control-1B-L1	6	Control-3B-L1	4
Control-2B-R1	1	Control-1B-R1	1	Control-3B-R1	3
Control-2B-B1	2	Control-1B-B1	4	Control-3B-B1	2

7.13 **Experimental Design:**

Immunization, weight measurement and observations were performed in the animal procedure room, and the study calendar was followed (Table 7.13.1).

Table 7.13.1. Study calendar+																			
Test article	EVENT	Doses Per mouse	STUDY WEEK																
			1	2	3	4	5	6	7	8	9	10	11	12	13-17	18	19	20	21
P10s-PADRE	Immunization	500 µg	24	24	16				16								8		
		300 µg	24	24	16				16								8		
		100 µg	24	24	16				16								8		
		QS-21	24	24	16				16								8		
	Euthanasia	500 µg			8						8								8
		300 µg			8						8								8
		100 µg			8						8								8
		QS-21			8						8								8
	Tail Bleeding	500 µg						16								8			
		300 µg						16								8			
		100 µg						16								8			
		QS-21						16								8			

Ninety six (96) animals were used. Animals in each dose group were injected subcutaneously with control (20µg/mouse QS-21) or test material (P10s-PADRE/QS-21) on weeks 1, 2, 3, 7, and 19 of the study. The "+" sign in table 7.13.1 indicates that the Protocol was amended after the first necropsy to allow collection of additional blood via tail vein. Therefore, tail bleeding was added to this table.

7.14 **Administration of Test Materials:**

Lab personnel wore suitable protective clothing such as laboratory coat, and gloves according to UAMS policy.

The vaccine was prepared according to the Standard Operating Procedure IMM002. 20µg of QS-21 per mouse and mixed with the appropriate quantity of peptide (100µg, 300µg and 500µg/mouse) in sterile phosphate buffered saline. The syringe was loaded with the vaccine mixture in the animal procedure room just before its use.

Immunization, weight measurement and observations were done in the animal procedure room and followed the study calendar (Table 7.12.1).

Three doses of vaccine were used: 100 µg; 300 µg; 500 µg. All immunizations were performed according to the Standard Operating Procedure IMM001. After the immunizations were completed, syringes were discarded in a biohazard disposal container. (Standard Operating Procedure SAF001).

8 Experimental Procedures

8.1 Mortality/Morbidity Checks and Clinical Observations

General health monitoring

Animals were monitored daily by experienced VMU animal care staff to assess their health and well-being. Visual inspections, as to general appearance of mice and condition of bedding, were performed per Standard Operating Procedure ANCA002. The research assistant monitored the animals twice a week for injection-site redness, swelling, heat, ulceration, or hair loss during two weeks following the immunization, then weekly until the next injection. Any abnormality noted for site injection reaction was noted on Form H from the protocol (Observation Form for Site Injection).

Mortality/Morbidity checks

Appropriate assessment techniques were performed as the following: evaluation of overall clinical condition including appearance, posture, body temperature, behavior and physiological responses; assessment of food and water intake; and weighing to determine changes in body weight. We followed a protocol that mandated euthanizing and performing necropsy on animals that became moribund or lost greater than 10% of body weight over a 2 weeks' period during the study.

8.2 Body Weights

Upon arrival at the animal care facility from the Charles River Laboratories Inc., mice were weighed according to Standard Operating Procedure ANCA003. Animals were weighed on a weekly basis thereafter on a calibrated scale per Standard Operating Procedure ANCA011 and weights were recorded.

8.3 Clinical Pathology:

Urine was collected for three days in individual metabolic cages according to Standard Operating Procedure EQU007 and prior to scheduled necropsy for complete urinalysis. Five out of eight mice per group were chosen randomly using a Microsoft® Office Excel 2003 Randomization spreadsheet for urinalysis testing (Table 7.12.3).

Appearance, volume, specific gravity, pH, Ketones, Bilirubin, Glucose, occult blood, and Urobilinogen of urine samples were evaluated under GLP conditions at: Rodent Clinical Pathology Core Laboratory Central Arkansas Veterans Healthcare System Research Services, (Little Rock, AR).

Blood was collected via cardiac puncture immediately postmortem, according to Standard Operating Procedure ANCA014 and via tail vein one week before 4^{th} and 5^{th} immunization according to Standard Operating Procedure ANCA016.

The hematology (Table 8.3.1) and serum chemistry (Table 8.3.2) parameters were evaluated under GLP conditions at the Rodent Clinical Pathology Core Laboratory: Central Arkansas Veterans Healthcare System, Little Rock, AR.

Table 8.3.1. Hematology parameters
Leukocyte count, total and differential
Erythrocyte count
Hematocrit
Hemoglobin
Mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration (calculated)
Platelet count

Table 8.3.2. Blood chemistry parameters
Alkaline phosphatase
Aspartate Aminotransferase
Bilirubin, total
Calcium
Chloride
Creatinine
Gamma glutamyl transferase
Glucose
Lactate Dehydrogenase
Magnesium
Phosphorus
Potassium
Sodium
Total Protein
Urea Nitrogen

8.4 **Gross Necropsy**, **Tissue Collection and Preservation:**
Necropsy

On Week 3, prior to injection, and on weeks 9 and 21, two cages of 4 mice per group were chosen according to the Microsoft® Office Excel 2003 randomization spreadsheet (Table 7.12.2) to be euthanized. The sacrifice and necropsy schedule were summarized in Table 7.13.1. Mice were euthanized via an overdose of CO_2 (Standard Operating Procedure ANCA006) until movement and respiration ceased. Death was determined by lack of movement and respiration. Animals were necropsied according to Standard Operating Procedure ANCA013.

Necropsy performed upon sacrifice or unscheduled death with recording of organ weights and gross pathology (Table 8.4.1) and preservation of a complete list of tissues at necropsy under Standard Operating Procedure ANCA013.

Kidney (paired), liver, spleen and heart tissues were evaluated for organ weight

Table 8.4.1. Gross pathology performed on these organs

Mesenteric lymph nodes	Ovaries
Brain and pituitary gland	Pancreas
Cecum	Rectum
Colon	Salivary glands, left and right Parotid, Sublingual, Submaxillary
Duodenum	
Esophagus	Skeletal muscle, quadriceps, left
Eyes, left and right	Skin, ventral and dorsal
Femur, left	Spinal cord in vertebral column
Injection site(s)	Spleen
Heart	Stomach
Ileum	Submandibular lymph nodes, left and right
Jejunum	Thymus
	Thyroids, left and right
Adrenal glands left and right	Tongue
Kidneys left and right	Trachea
Liver	Urinary Bladder
Lungs	Uterus
Mammary gland	Vagina

Tissues were evaluated for gross lesions and preserved in 10% neutral formalin.

8.5 **Histology and Histopathology:**

Tissues were collected and processed according to Standard Operating Procedure HIST004 and HIST006. All harvested organs were embedded in paraffin blocks according to Standard Operating Procedure HIST003. Tissue from the control and high dose (500 µg) group were sectioned according to Standard Operating Procedure HIST005 and then stained with hematoxylin and eosin (H&E) according to Standard Operating Procedure HIST001. Slides were identified according to Standard Operating Procedure ANCA001 and examined by a veterinary pathologist. All gross lesions and target tissues were evaluated in the mid- (300 µg) and low-dose (100 µg) groups, while detailed analysis was performed for Control and 500 µg groups.

9 **Data Acquisition and Analysis:**

Appropriate entries in the experimental record were made after each procedure and according to Standard Operating Procedure AM007.

a. Protocol and Standard Operating Procedure Deviation

All deviations to the Protocol or Standard Operating Procedure were reported immediately to the Study Director. All study staff were required to fill out a Protocol or Standard Operating Procedure Deviation form, if he/she deviated from the implemented Protocol or Standard Operating Procedures. All Protocol and Standard Operating Procedure Deviation forms were sent to the Study Director and the Administration Manager according to Standard Operating Procedure AM005.

b. Statistical Analysis

See section 11 for detail of statistical analysis

10 Maintenance of Raw Data, Records, and Specimens

All raw data, records, protocol and report copies were maintained according to standard operating procedure Standard Operating Procedure AM003. This study was conducted in compliance with the FDA Good Laboratory Practice Regulations (21CFR58) and according to standard operating procedures.

11 Results:

11.1 Survival:

Survival was decreased in the 100µg dose group, with one early death prior to the first necropsy and 2 early-removal animals prior to the second necropsy.

Cause of death was not established for animal 100-1B-0 due to postmortem decomposition of tissues.

Animals 100-1A-L1 and 100-1A-B1, housed in the same cage, suffered from edema and loss of limb of unknown origin, respectively, of the distal right hindlimb. Serum from these animals was submitted for virology to Charles River Laboratories. There were no significant viral titres noted associated with Ectromelia, ruling out this possibility for the limb loss. Other attributes ruling out viral infection included no significant animal mortality within the colony.

Mouse, 500-2A-0 died during the 2nd tailbleed. Cause of death was determined to be suffocation due to thoracic compression in the bleeding chamber.

11.2 Clinical Observations:

Injection site reactions were mild and transient in all mice, and were present in mice from both control and high-dose groups. Reactions were most common during weeks 3-9 of the trial. This represents the time between the 3rd and 4th immunizations. Reactions were noted in 3 animals at 20 weeks, the week following immunization number 5. The most common reaction was hair loss, followed by ulceration, redness, and swelling at the injection site. Within ½ hour of immunization 5, 1 animal from each of 3 cages, 500-2B, 300-2B, 300-2A exhibited decreased motility, hunched posture, and rapid breathing. This reaction was transient and mice recovered within 1 day without incident.

11.3 Clinical Pathology:

Statistical Analysis Report of GLP Mouse Vaccination Study of P10s-PADRE: Hemavet Hematology, Vetscan Blood-Serum Chemistry, and Urinalysis.

Data collection: Mice were randomly allocated to four dose groups: the control group (0 µg), and vaccine groups of 100 µg, 300 µg, and 500 µg (at 32 mice per dose group), and vaccinated according to the protocol. During the study, 8 mice per dose group were randomly selected for sacrifice and necropsy at three scheduled times according SOPs and the protocol. Prior to necropsy, 5 of the 8 mice per dose group were housed singly for urine collection and urinalysis. At necropsy, blood was collected from all selected animals for measurement of hematology parameters and serum chemistry. After the first scheduled necropsy, the protocol was amended to allow collection of additional blood via tail-vein bleed for determination of hematology parameters between the first and second necropsy, and between the second and third necropsy. Thus, hematology was measured five times during the study, while serum chemistry and urinalysis parameters were measured three times during the study. Mice were weighed weekly during the course of the study; body weights at the times of necropsies and tail bleeds were used for statistical analysis.

Statistical analysis: Analysis was performed on all data collected during scheduled procedures. All numeric variables were summarized by dose group as the mean, standard deviation (SD), median, minimum, and maximum, and displayed as scatterplots versus dose group clustered by procedure. All character variables were tabulated as frequency per dose group. Within each procedure event, the Kruskal-Wallis (K-W) test was used to compare dose groups for any difference in numeric variables, while Spearman Correlation analysis was used to test for their net trend with dose. Character variables were re-coded as normal versus abnormal, and tested for dose-group differences within procedure via Fisher exact test.

Statistical significance and its interpretation: For body weights, the most comprehensive measure of overall animal health, differences and net trends were considered statistically significant if $P<0.05$. For all other measures, it was necessary to adjust for the large number of multiple comparisons without unduly inflating Type II error; accordingly, differences and net trends were considered statistically significant if $P<0.01$. Statistically significant *P* values were not considered definitive. Rather, they were considered to be indicators that closer scrutiny should be paid to the scatterplots presented in Appendices #11.3.1 through #11.3.4. The results in the plots and tabulations were considered informative for determining whether a statistically significant result constituted detection of a treatment-related biological signal.

Summary of results:

- **Body weights:** Body weights showed no statistically significant group differences, and no net trends with dose. This was true for all five procedure times. Appendix #11.3.1 shows the statistical results and scatterplot.

- **Hematology (Hemavet):** Table 11.3.1 provides an overview of statistical results. At the first tail-bleed, 15 of the 20 Hemavet measures produced statistically significant K-W test results, but only one of the 15 (Monocyte %(MO %)) also yielded a statistically significant net trend (negative) with dose. This measure also yielded a significant K-W test and trend at the third necropsy, but the trend with dose changed from negative to positive. Monocyte (MO) concentrations also yielded numerous statistically significant results that did not, however, coincide in time with MO %. Neutrophils (NE) showed a number of significant test results in the concentration measure but none in the percentage measure. Apart from the first tail-bleed, the other Hemavet measures showed only sporadic statistically significant results that were not sustained over time. **The overall conclusion was that the results for first tail-bleed did not reflect a test articule-related biological signal, and that results at other times were consistent with study variability.** Plots and more detailed statistical results are shown in Appendix #11.3.2.

- **Serum chemistry (Vetscan):** Table 11.3.2 shows an overview of statistical results. At 2nd necropsy, two Vetscan measures produced a statistically significant K-W test accompanied by a statistically significant net trend with dose. Page 9 of Appendix #11.3.3 shows that the net negative trend in serum glucose arises from a discontinuous drop between the lower two and upper two doses, while Page 12 of Appendix #11.3.3 shows that the net positive trend in serum phosphate similarly arises from a discontinuous rise between the lower two and upper two doses. For both these parameters, the dose discontinuities disappeared by 3rd necropsy (pages 9 and 12 of Appendix #11.3.3). At 1st necropsy, two significant K-W tests unaccompanied by significant trends were seen, and three significant trends were seen that were unaccompanied by significant K-W tests. **The overall conclusion was that serum levels of glucose and phosphate showed transient changes that possibly could have been related to the vaccination dose, but that other Vetscan measures showed no evidence of a biological signal.** Plots and more detailed statistical results are shown in appendix #11.4.3.

- **Urinalysis:** Table 11.3.3 shows an overview of statistical results. Leukocytes produced the only significant test result, which was at 3rd necropsy. Total bilirubin was noted to be at abnormal levels in all animals at first necropsy, and at abnormal levels in many animals at subsequent times. All measurements of urine ketone and urine protein were high enough to be numeric, which meant that the urine of all mice tested had abnormal levels of ketone and protein. No dose-group differences in prevalence of urinary abnormalities were detected. **The overall conclusion was that the leukocyte result was not a biological signal, because urinary tract infection was not confirmed by histology. Likewise, renal lesions consistent with development of proteinuria were also lacking. Urinary bilirubin and protein results likely reflected fecal contamination of the urine. A biological explanation is lacking for elevated ketones in these mice, as there is no evidence of renal disease or diabetes.** Plots and more detailed statistical results are shown in Appendix #11.3.4.

(Tables 11.3.1, 11.3.2, and 11.3.3 are on the next two pages.)

TABLE 11.3.1: Overview of statistical test results for Hemavet hematology measures shown in Appendix #11.3.2.											Appendix page #
Appendix #11.3.2:	**1st Necropsy**		**1st Tail-bleed**		**2nd Necropsy**		**2nd Tail-bleed**		**3rd Necropsy**		
HEMAVET	Overall	Trend,±	Overall	Trend, ±	Overall	Trend, ±	Overall	Trend, ±	Overall	Trend, ±	
Erythrocytes											
HCT %	---	---	<0.01	---							**1**
Hb g/dL	---	---	<0.01	---							**2**
MCH pg	---	---	<0.01	---							**3**
MCHC g/dL	---	---	<0.01	---							**4**
MCV fL	---	---	---	---							**5**
RBC M/uL	---	---	<0.01	---							**6**
RDW %	---	---	---	---							**7**
Leukocytes											
BA %	---	---	<0.01	---		<0.01,+	---	---			**8**
BA k/uL	---	---	<0.01	---							**9**
EO %	---	---	<0.01	---							**10**
EO k/uL	---	---	<0.01	---							**11**
LY %	---	---	<0.01	---					<0.01	<0.01,-	**12**
LY k/uL	---	<0.01,-	<0.01	---							**13**
MO %	---	---	<0.01	<0.01,-	---	---			<0.01	<0.01,+	**14**
MO k/uL	---	---	<0.01	---	<0.01	<0.01,-	<0.01	---			**15**
NE %	---	---	---	---							**16**
NE k/uL	<0.01	<0.01,-	<0.01	---	<0.01	<0.01,-	<0.01	---	---	---	**17**
WBC k/uL	---	<0.01,-	<0.01	---			<0.01	---			**18**
Thrombocytes											
MPV fL	<0.01	---	---	---				<0.01,-	---	---	**19**
PLT K/uL	---	---	---	---							**20**

The Kruskal-Wallis test was for detecting Overall differences, while Spearman correlation was for detecting net Trend with dose. Statistical test results are shown as "---" if not significant, or as "<0.01" if significant. The "+" or "-" after a significant Trend test indicates positive or negative trend.

TABLE 11.3. 2: Overview of statistical test results for Vetscan serum chemistry measures shown in Appendix #11.3.3.

Appendix #11.3.3:	1st Necropsy	2nd Necropsy	3rd Necropsy	Appendix
VETSCAN	Overall Trend	Overall Trend	Overall Trend	page #
ALB g/dl	<0.01 --- --- --- --- ---			1
ALP U/L	--- --- --- --- --- ---			2
ALT U/L	--- --- --- --- --- ---			3
AMY U/L	--- --- --- --- --- ---			4
BUN mg/dl	--- --- --- --- --- ---			5
CA mg/dl	--- --- --- --- --- ---			6
CRE mg/dl	--- <0.01,+	--- --- --- ---		7
GLOB g/dl	--- <0.01,-	--- --- --- ---		8
GLU mg/dl	<0.01 ---	<0.01 <0.01,-	--- ---	9
K+ mmol/L	--- --- --- --- --- ---			10
NA+ mmol/L	--- --- --- --- --- ---			11
PHOS mg/dl	--- ---	<0.01 <0.01,+	--- ---	12
TBIL mg/dl	--- --- --- --- --- ---			13
TP g/dl	--- <0.01,-	--- --- --- ---		14
QC	All OK	All OK	All OK	15
HEM	No Abnormals	No Abnormals	No Abnormals	16
ICT	No Abnormals	No Abnormals	No Abnormals	17
LIP	No Abnormals	No Abnormals	No Abnormals	18

The Kruskal-Wallis test was for detecting Overall differences, while Spearman correlation was for detecting net Trend with dose. Statistical test results are shown as "---" if not significant, or as "<0.01" if significant. The "+" or "-" after a significant Trend test indicates positive or negative trend.

TABLE 11.3.3: Overview of statistical test results for Urinalysis measures shown in Appendix #11.3.4.

Appendix #11.3.4:	1st Necropsy	2nd Necropsy	3rd Necropsy	Appendix
URINALYSIS	Overall Trend	Overall Trend	Overall Trend	page #
Ketone	--- --- --- --- --- ---			1
Protein	--- --- --- --- --- ---			2
Specific Gravity	--- --- --- --- --- ---			3
pH	--- --- --- --- --- ---			4
Bilirubin All	Abnormal	---	---	5
Blood ---		---	No Abnormals	6
Glucose No	Abnormals	No Abnormals No	Abnormals	7
Leukocytes ---		---	<0.01	8
Nitrite No	Abnormals	---	---	9
Urobilinogen	No Abnormals	No Abnormals	No Abnormals	10

The Kruskal-Wallis test was for detecting Overall differences, while Spearman correlation was for detecting net Trend with dose, and Fisher exact test was for detecting differences in categorical data. Statistical test results are shown as "---" if not significant, or as "<0.01" if significant. The "+" or "-" after a significant Trend test indicates positive or negative trend.

Discussion:
CBC: Elevated RBC (red blood cell) parameters including RBC M/µl, Hb g/dl (hemoglobin), HCT% (hematocrit) were noted in all groups, both Control and treatment groups. There were no significant differences in RBC parameters among groups. Thrombocyte numbers (PLT K (1000)/µl) were variably decreased in dose 100µg, 300µg, and 500µg mice at all timepoints. Thrombocytopenia was noted in only 1 control mouse sampled at the second tail bleed. No animals exhibited clinical bleeding, and no statistically significant dose response was evident.

White blood cell parameters fluctuated throughout the study. Transient leukocytosis, including neutrophilia in animals from all groups suggests mild intercurrent disease. This finding is consistent with mild liver lesions.

11.4 Gross Pathology

Gross lesions were limited to uterine enlargement (dilatation) and were present in both control and treatment groups in the first necropsy. Uterine dilatation is common and a normal change attributed to the estrous cycle. It may be more pronounced in young mice around the onset of sexual maturity, and lesions in this study were indeed prevalent at the first sacrifice.

Gross pathology of early removal mice 100-1A-L1 and 100-1A-B1:
Mice 100-1A-L1 and 100-1A-B1 were housed in the same cage and developed similar lesions. Mouse 100-1A-L1 developed severe edema of the distal right hindlimb with well-demarcated alopecia, crusting, and ulceration just proximal to the hock joint. Crusting in a similar location, with limb loss of the distal right hindlimb just proximal to the hock joint was also present in mouse 100-1A-B1.

11.5 Histopathology

Background and purpose: Mice were randomly allocated to four dose groups: the control group (0 mg), 100 µg, 300 µg, and 500 µg (at 24 mice per dose group), and vaccinated according to the protocol schedule. During the study, 8 mice per dose group were randomly selected for sacrifice and necropsy at three scheduled times. At necropsy, tissues and organs were harvested from all animals, evaluated for gross lesions, preserved in 10% formalin, and embedded into paraffin blocks. For the control group and the high-dose (500µg) group only, paraffin-embedded tissue was sectioned and stained with H&E for evaluation by a veterinary pathologist. Observed lesions were given a diagnosis and a score from 0 (none) to 4 (severe); results were recorded in Microsoft Excel for subsequent statistical analysis.

Methods: All control and 500 µg group slides were initially evaluated in a non-masked fashion using an organ-by-organ method (each organ examined across all animals in all groups) to allow for the most consistent lesion grading within an organ. Where lesions were noted, grade was assigned on a 5 point scale (0, or NSL in data table- no lesion; 1-minimal changes; 2-mild changes; 3- moderate changes; and 4-

severe changes. Where lesions within an organ were noted, re-evaluation of that organ was performed in a randomized, masked fashion on 100% of slides from both groups to optimize consistency and objectivity of grade assignment. All data was directly recorded in Microsoft Excel software at the microscope. All evaluations were made by a single pathologist (LH).

Slides from early-death animals were evaluated separately.

Identified lesions are defined in Definitions, Page 33.

Results:

Lesions noted in this study were generally minimal to mild. Only one lesion, alveolar septal edema differed significantly ($p<0.01$) between control and 500 µg dose groups. The 6 mice with alveolar septal edema were all in the high-dose group, yielding a statistically significant dose-group difference overall ($p<0.01$), but not at individual necropsy times. Five of the six were in Necropsy #2, while the sixth was in Necropsy #1; Appendix 11.5.1 Lung shows that all six had a score of only 1 (minimal) for severity. Alveolar septal edema is an acute change, and is unlikely to be treatment-related, as no evidence of chronic inflammation or edema was present in any animal. This change is therefore interpreted as agonal. Acute thrombi in small pulmonary arteries, noted in all groups, is of unknown etiology. These lesions are defined as fibrin thrombi, and graded only as to presence or absence. Fibrin is not confirmed via special stain; however, morphology is consistent with fibrin or fibrinogen, either of which is consistent with acute thrombosis, suggesting that thrombosis is occurring near death. This lesion is temporally removed from vaccination and not significantly different between groups; therefore, it is unlikely to be treatment-related. Acute hepatocellular necrosis, noted in animals from both groups at all necropsies, strongly suggests the presence of intercurrent disease in these animals. Chronic dermal inflammation may be due to injection of adjuvant, as it is present in both groups.

The central and peripheral nervous system and hematopoietic system are considered likely target organs for treatment toxicity due to widespread expression of the target glycosaminoglycans on neural and bone-marrow cells (unpublished data). No neural lesions were observed in any animal. No changes were noted in bone marrow, and extramedullary hematopoeisis was observed at similar levels in the livers of both control and treated groups.

Early deaths/early removal:

No cause of death was established for mouse100-1B-0 due to extensive postmortem decomposition.

Mice 100-1A-L1 and 100-1A-B1 were housed in the same cage and developed similar lesions. Mouse 100-1A-L1 developed severe edema of the distal right hindlimb with well-demarcated alopecia, crusting, and ulceration just proximal to the hock joint.

Crusting in a similar location, with loss of the distal right hindlimb just proximal to the hock joint was also present in mouse 100-1A-B1. Histologically, lesions consisted of severe subcutaneous edema with multifocal degeneration of epithelial cells, mixed vasculitis, and periosteal hyperplasia (Figure 11.5.1). Serologic testing did not reveal significant titers to poxviral virus or other viruses, which might have caused the limb loss. No foreign bodies were present in the cage or on the limbs. Presence of periosteal proliferation indicates chronicity of the limb edema. Bacterial colonies were present superficially, but are interpreted as secondary invaders.

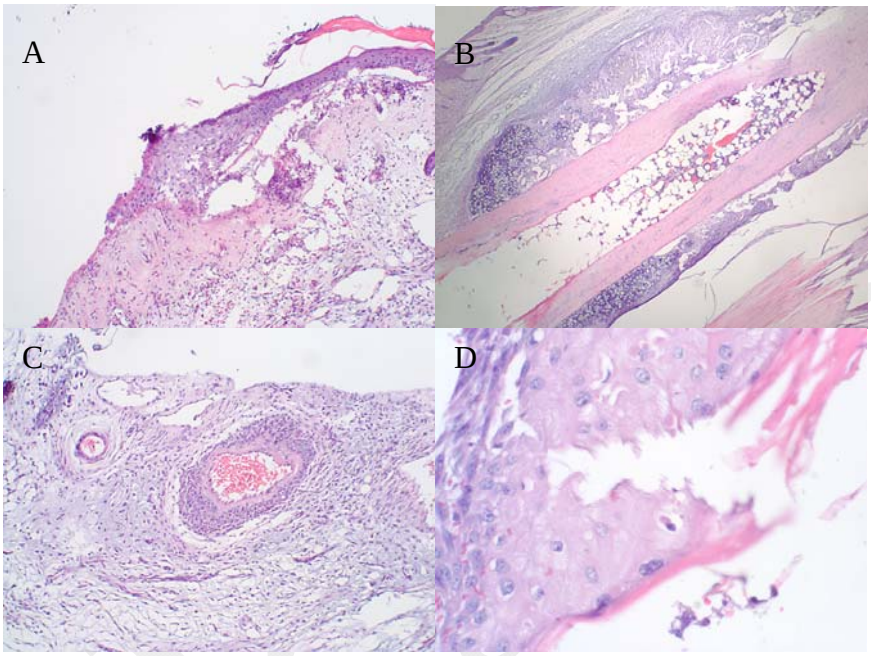


Figure 11.5.1. Histology, animal #100-1A-L1:
A, Epidermal necrosis, ulceration, and subcutaneous edema.
B, Periosteal proliferation. C, Vasculitis.D, Epidermal degeneration.

Mouse 500-2A-0 died during the second tail bleed, and death was attributed to compression of the thorax while the mouse was in the restrainer. Because mice are compressed in a cranio-caudal direction by the plunger within the restrainer, it is likely that significant pulmonary compression occurred in this mouse, leading to compromised respiration in the lungs. Histologically, mild hemorrhage and two small acute fibrin arterial thrombi were noted. Similar thrombi were noted in other animals, including controls, and are therefore unlikely to be contributory to death. Thrombosis was not observed in other organs.

Statistical analysis:

Statistical Analysis Report of GLP Mouse Vaccination Study of P10s-PADRE: Lesion Assessments in Organs and Tissues Harvested at Necropsy.

According to Section 9c of the protocol under which this study was conducted, if statistical analysis shows significantly more pathology in the high-dose group than the

control group, then organs from the 300-μg group will be evaluated. The purpose of this report is to disseminate the results of the statistical analysis.

Statistical analysis: In both control and treatment animals, liver and lung were the only two organs that frequently had two or more lesions in the same animal. In these two organs, lesions were classified and analyzed by diagnosis. In the remaining 40 organs, it was rare to see more than one lesion in the same animal, and more than two were never seen. Lesions in these other organs were accordingly classified and analyzed by organ, using the organ's maximum lesion score if the organ had more than one lesion. At individual necropsy times, scores were classified as negative (if =0) or positive (if >0), and analyzed for dose-group differences via Fisher's exact test. Then the scores themselves were gathered from all necropsy times and compared for overall difference in mean scores between dose groups, using a stratified version of the Cochran-Mantel-Haenszel Correlation Chi-square test in which the three necropsy times were the strata. All statistical tests were two-sided.

Statistical significance and its interpretation: In order to adjust for the large number of multiple comparisons without inflating the false-negative-finding rate unduly, test results were considered statistically significant only if $P<0.01$. Statistically significant test results were not considered definitive. Rather, they were to be considered as indicators that the type of lesion, as well as its severity score, should be evaluated more closely for biological and/or pathological significance.

Results: One animal in the high-dose Necropsy #3 group was excluded from analysis because it died early. Animal totals (control/high dose) were thus 16 (8/8), 16 (8/8), and 15 (8/7) at necropsy times #1, #2, and #3, respectively.

- Lung: The number of lesions in a lung ranged from zero to four. Table 11.5.1 gives an overview of specific lesion diagnoses at each necropsy time. Grand totals (%) out of 47 animals were: 12 (26%) with acute hemorrhage, 7 (15%) with acute fibrin thrombosis artery, 6 (13%) with alveolar septal edema, 4 (9%) with alveolar histiocytosis, 3 (6%) with lymphoid hyperplasia peribronchiolar, and 1 (2%) with acute hemorrhage peritumoral or hyperplasia bronchial epithelium. The total number of lung lesions was thus 34 in 47 animals, for an average of 0.72 lung lesions per animal. The 6 alveolar septal edemas were all in the high-dose group, yielding a statistically significant dose-group difference overall, but not at individual necropsy times. Five of the six were in Necropsy #2, while the sixth was in Necropsy #1; Appendix 11.5.1 Lung shows that all six had a score of only 1 for severity. None of the other lesion types showed statistically significant group differences either overall or at individual necropsy times. **The overall conclusions are: (1) because the alveolar septal edemas were mild and largely confined to one necropsy time, their apparent dose dependence was regarded as noteworthy but not likely to be biologically important; and (2) none of the other lung lesions showed an evident dependence on dose.** Appendix 11.5.1 Lung gives detailed tabulations of the number of positive lesions of each diagnostic

type, as well as the numbers scored as 1 or 2 for lesion severity; only the acute hemorrhages yielded scores as high as 2.

- Liver: All animals, control and treatment animals had at least one lesion in the liver; some had as many as four. Table 11.5.2 gives an overview of specific lesion diagnoses at each necropsy time. Grand totals (%) out of 47 animals were: 36 (77%) with **Extramedullary hematopoiesis (**EMH), 33 (70%) with chronic inflammation lobular, 18 (38%) with acute hepatocellular necrosis, 5 (11%) with chronic inflammation periportal or chronic-active inflammation portal, 2 (4%) with chronic inflammation portal, and 1(2%) with chronic-active inflammation periportal, lymphoplasmacytic inflammation periportal, suppurative inflammation lobular, or suppurative inflammation periportal. The total number of liver lesions was thus 103 in 47 animals, for an average of 2.19 liver lesions per animal. No statistically significant group differences were seen either overall or at individual necropsy times. **The overall conclusion is that liver lesions showed no evident dependence on dose.** Appendix 11.5.2 Liver gives detailed tabulations of the number of positive lesions of each diagnostic type, as well as the number scored as 1, 2, or 3 for lesion severity; only EMH yielded scores as high as 3.

- Other Organs: Table 11.5.3 gives an overview by organ of the number of animals showing lesions in the 40 other organs examined. Organs in which 5 or more (>10%) out of 47 animals had a lesion were: salivary (22), cecum (13), duodenum (13), skin ventral (13), kidney (12), uterus (11), jejunum (8), rectum (7), urinary bladder (7), and skin dorsal (6). Organs that showed zero lesions among 47 animals were: Bone marrow, eye, femur, lacrimal gland, optic nerve, ovary, oviduct, pituitary, sciatic nerve, skeletal muscle, spinal cord, trachea, and vagina. None of the 40 organs showed a statistically significant dose-group difference either overall or at individual necropsy times. **The overall conclusion is that, among the 40 other organs examined, none showed evidence of a dose dependency in either the number or severity of lesions seen.**

(Tables 11.5.1, 11.5.2, and 11.5.3 are on the next two pages.)

TABLE 11.5.1: Overview of statistical test results for the indicated lesions displayed in Appendix 11.5.1 Lung

Lesions in the Lungs	Necropsy#1		Necropsy#2		Necropsy#3		Overall		Appendix
Diagnosis (Description of lesion)	Npos	test	Npos	test	Npos	test	Total	test	pages
acute fibrin thrombosis, artery	0	---	4	---	3	---	7	---	1,2
acute hemorrhage	6	---	6	---	0	---	12	---	3,4
acute hemorrhage, peritumoral	0	---	0	---	1	---	1	---	5,6
alveolar histiocytosis	0	---	2	---	2	---	4	---	7,8
alveolar septal edema	1	---	5	---	0	---	6	<0.01	9,10
hyperplasia, bronchial epithelium	1	---	0	---	0	---	1	---	11,12
lymphoid hyperplasia, peribronchiolar	0	---	1	---	2	---	3	---	13,14

Npos = number of lesions seen among 16, 16, and 15 mice in Necropsies #1, #2, and #3, respectively; total = total lesions seen among all 47 mice. Tests were Fisher's exact tests for individual necropsies, and the stratified correlation chi-square test with all three necropsies as the strata.
Detailed results can be found at the indicated Appendix pages in "Appendix 11.5.1 Lung, lesion details".

TABLE 11.5.2: Overview of statistical test results for the indicated lesions displayed in Appendix 11.5.2 Liver

Lesions in the Liver	Necropsy#1		Necropsy#2		Necropsy#3		Overall		Appendix
Diagnosis (Description of lesion)	Npos	test	Npos	test	Npos	test	Total	test	pages
EMH	16	---	8	---	12	---	36	---	1,2
acute hepatocellular necrosis	4	---	7	---	7	---	18	---	3,4
chronic inflammation, lobular	9	---	12	---	12	---	33	---	5,6
chronic inflammation, periportal	0	---	4	---	1	---	5	---	7,8
chronic inflammation, portal	1	---	1	---	0	---	2	---	9,10
chronic-active inflammation, periportal	0	---	1	---	0	---	1	---	11,12
chronic-active inflammation, portal	5	---	0	---	0	---	5	---	13,14
lymphoplasmacytic inflamm., periportal	0	---	0	---	1	---	1	---	15,16
suppurative inflammation, lobular	0	---	1	---	0	---	1	---	17,18
suppurative inflammation, periportal	0	---	1	---	0	---	1	---	19,20

Npos = number of lesions seen among 16, 16, and 15 mice in Necropsies #1, #2, and #3, respectively; total = total lesions seen among all 47 mice. Tests were Fisher's exact tests for individual necropsies, and the stratified correlation chi-square test with all three necropsies as the strata.
Detailed results can be found at the indicated Appendix pages in "Appendix 1.5.2 Liver, lesion details

TABLE 11.5.3: Overview of statistical test results on lesions found in organs other than Liver or Lung, as displayed in Appendix 11.5.3 Other Organs

Lesions in Other Organs	Necropsy#1	Necropsy#2	Necropsy#3	Overall	Appendix
Organ or Site	Npos test	Npos test	Npos test	Total test	pages
adrenal	1 ---	1 ---	2 ---	4 ---	1,2
bone marrow	0 ---	0 ---	0 ---	0 ---	3,4
brain	2 ---	1 ---	0 ---	3 ---	5,6
cecum	4 ---	7 ---	2 ---	13 ---	7,8
cervix	0 ---	0 ---	1 ---	1 ---	9,10
colon	1 ---	0 ---	1 ---	2 ---	11,12
duodenum	2 ---	4 ---	7 ---	13 ---	13,14
esophagus	0 ---	0 ---	4 ---	4 ---	15,16
eye	0 ---	0 ---	0 ---	0 ---	17,18
femur	0 ---	0 ---	0 ---	0 ---	19,20
heart	0 ---	2 ---	0 ---	2 ---	21,22
ileum	1 ---	1 ---	1 ---	3 ---	23,24
jejunum	1 ---	6 ---	1 ---	8 ---	25,26
kidney	2 ---	9 ---	1 ---	12 ---	27,28
lacrimal gland	0 ---	0 ---	0 ---	0 ---	29,30
mammary gland	2 ---	0 ---	0 ---	2 ---	31,32
mandibular lymph node	0 ---	3 ---	1 ---	4 ---	33,34
mesenteric lymph node	2 ---	0 ---	0 ---	2 ---	35,36
optic nerve	0 ---	0 ---	0 ---	0 ---	37,38
ovary	0 ---	0 ---	0 ---	0 ---	39,40
oviduct	0 ---	0 ---	0 ---	0 ---	41,42
pancreas	0 ---	1 ---	0 ---	1 ---	43,44
parathyroid	0 ---	0 ---	1 ---	1 ---	45,46
pituitary	0 ---	0 ---	0 ---	0 ---	47,48
rectum	3 ---	0 ---	4 ---	7 ---	49,50
salivary	4 ---	8 ---	10 ---	22 ---	51,52
sciatic nerve	0 ---	0 ---	0 ---	0 ---	53,54
skeletal muscle	0 ---	0 ---	0 ---	0 ---	55,56
skin dorsal	3 ---	0 ---	3 ---	6 ---	57,58
skin ventral	7 ---	3 ---	3 ---	13 ---	59,60
spinal cord	0 ---	0 ---	0 ---	0 ---	61,62
spleen	1 ---	0 ---	0 ---	1 ---	63,64
stomach	3 ---	2 ---	2 ---	7 ---	65,66
thymus	0 ---	1 ---	0 ---	1 ---	67,68
thyroid	1 ---	0 ---	0 ---	1 ---	69,70
tongue	0 ---	1 ---	0 ---	1 ---	71,72
trachea	0 ---	0 ---	0 ---	0 ---	73,74
urinary bladder	1 ---	3 ---	3 ---	7 ---	75,76
uterus	6 ---	4 ---	1 ---	11 ---	77,78
vagina	0 ---	0 ---	0 ---	0 ---	79,80

Npos = number of lesions seen among 16, 16, and 15 mice in Necropsies #1, #2, and #3, respectively; total = total lesions seen among all 47 mice. Organs and totals are underlined if the totals are 5 or more (>10%) out of 47 animals. Tests were Fisher's exact tests for individual necropsies, and the stratified correlation chi-square test with all three necropsies as the strata.

Detailed results can be found at indicated Appendix pages in "Appendix 11.5.3 Other Organs,

11.6. **Body Weights and Body Weight Changes**:

Data collection: Mice were randomly allocated to four dose groups: the control group (0 μg), 100 μg, 300 μg, and 500 μg (at 24 mice per dose group), and vaccinated according to the protocol schedule. Mice were weighed on an approximately weekly basis commencing on 05/27/08 (hereinafter Day 1). During the study, 8 mice per dose group were randomly selected for sacrifice and necropsy at three scheduled times: 06/09/08 (Day 14), 07/21/08 (Day 56), and 10/13/08 (Day 140). Mice were weighed on 20 weighing days and the three necropsy days to yield a total of 23 time points. Weights were recorded, subjected twice to Quality Assurance inspection, and made available for statistical analysis.

Statistical analysis: At each timepoint, the mouse's change in weight was calculated as its current weight minus its previously recorded weight; positive numbers thus represent weight gain while negative numbers represent weight loss. Weights and weight changes (both in grams) were summarized by day and dose group as the mean, standard deviation (SD), median, minimum, and maximum. Means ±1SD of weights and weight changes were plotted versus the day of study. Data were then analyzed via Repeated Measures ANOVA, using a mixed-models approach to handle data that was missing by design because of the necropsies. The within-subject covariance was modeled as having first-order autoregressive correlation structure, using the Spatial Power model to adjust for the sometimes-unequal number of days between successive weighings. Fixed effects in the Repeated Measures ANOVA were Dose group, Day, and their interaction.

Statistical significance and its interpretation: Because mouse weights over time are the most reliable measure of overall health, fixed effects in the Repeated Measures ANOVA were considered statistically significant if $P<0.05$. Statistically significant results were interpreted for potential biological meaning using the plots of means by day of study.

Summary of results:

- **Anomalies:** Pages 1 through 8 of "Appendix 11.6.1 Weekly Mouse Weights, 03-25-09.doc" give the summaries of weights and weight changes by day and dose group, while Page 9 shows the plots of their means±1SD by day. At Day 56 (the second necropsy), all four dose groups show a negative mean weight change relative to the Day 51 weighing, but at Day 58, all four dose groups show a positive mean weight change relative to Day 56. The drop at Day 56 is clearly visible in both plots of the appendix 11.6.1. On Day 113, the maximum weight gain was 8.9 grams, and was followed on Day 120 by a maximum weight loss of 8.6 grams; this pair of extreme changes was traced to one mouse (Cont-1A-B1) whose weight was recorded as 20.65 grams on 09/09/08, 29.59 grams on 09/16/08, and 20.95 grams on 09/23/08. The effect of this one mouse's transient weight change shows as large control-group SDs on days 113 and 120 in the plots of the appendix 11.6.1.

- **Repeated Measures ANOVA on mouse weights:** Page 10 of "Appendix 11.6.1 Weekly Mouse Weights, 03-25-09.doc" shows the results of Repeated Measures ANOVA on mouse weights. The coefficient of within-subject correlation was estimated as 92.8% for weights taken one day apart, and is equivalent to a 59.4% correlation between the majority of weights taken seven days apart. Dose group was not statistically significant (P=0.38). Day was highly significant (P<0.0001), and indicative of the generally upward trend in weights over the study period. The Dose-group-by-Day interaction was not statistically significant (P=0.072).

- **Repeated Measures ANOVA on changes in mouse weights:** Page 11 of "Appendix 11.6.1 Weekly Mouse Weights, 03-25-09.doc" shows the results of Repeated Measures ANOVA on mouse weights. The coefficient of within-subject correlation was estimated as 1.9% for weights taken one day apart, and is equivalent to a correlation of 10^{-12} (essentially zero) between the majorities of changes calculated on weights taken seven days apart. Dose group was not statistically significant (P=0.91). Day was highly significant (P<0.0001), and probably reflective of the sharp negative weight change at second necropsy as well as a more gradual change from mostly positive the first 100 days to net negative thereafter (bottom plot on page 9 of the appendix 11.6.1). The Dose-group-by-Day interaction was statistically significant at P=0.023, but the bottom plot on page 9 of the appendix 11.6.1 shows that the significant interaction reflects that the dose-group curves merely cross each other frequently without net divergence over time.

- **Conclusions:** Although something appears to have happened to mouse weights at Day 56, it was transient, and affected all dose groups in a similar manner. Therefore, the weight loss was not considered to be related to the vaccine. The one mouse with the transient 9-gram weight gain was in the control group, so its weight changes were not vaccine-related. The Repeated Measures ANOVA produced no evidence for dose-related effects on weights or weight changes. **The overall conclusion is that no evidence was seen for an effect of vaccine on mouse weights measured regularly over the 140-day course of the study.**

11.7. Analysis of organ weight

Data collection: Mice were randomly allocated to four dose groups: the control group (0 mμg), 100 μg, 300 μg, and 500 μg (at 24 mice per dose group), and vaccinated according to the protocol schedule. During the study, 8 mice per dose group were randomly selected for sacrifice and necropsy at three scheduled times: 06/09/08, 07/21/08, and 10/13/08. The heart, kidneys (left and right together), liver, and spleen of each mouse were weighed, and the weights were recorded for subsequent statistical analysis.

Statistical analysis: Analysis was performed on all organ weights collected during the three scheduled procedures. Organ weights in grams were divided by mouse body weights at necropsy and expressed as percent weights. Gram weights and percent weights were summarized by dose group as the mean, standard deviation (SD), median,

minimum, and maximum, and displayed as scatterplots versus dose group clustered by procedure date. Within each procedure event, the Kruskal-Wallis (K-W) test was used to compare dose groups for any difference in gram weights or percent weights, while Spearman Correlation analysis was used to test for their net trend with dose.

Statistical significance and its interpretation: In order to adjust for the large number of multiple comparisons without unduly inflating Type II error, differences and net trends were considered statistically significant if $P<0.01$. Statistically significant *P* values were not considered definitive. Rather, they were considered to be indicators that closer scrutiny should be paid to the scatterplots presented in Appendix 11.7.1 Organ Weights. The results in the plots were considered informative for determining whether a statistically significant result constituted detection of a treatment-related biological signal.

Summary of results:

- One dose 100-μg mouse died before the second scheduled necropsy and had no organ weights to analyze. Two dose 100-μg mice were sacrificed three weeks before the third scheduled necropsy, and their organ weights were excluded from analysis.

- **Heart:** Page 1 of Appendix 11.7.1 Organ Weights shows the descriptive statistics, while Page 2 of this appendix shows the K-W test results and Spearman correlations, and Page 3 shows the scatterplots. No statistically significant differences or trends were seen at any of the three necropsy times.

- **Kidney:** Page 4 of Appendix 11.7.1 Organ Weights shows the descriptive statistics, while Page 5 of this appendix shows the K-W test results and Spearman correlations, and Page 6 shows the scatterplots. No statistically significant differences or trends were seen at any of the three necropsy times.

- **Liver:** Page 7 of Appendix 11.7.1 Organ Weights shows the descriptive statistics, while Page 8 of this appendix shows the K-W test results and Spearman correlations, and Page 9 shows the scatterplots. At second and third necropsies, liver percent weights showed significant K-W test results, and second necropsy also showed significant negative trends with dose in both gram weights and percent weights. The scatterplots show that similar but non-significant trends may be present at first necropsy, but do not show trend-like behavior at third necropsy.

- **Spleen:** Page 10 of Appendix 11.7.1 Organ Weights shows the descriptive statistics, while Page 11 of this appendix shows the K-W test results and Spearman correlations, and Page 12 shows the scatterplots. First necropsy yielded a statistically significant K-W test results for both gram weight and percent weight that were not accompanied by significant evidence for trend.

12 **Conclusion**

The statistically significant trends with dose in the liver gram weight and percent weight at second necropsy are noteworthy. But in other analyses reported elsewhere (see Report GLP Mouse Organ Harvests, 03-06-09.doc), liver lesions showed no evident dependence on dose. Moreover, the significant trends at second necropsy were not significant at first necropsy, and not evident in the scatterplots at third necropsy. Statistically significant decrease in liver size was not correlated with any histologic evidence of hepatic atrophy or fibrosis, which are common causes for decreased liver weight. **For these reasons, the liver trends with dose are regarded as unlikely to be biologically meaningful.** The significant K-W test result for liver at third necropsy was not accompanied by scatterplot evidence for a trend with dose, and thus does not appear to be biologically meaningful. The statistically significant spleen results at first necropsy did not manifest as significant trends with dose, and therefore do not appear to be biologically meaningful. The other two organs did not show evidence at any necropsy for a treatment-related effect on organ weight.

13. Definitions:

1. **Chronic inflammation:** Lymphoplasmacytic infiltrate with or without macrophages.
2. **Hemorrhage, acute:** Presence of red blood cells in extravascular areas with no evidence of hemoglobin degradation pigments.
3. **Hyperkeratosis:** Increase in thickness of the superficial keratin in esophagus or non-glandular stomach.
4. **Lymphoplasmacytic infiltrate:** Diffuse increase in lymphocytes and plasma cells in gastrointestinal lamina propria.
5. **Lymphoid hyperplasia:** Increase in size of lymphoid nodules in lymph nodes, mucosal-associated lymphoid tissue, or bronchial associated lymphoid tissue.
6. **Extramedullary hematopoiesis (EMH)**: Presence of hematopoietic cells forming clusters of single-origin (red cell or granulocyte), immature blood cells within the liver.
7. **Acute hepatocellular necrosis:** Ballooning or fragmentation of clusters of hepatocytes with neutrophilic or mixed inflammatory infiltrate.
8. **Tubular hyperplasia (regeneration):** Enlargement of renal tubular cells with prominent nuclei and basophilic cytoplasm which suggests regenerative response to injury.
9. **Suppurative inflammation:** Focal infiltrate of neutrophils.
10. **Chronic-active inflammation:** Infiltrate of neutrophils along with lymphocytes and plasma cells; generally seen in portal regions of the liver in this study.
11. **Vasculitis:** Perivascular inflammation extending into the vessel wall.
12. **Ultimobranchial cyst**: Developmental remnant of the branchial arch found in or adjacent to thyroid or parathyroid gland. Generally mucus-filled cyst lined by ciliated columnar epithelium.
13. **Atrophy:** Decrease in normal size of an organ or cellular size in a tissue.
14. **Squamous cyst:** Cyst filled with keratin and lined by squamous epithelium. Developmental abnormality.
15. **Mineralization, epicardial:** Presence of calcium salts indicated by amorphous basophilic deposits on epicardial surface.

16. **Angiectasis:** Dilation of blood vessels.
17. **Pulmonary adenocarinoma:** Adenocarcinoma of the alveolar epithelium.
18. **Alveolar histiocytosis:** Increased numbers of alveolar histiocytes within alveolar spaces.
19. **Type II pneumocyte hyperplasia:** Increased size and basophilia of alveolar type II epithelial cells.
20. **Fibrin thrombus:** Eosinophilic fibrin strands occlude arterial lumen. Erythrocytes or white blood cells may be entrapped in fibrin, but macrophages, fibroblasts, and mature collagen are not present.
21. **Alveolar septal edema:** Alveolar septa are thickened with hyaline material.
22. **Dilatation:** Distension of tubular organ. Observed in the uterus of this study. Mild dilatation consistent with oestrus cycle changes is not included in this definition.
23. **Corneal dystrophy:** Epidermal inclusion in corneal stroma.
24. **Follicular cyst:** Ovarian cyst containing variable amounts of fluid, not lined by ciliated epithelium.
25. **Endometrial polyp:** Polypoid hyperplasia of benign endometrial mucosa with a central connective tissue stalk.
26. **Hyperplasia:** Cellular enlargement and increase in cell number with no features of malignancy.
27. **Mast cell infiltrate**: Diffuse increase in numbers of mast cells.
28: **Lymphoplasmacytic dermatitis**: Lymphoplasmacytic infiltrate in the dermis.
29: **Sinus histiocytosis**: Increased number of histiocytes in medullary or subcapsular sinus of lymph node.
30: **Chronic-active pyelonephritis**: Chronic-active inflammation of the renal pelvis and medullary tubules.
31: **Nodular hyperplasia**: Hyperplastic nodules in the adrenal cortex.
32: **Chronic-active gastritis**: Chronic-active inflammation in the stomach.
33: **Ectromelia:** Loss of limb

14 References

[1] Kieber-Emmons T, Luo P, Qiu J, Chang TY, O I, Blaszczyk-Thurin M, et al. Vaccination with carbohydrate peptide mimotopes promotes anti-tumor responses. Nat Biotechnol 1999;17(7):660-5.

[2] Monzavi-Karbassi B, Artaud C, Jousheghany F, Hennings L, Carcel-Trullols J, Shaaf S, et al. Reduction of spontaneous metastases through induction of carbohydrate cross-reactive apoptotic antibodies. J Immunol 2005; 174(11):7057-65.

[3] Monzavi-Karbassi B, Luo P, Jousheghany F, Torres-Quinones M, Cunto-Amesty G, Artaud C, et al. A mimic of tumor rejection antigen-associated carbohydrates mediates an antitumor cellular response. Cancer Res 2004; 64(6):2162-6.

[4] Monzavi-Karbassi B, Hennings LJ, Artaud C, Liu T, Jousheghany F, Pashov A, et al. Preclinical studies of carbohydrate mimetic peptide vaccines for breast cancer and melanoma. Vaccine 2007; 25(16):3022-31.

[5] Wondimu A, Zhang T, Kieber-Emmons T, Gimotty P, Sproesser K, Somasundaram R, et al. Peptides mimicking GD2 ganglioside elicit cellular, humoral and tumor-protective immune responses in mice. Cancer Immunol Immunother 2008; 57(7):1079-89.

[6] Monzavi-Karbassi B, Cunto-Amesty G, Luo P, Shamloo S, Blaszcyk-Thurin M, Kieber-Emmons T. Immunization with a carbohydrate mimicking peptide augments tumor-specific cellular responses. Int Immunol 2001; 13(11):1361-71.

Appendices

Vaccination of High-Risk Breast Cancer patients with Carbohydrate Mimicking Peptide
Grant Period: 04/24/2006 – 04/23/2011
Standard Operating Procedures (SOPs) Master List

Administration

☒ AM001 - Creation, Revision, and Implementation of SOPs Rev 3
☒ AM002 - Personnel Records Rev 1
☒ AM003 - Indexing, Handling, Storage, and Retrieval of Records and Data Rev 2
☒ AM004 - Study Personnel Training Rev 3
☒ AM005 - Protocol and SOP Deviation Rev 2
☒ AM006 - Error in GLP Source Data
☒ AM007 - Data Recording
☒ AM008 - **Obsolete**
☒ AM009 - Management Responsibilities
☒ AM010 - Archiving Original Paper Documents Electronically
☒ AM011 - Electronic Records & Signatures

Animal Care

☒ ANCA001 – Specimen Handling and Tracking Rev 1
☒ ANCA002 – Animal Care and Husbandry Rev 2
☒ ANCA003 – Animal Receiving Rev 3
☒ ANCA004 – **Obsolete**
☒ ANCA005 – Preparing an Animal room for receiving animals
☒ ANCA006 – CO2 Euthanasia Rev 1
☒ ANCA007 – Rodent Quarantine Rev 1
☒ ANCA008 – Animal Feeding & Bedding
☒ ANCA009 – Rodent Ear Marking Rev 2
☒ ANCA010 – Handling dead or moribund animal Rev 1
☒ ANCA011 – Rodent Weighing
☒ ANCA012 – Rodent Daily room log
☒ ANCA013 – Necropsy Rev 1
☒ ANCA014 – Cardiac Rev 3
☒ ANCA015 – **Obsolete**
☒ ANCA016 – Tail Bleed Rev 1
☒ ANCA017 – Mouse Restrainer

Equipment

☒ EQU001 - Balance Calibration PB602-2
☒ EQU002 - Balance Maintenance
☒ EQU003 - Balance Operation PB602-2
☒ EQU004 - Autoclave/sterilization Rev 1
☒ EQU005 - Cage Wash Operations Rev 1
☒ EQU006 - Cage washroom
☒ EQU007 - Metabolic cages Rev 2
☒ EQU008 - Disinfection of a reagent
☒ EQU009 - Water Deliver
☒ EQU010 - Microscope Maintenance
☒ EQU011 - Maintenance refrigerator Rev 2
☒ EQU012 - Maintenance Tissue TEK VIP 3000
☒ EQU013 - Wet Tissue Storage Rev 2
☒ EQU014 -Thermometer Operation Calibration
☒ EQU015 - Glassware Washer
☒ EQU016 - Microbiologic Monitoring
☒ EQU017 - **Obsolete**
☒ EQU018 - Autoclave AMSCO
☒ EQU019 - Microcentrifuge
☒ EQU020 - Microm Autostainer maintenance Rev 1
☒ EQU021 - Boekel Scientific Lab Oven Rev 1
☒ EQU022 - Lipshaw Model 374 Rev 1
☒ EQU023 - Thelco GCA Precision Scientific Oven
☒ EQU024 - Maintenance Embedder
☒ EQU025 - Vacuum sealer
☒ EQU026 - Balance calibration accu-413 Rev 1
☒ EQU027 - Operation of the ACCC-413 Balance
☒ EQU028 - Balance Accu-413 Maintenance
☒ EQU029 - Labconco Fume Adsorber Maintenance
☒ EQU030 - Balance calibration AG104
☒ EQU031 - Balance Operation AG104
☒ EQU032 - Mettler Toledo AG104 maintenance
☒ EQU033 - Maintenance REVCO Freezer
☒ EQU034 - Labe Label Mini
☒ EQU035 - Maintenance Coverslipper
☒ EQU036 - Cryostat Maintenance
☒ EQU037 - HEMAVET HV950 FS
☒ EQU038 - VETSCAN VS2 Rev 1
☒ EQU039 - Maintenance Floatation Bath
☒ EQU040 - Microplate Reader Calibration ELX808
☒ EQU041 - Microplate Reader Decontamination
☒ EQU042 - Automated Microplate Reader ELX808
☒ EQU043 - Operation of Isotemp Laboratory CO2 Incubator
☒ EQU044 - CO2 incubator Maintenance

Histology

☒ HIST001 – H&E Autostaining
☒ HIST002 – Coverslipping Rev 1
☒ HIST003 – Embedding
☒ HIST004 – Tissue Collection Rev 1
☒ HIST005 – Microtome Rev 5
☒ HIST006 – Tissue processing
☒ HIST007 – Urinalysis Rev 1
☒ HIST009 – Trimming Rev 1
☒ HIST010 – QA Histology Rev 1
☒ HIST011 – Block Sealing
☒ HIST012 - Block and Slide Storage

Immunology

☒ IMM001 – Immunizationca Rev 2
☒ IMM002 – Vaccine Mixture Rev 2
☒ IMM003 – Test and Control Articles Rev 2
☒ IMM004 – FACS Protocol Rev1
☒ IMM005 – FACS maintenance
☒ IMM006 – **Obsolete**
☒ IMM007 – ELIZA

Quality Assurance

☒ QAU001 - Quality Assurance Responsibilities
☒ QAU002 - QAU Personnel Training Rev 1
☒ QAU003 - Maintaining the Master Schedule Rev 1
☒ QAU004 - Filing and Indexing QAU Study Records Rev 2
☒ QAU005 - QAU Nonclinical Inspections

Safety

☒ SAF001 – Handling Syringes, Needles & Sharps
☒ SAF002 – Reporting-Tracking Work Relate Injury-illness

UAMS

Office of Breast Cancer Research

Quality Assurance Statement

The Quality Assurance Unit of the UAMS Research Support Center (RSC), formerly the office of Research Support and Regulatory Affairs, was designated by study management to perform the duties of the quality assurance unit for the non-clinical laboratory study entitled "*Determination of the Safety and Tolerability of Immunization with a LeY Peptide Mimotope Vaccine in Mice.*"

In-Phase inspections were conducted on seven separate occasions throughout the study to assure the integrity of the data collected and to confirm that the study protocol and standard operating procedures were followed as written. Written reports of each inspection were forwarded to the study director and study management indicating any findings resulting from the inspection. No deviations from approved protocols or standard operating procedures were made without proper authorization and documentation.

The inspection in-phase inspection calendar is shown below:

UAMS QAU In-Phase Inspection Calendar

Date of Inspection	Phase of Study Inspected	Person Performing Inspection	Date of Inspection Report
5/23/08	Week -1: Quarantine	R.C. Anderson	5/29/08
5/27/08	Week 1: Immunization I	R.C. Anderson	5/30/08
6/09/08	Week 3: Necropsy I	R.C. Anderson	6/09/08
7/8-9/08	Week 7: Immunization IV	Tracy L. Gatlin	7/10/08
7/21/08	Week 9: Necropsy II	Tracy L. Gatlin	7/21/08
9/30/08	Week 19: Immunization V	Tracy L. Gatlin/ R.C. Anderson	10/01/08
10/13/08	Week 21: Necropsy III	Tracy L. Gatlin	10/15/08

R.C. Anderson 5/19/09

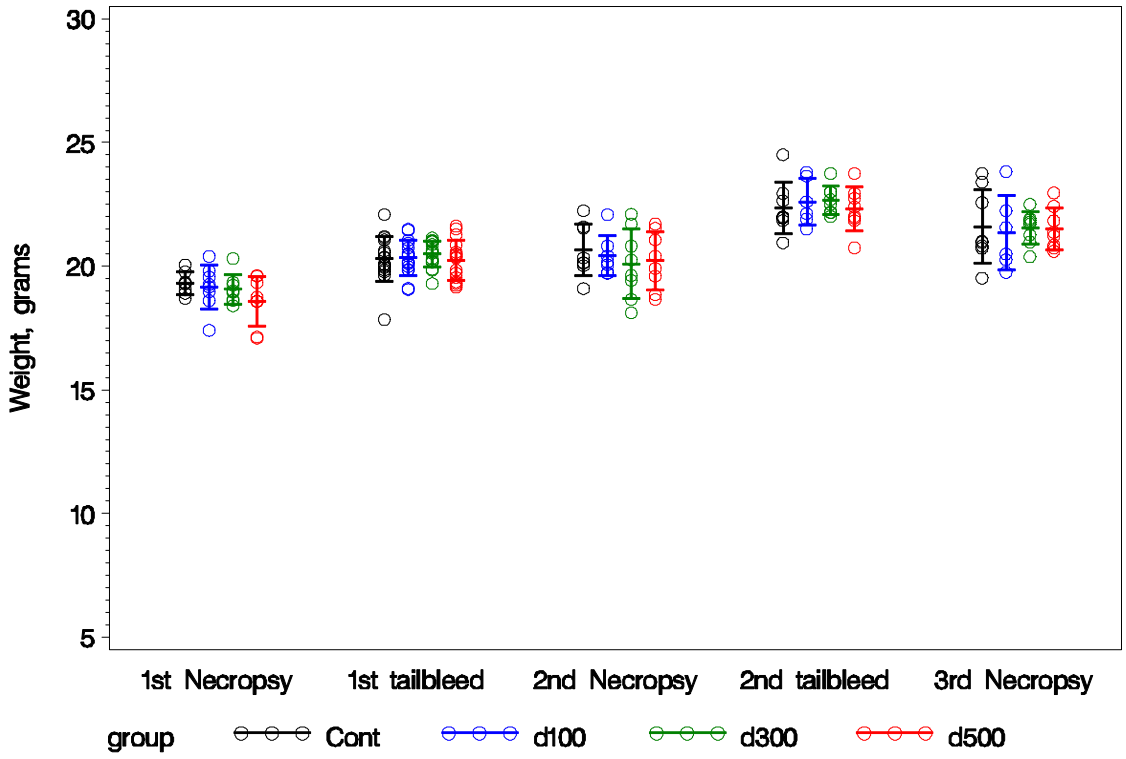
R.C. Anderson Date
Research Support Center
Quality Assurance Manager

Tracy L. Gatlin 5/19/08

Tracy L. Gatlin Date
Research Support Center
Good Tissue Practice Specialist

APPENDIX #11.3.1, Summary of Mouse Weights at All Necropsies and Tail-bleeds

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st tailbleed	1.372	3	0.7122	Dose	-0.016	0.8985
2nd tailbleed	2.308	3	0.5110	Dose	0.063	0.7408
1st Necropsy	2.989	3	0.3934	Dose	-0.309	0.0855
2nd Necropsy	1.032	3	0.7934	Dose	-0.166	0.3720
3rd Necropsy	0.597	3	0.8970	Dose	0.046	0.8112

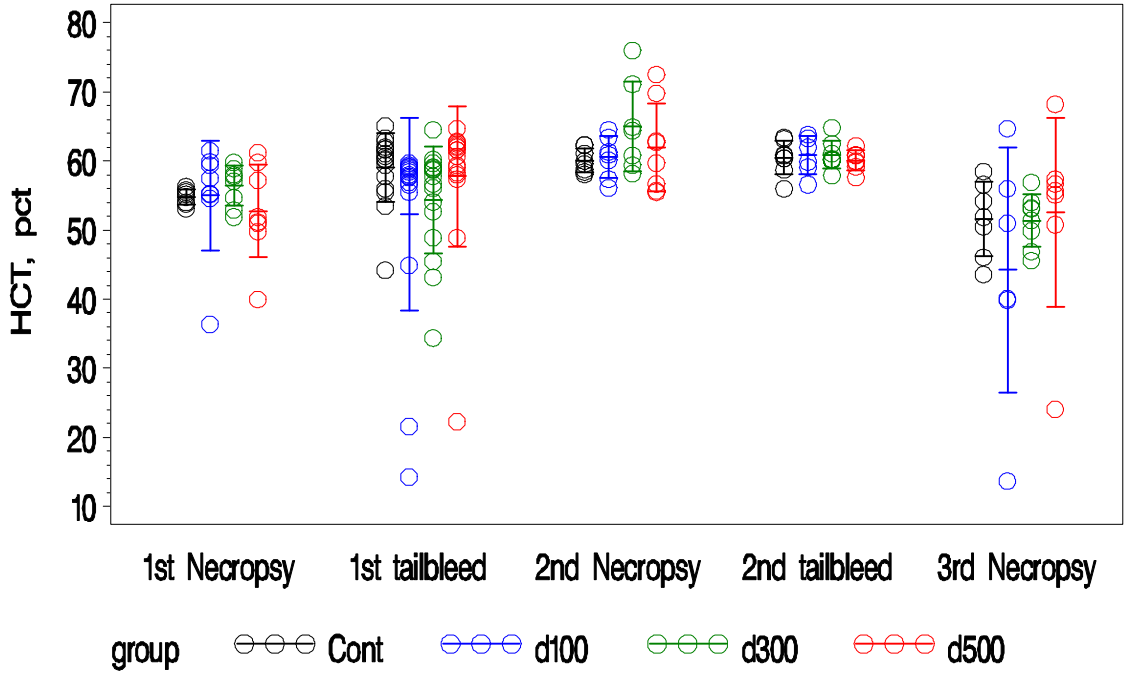


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/08	07/02/08	07/21/08	09/23/08	10/13/08

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Erythrocytes'; Measurement='HCT, pct'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	3.113	3	0.3745	HCT, pct	Dose	-0.074	0.6866
1st tail-bleed	12.558	3	**0.0057**	HCT, pct	Dose	0.023	0.8563
2nd Necropsy	2.636	3	0.4512	HCT, pct	Dose	0.134	0.4811
2nd tail-bleed	0.763	3	0.8582	HCT, pct	Dose	-0.118	0.5406
3rd Necropsy	2.490	3	0.4770	HCT, pct	Dose	0.184	0.3492

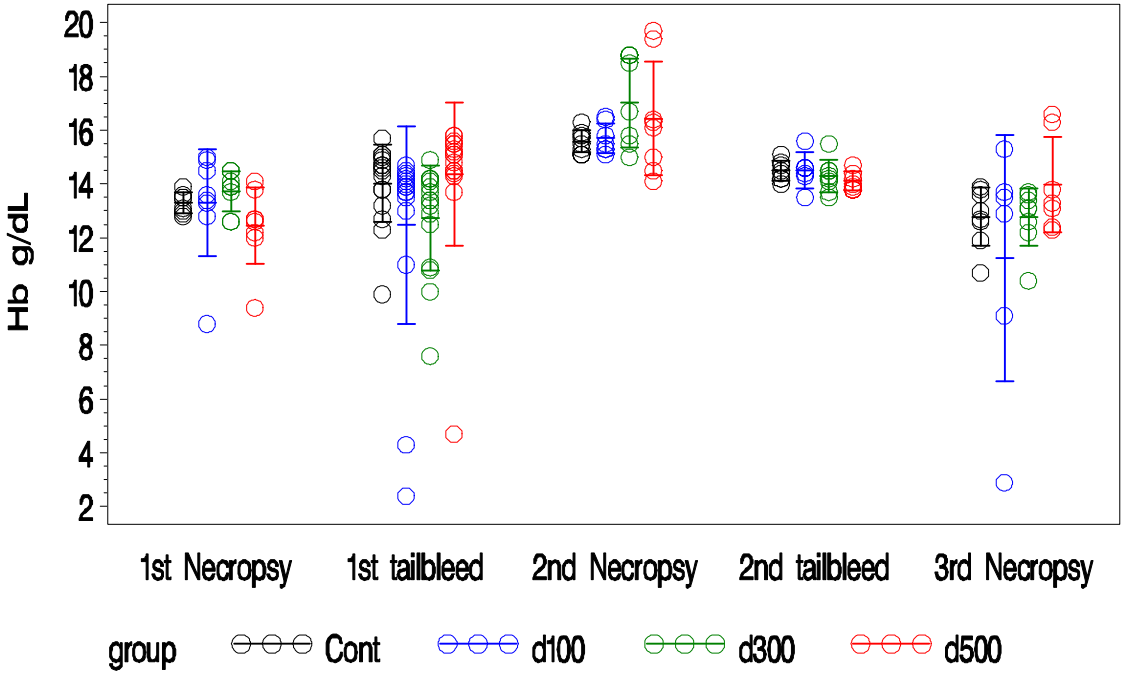


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Erythrocytes'; Measurement='Hb g/dL'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	6.091	3	0.1073	Hb g/dL	Dose	-0.199	0.2759
1st tail-bleed	20.762	3	**0.0001**	Hb g/dL	Dose	0.180	0.1542
2nd Necropsy	2.582	3	0.4606	Hb g/dL	Dose	0.195	0.3029
2nd tail-bleed	3.748	3	0.2900	Hb g/dL	Dose	-0.352	0.0608
3rd Necropsy	1.451	3	0.6936	Hb g/dL	Dose	0.159	0.4089

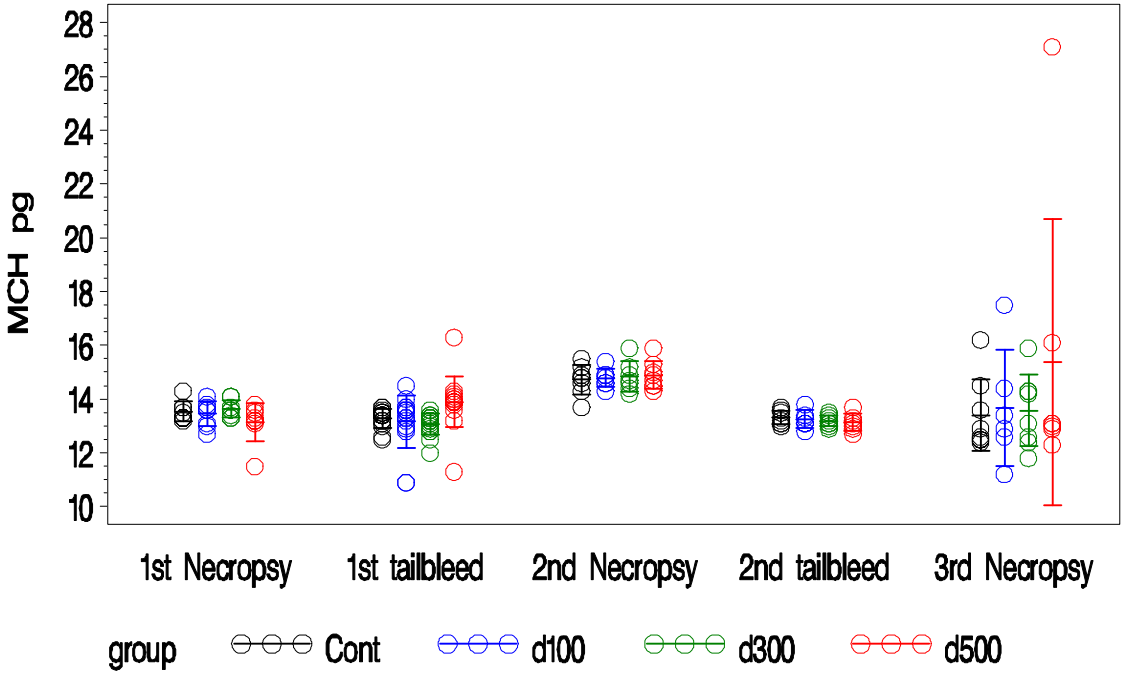


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Erythrocytes'; Measurement='MCH pg'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	3.383	3	0.3362	MCH pg	Dose	-0.154	0.4002
1st tail-bleed	22.068	3	**<.0001**	MCH pg	Dose	0.318	0.0104
2nd Necropsy	0.118	3	0.9896	MCH pg	Dose	0.053	0.7824
2nd tail-bleed	1.779	3	0.6196	MCH pg	Dose	-0.252	0.1877
3rd Necropsy	0.471	3	0.9252	MCH pg	Dose	0.121	0.5331

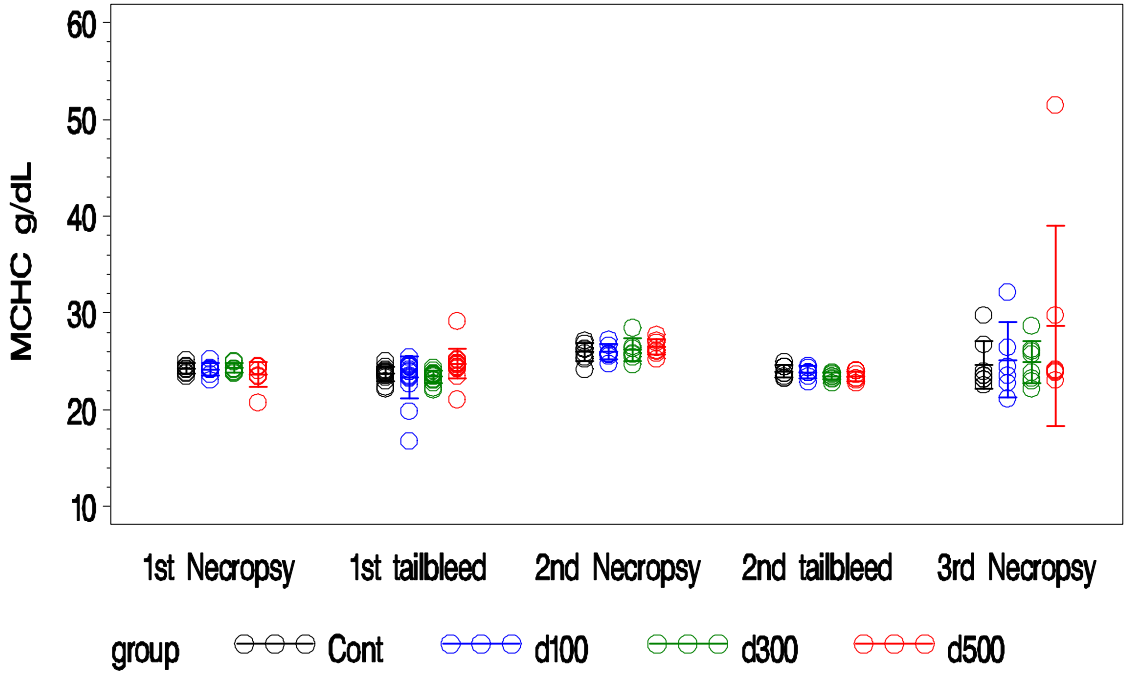


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Erythrocytes'; Measurement='MCHC g/dL'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	1.501	3	0.6821	MCHC g/dL	Dose	-0.149	0.4160
1st tail-bleed	18.756	3	**0.0003**	MCHC g/dL	Dose	0.301	0.0156
2nd Necropsy	1.853	3	0.6035	MCHC g/dL	Dose	0.210	0.2644
2nd tail-bleed	4.255	3	0.2352	MCHC g/dL	Dose	-0.344	0.0677
3rd Necropsy	1.216	3	0.7492	MCHC g/dL	Dose	0.193	0.3171

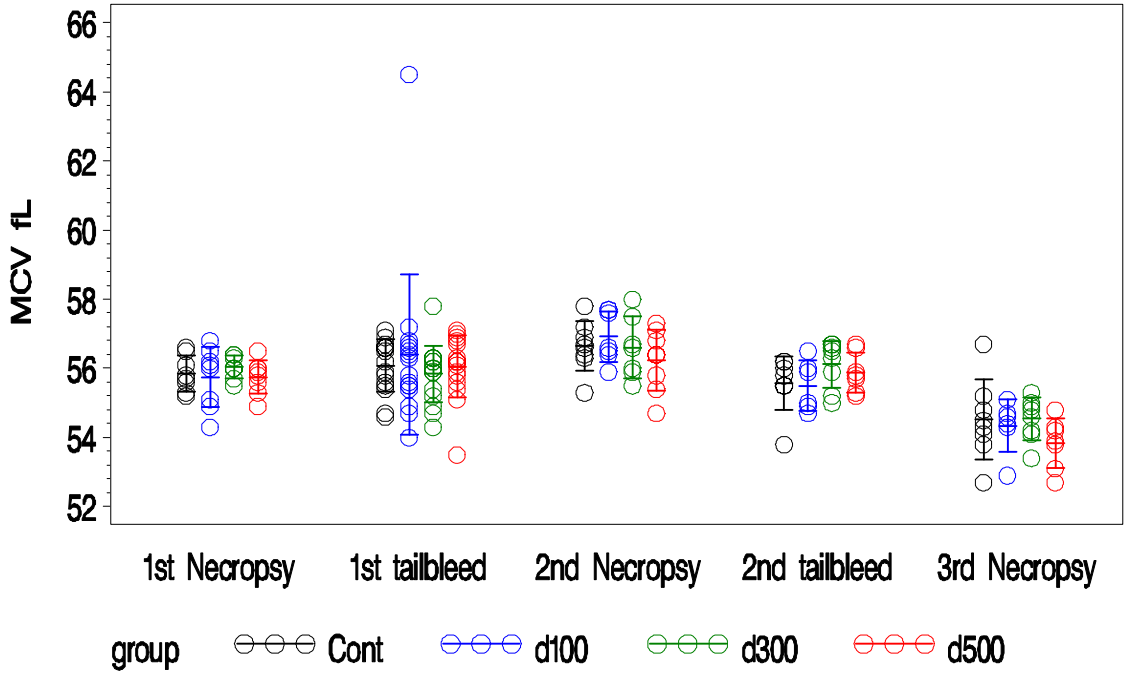


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Erythrocytes'; Measurement='MCV fL'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	1.170	3	0.7603	MCV fL	Dose	-0.041	0.8235
1st tail-bleed	1.636	3	0.6512	MCV fL	Dose	-0.029	0.8213
2nd Necropsy	1.931	3	0.5869	MCV fL	Dose	-0.196	0.2996
2nd tail-bleed	3.655	3	0.3012	MCV fL	Dose	0.212	0.2698
3rd Necropsy	3.938	3	0.2683	MCV fL	Dose	-0.217	0.2591

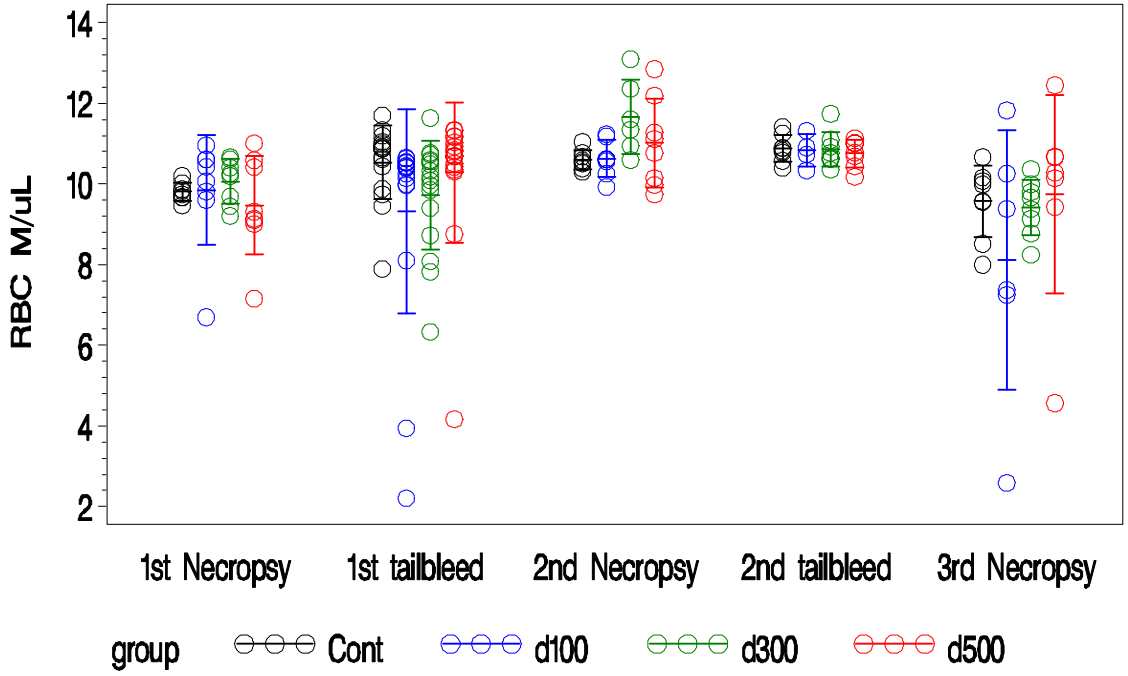


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Erythrocytes'; Measurement='RBC M/uL'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	2.853	3	0.4149	RBC M/uL	Dose	-0.073	0.6927
1st tail-bleed	12.335	3	**0.0063**	RBC M/uL	Dose	-0.005	0.9716
2nd Necropsy	6.700	3	0.0821	RBC M/uL	Dose	0.268	0.1597
2nd tail-bleed	0.305	3	0.9591	RBC M/uL	Dose	-0.100	0.6204
3rd Necropsy	3.596	3	0.3085	RBC M/uL	Dose	0.189	0.3273

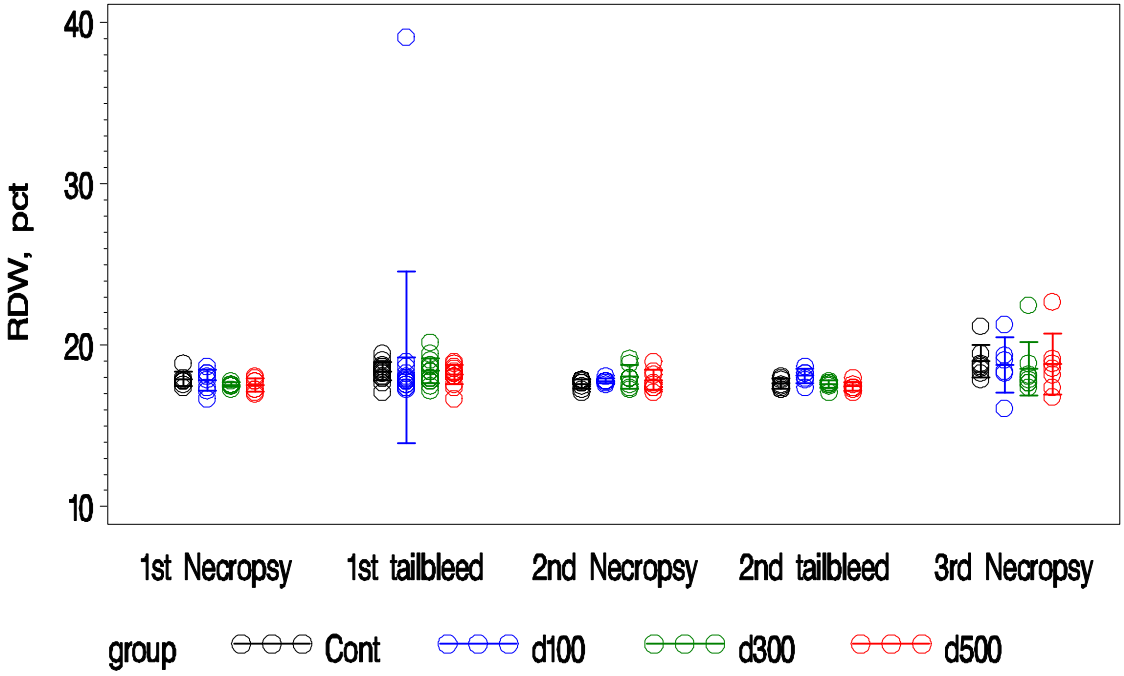


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Erythrocytes'; Measurement='RDW, pct'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	4.114	3	0.2494	RDW, pct	Dose	-0.324	0.0703
1st tail-bleed	5.526	3	0.1371	RDW, pct	Dose	-0.016	0.9006
2nd Necropsy	1.646	3	0.6489	RDW, pct	Dose	0.098	0.6069
2nd tail-bleed	8.076	3	0.0445	RDW, pct	Dose	-0.266	0.1635
3rd Necropsy	2.665	3	0.4462	RDW, pct	Dose	-0.197	0.3066

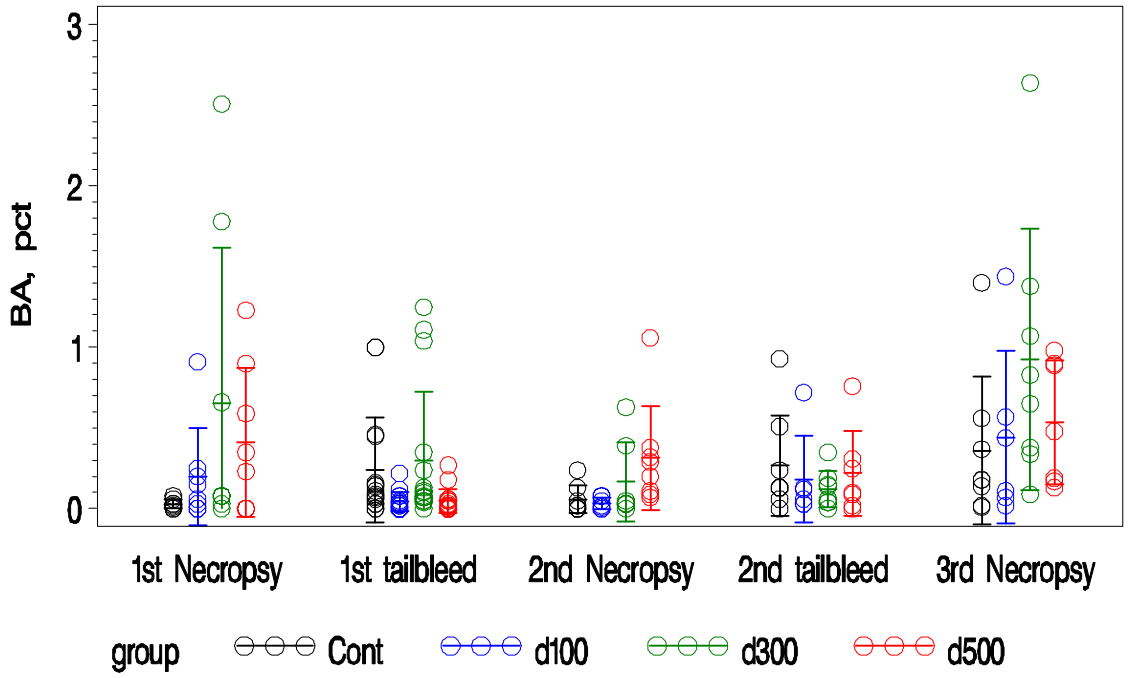


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Leukocytes'; Measurement='BA, pct'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	4.546	3	0.2083	BA, pct	Dose	0.313	0.0808
1st tail-bleed	17.085	3	**0.0007**	BA, pct	Dose	-0.209	0.0969
2nd Necropsy	10.936	3	0.0121	BA, pct	Dose	0.565	**0.0012**
2nd tail-bleed	1.011	3	0.7985	BA, pct	Dose	-0.087	0.6526
3rd Necropsy	4.038	3	0.2573	BA, pct	Dose	0.289	0.1280

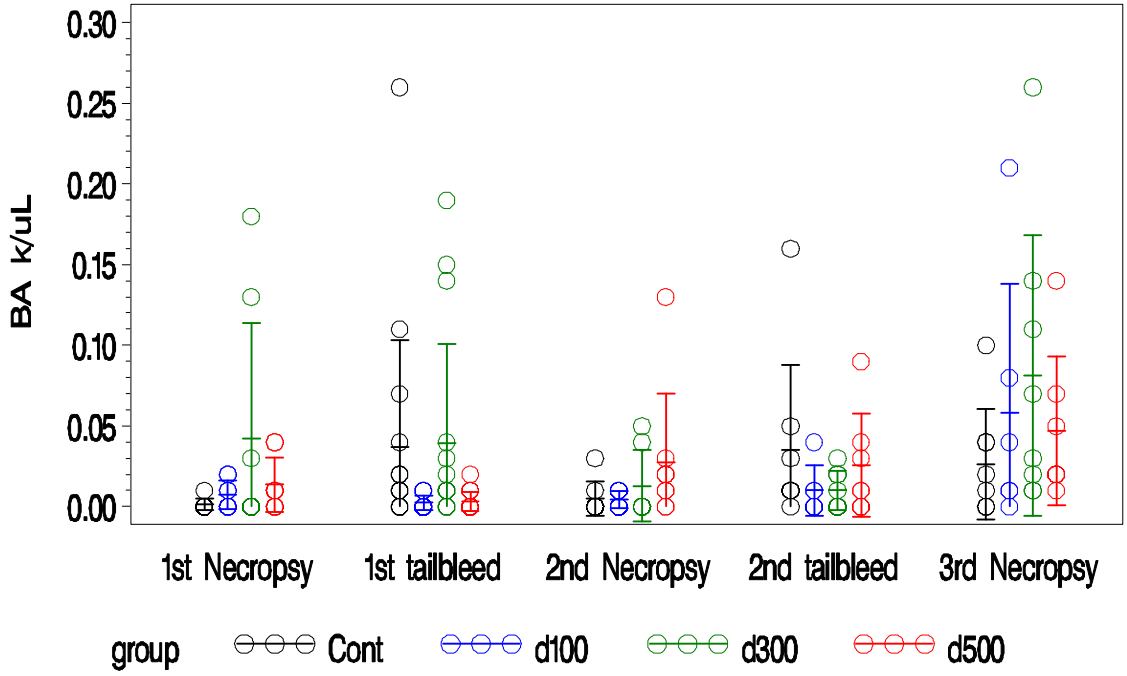


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Leukocytes'; Measurement='BA k/uL'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	4.125	3	0.2483	BA k/uL	Dose	0.333	0.0623
1st tail-bleed	19.116	3	**0.0003**	BA k/uL	Dose	-0.241	0.0547
2nd Necropsy	4.690	3	0.1960	BA k/uL	Dose	0.365	0.0476
2nd tail-bleed	2.919	3	0.4042	BA k/uL	Dose	-0.065	0.7371
3rd Necropsy	3.193	3	0.3628	BA k/uL	Dose	0.279	0.1423

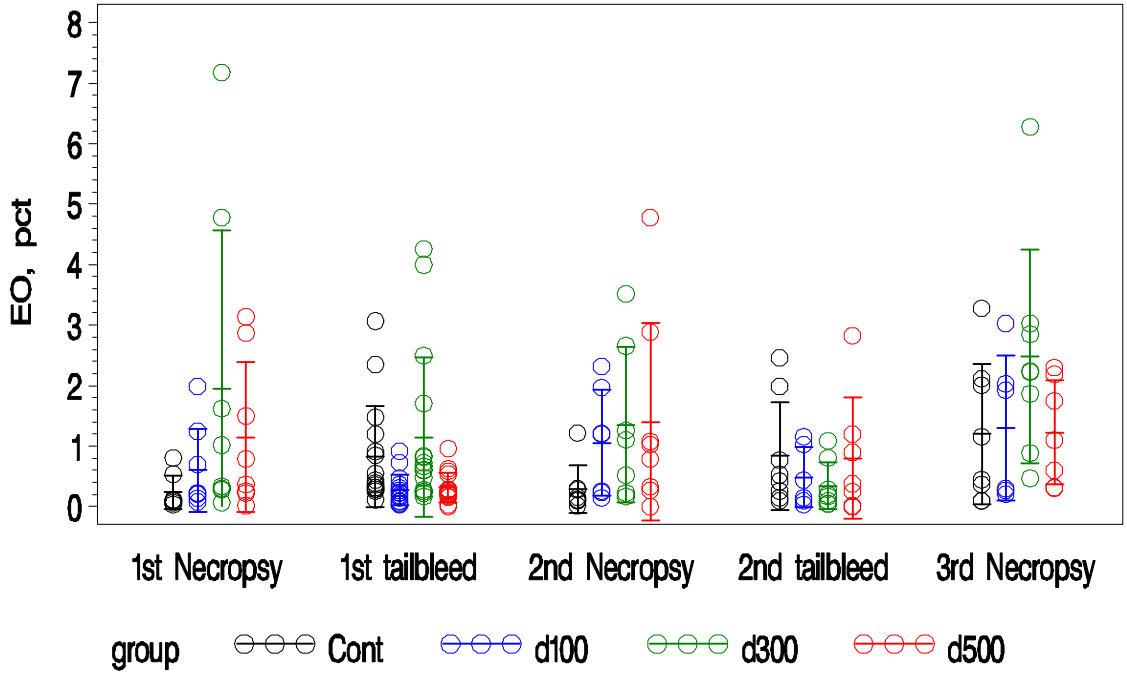


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Leukocytes'; Measurement='EO, pct'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	6.810	3	0.0782	EO, pct	Dose	0.415	0.0182
1st tail-bleed	17.068	3	**0.0007**	EO, pct	Dose	-0.176	0.1634
2nd Necropsy	6.338	3	0.0963	EO, pct	Dose	0.368	0.0454
2nd tail-bleed	1.638	3	0.6508	EO, pct	Dose	-0.122	0.5283
3rd Necropsy	4.633	3	0.2007	EO, pct	Dose	0.167	0.3857

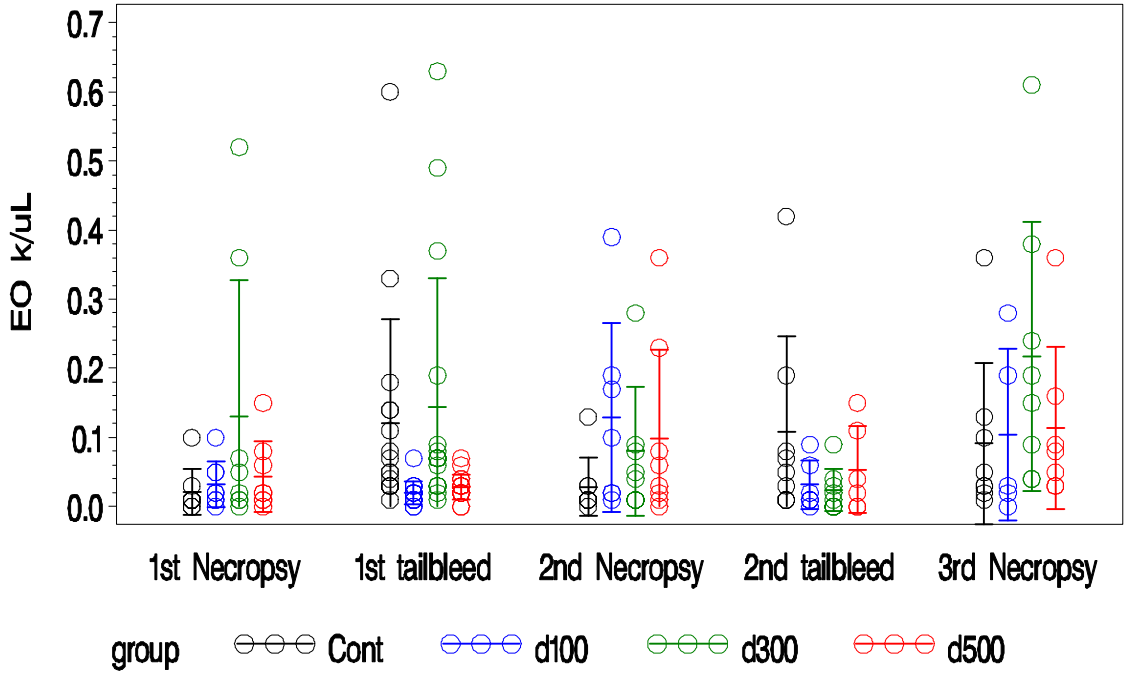


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Leukocytes'; Measurement='EO k/uL'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	2.630	3	0.4522	EO k/uL	Dose	0.236	0.1931
1st tail-bleed	26.253	3	**<.0001**	EO k/uL	Dose	-0.178	0.1583
2nd Necropsy	4.506	3	0.2117	EO k/uL	Dose	0.224	0.2334
2nd tail-bleed	3.815	3	0.2821	EO k/uL	Dose	-0.234	0.2311
3rd Necropsy	4.573	3	0.2059	EO k/uL	Dose	0.231	0.2369

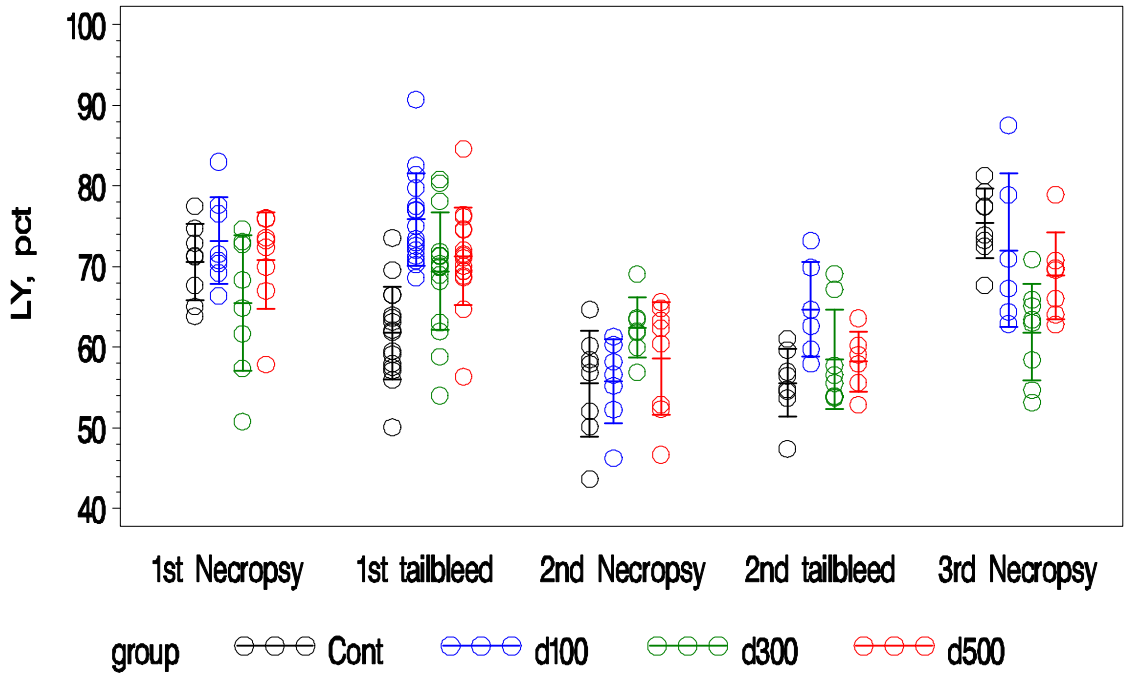


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Leukocytes'; Measurement='LY, pct'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	3.702	3	0.2955	LY, pct	Dose	-0.039	0.8307
1st tail-bleed	26.941	3	**<.0001**	LY, pct	Dose	0.300	0.0159
2nd Necropsy	7.108	3	0.0685	LY, pct	Dose	0.360	0.0507
2nd tail-bleed	8.348	3	0.0393	LY, pct	Dose	0.079	0.6885
3rd Necropsy	12.956	3	**0.0047**	LY, pct	Dose	-0.483	**0.0080**

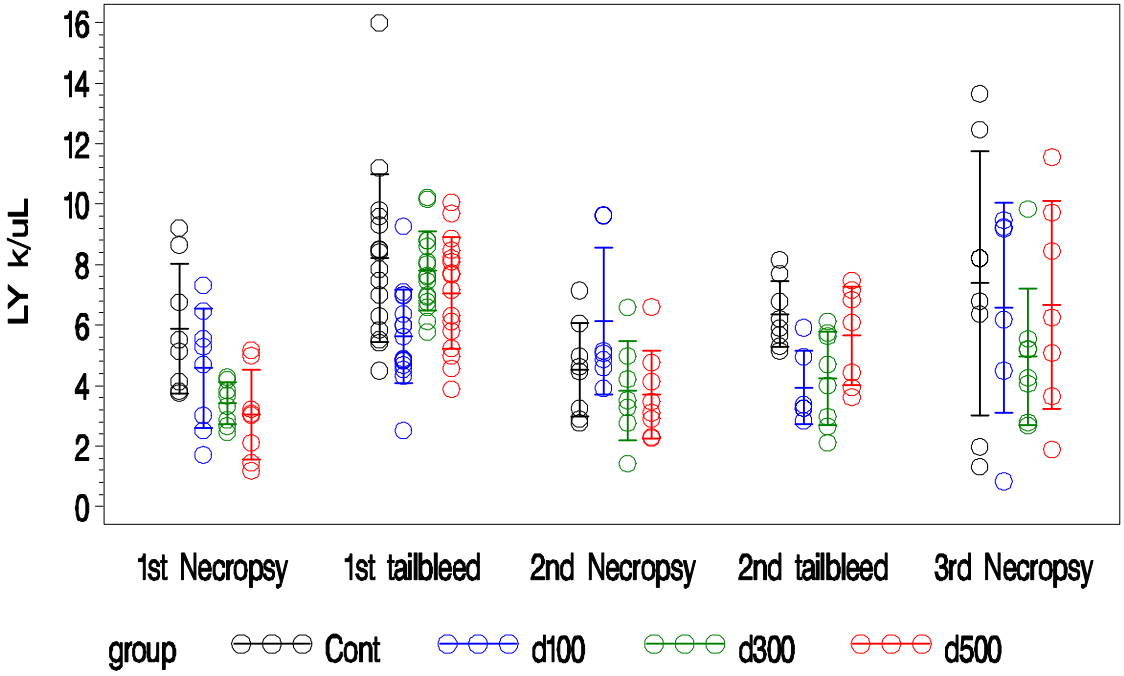


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Leukocytes'; Measurement='LY k/uL'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	9.389	3	0.0245	LY k/uL	Dose	-0.542	**0.0014**
1st tail-bleed	14.250	3	**0.0026**	LY k/uL	Dose	0.008	0.9503
2nd Necropsy	6.575	3	0.0867	LY k/uL	Dose	-0.293	0.1158
2nd tail-bleed	10.187	3	0.0170	LY k/uL	Dose	-0.137	0.4786
3rd Necropsy	1.862	3	0.6015	LY k/uL	Dose	-0.120	0.5355

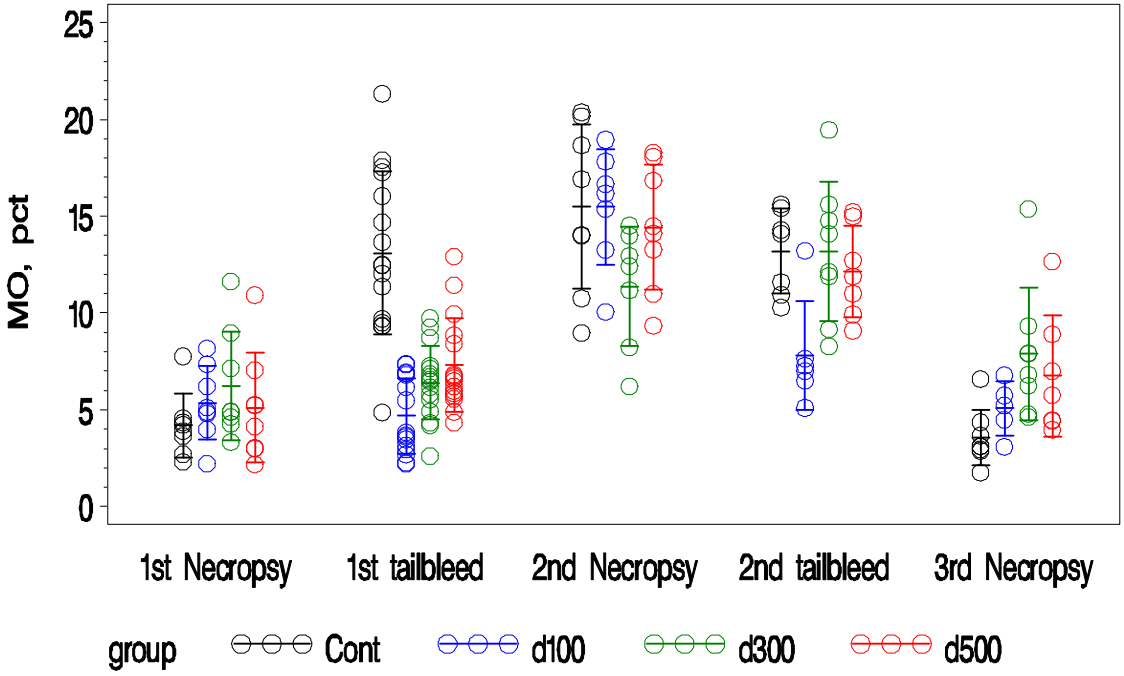


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Leukocytes'; Measurement='MO, pct'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	3.943	3	0.2677	MO, pct	Dose	0.161	0.3802
1st tail-bleed	30.843	3	**<.0001**	MO, pct	Dose	-0.323	**0.0091**
2nd Necropsy	5.508	3	0.1382	MO, pct	Dose	-0.192	0.3093
2nd tail-bleed	9.656	3	0.0217	MO, pct	Dose	0.042	0.8320
3rd Necropsy	13.183	3	**0.0043**	MO, pct	Dose	0.570	**0.0015**

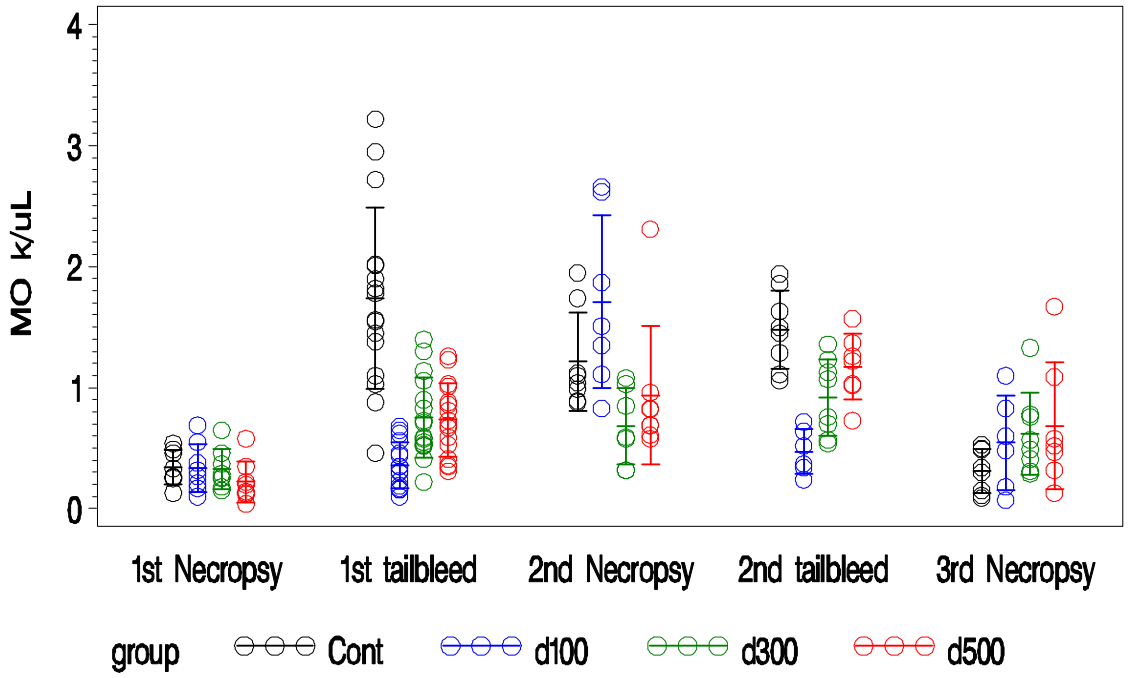


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Leukocytes'; Measurement='MO k/uL'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	3.642	3	0.3028	MO k/uL	Dose	-0.286	0.1122
1st tail-bleed	36.663	3	**<.0001**	MO k/uL	Dose	-0.283	0.0233
2nd Necropsy	13.772	3	**0.0032**	MO k/uL	Dose	-0.511	**0.0039**
2nd tail-bleed	17.546	3	**0.0005**	MO k/uL	Dose	-0.163	0.3977
3rd Necropsy	3.843	3	0.2789	MO k/uL	Dose	0.325	0.0855

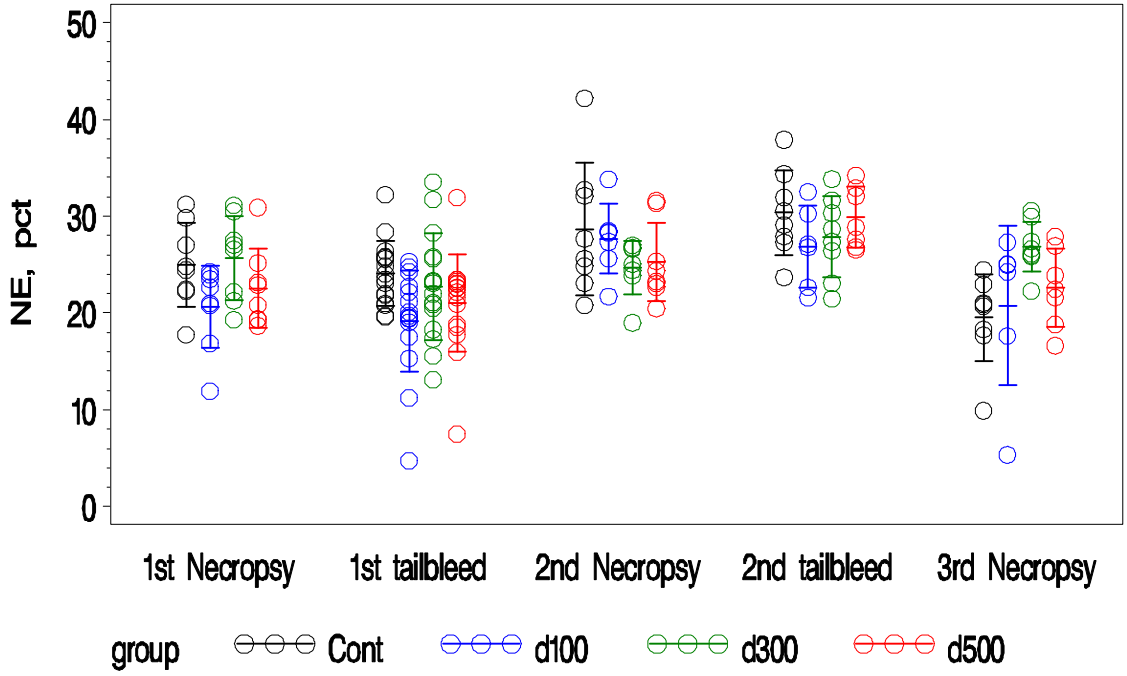


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Leukocytes'; Measurement='NE, pct'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	5.236	3	0.1553	NE, pct	Dose	-0.100	0.5864
1st tail-bleed	9.281	3	0.0258	NE, pct	Dose	-0.163	0.1970
2nd Necropsy	4.226	3	0.2381	NE, pct	Dose	-0.318	0.0868
2nd tail-bleed	3.487	3	0.3224	NE, pct	Dose	-0.039	0.8410
3rd Necropsy	10.717	3	0.0134	NE, pct	Dose	0.355	0.0587

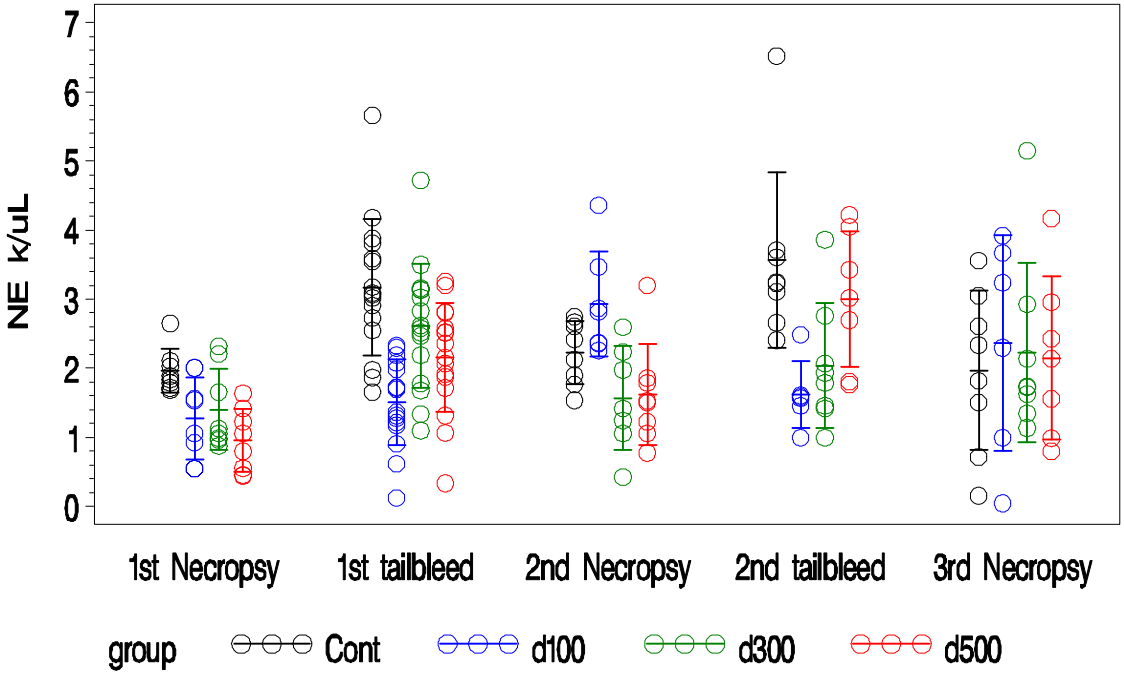


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Leukocytes'; Measurement='NE k/uL'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	12.244	3	0.0066	NE k/uL	Dose	-0.554	**0.0010**
1st tail-bleed	23.914	3	**<.0001**	NE k/uL	Dose	-0.177	0.1616
2nd Necropsy	12.971	3	**0.0047**	NE k/uL	Dose	-0.478	**0.0076**
2nd tail-bleed	13.210	3	**0.0042**	NE k/uL	Dose	-0.082	0.6734
3rd Necropsy	0.366	3	0.9472	NE k/uL	Dose	-0.002	0.9911

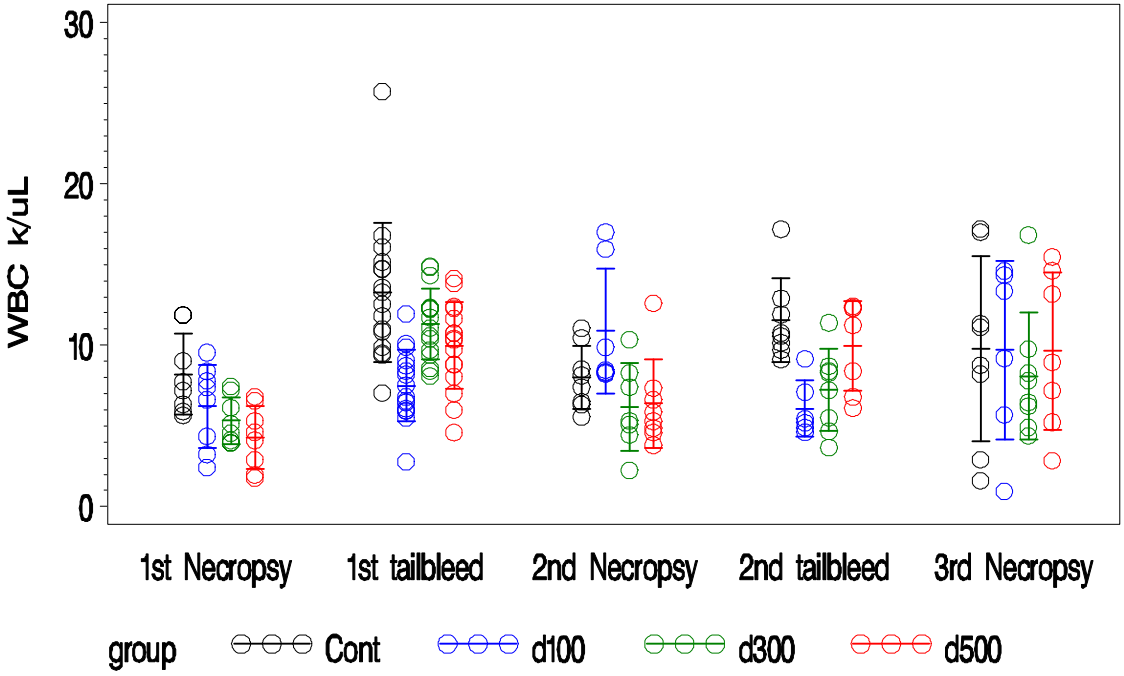


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Leukocytes'; Measurement='WBC k/uL'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	9.217	3	0.0265	WBC k/uL	Dose	-0.543	**0.0013**
1st tail-bleed	24.042	3	**<.0001**	WBC k/uL	Dose	-0.134	0.2914
2nd Necropsy	10.602	3	0.0141	WBC k/uL	Dose	-0.430	0.0178
2nd tail-bleed	13.417	3	**0.0038**	WBC k/uL	Dose	-0.131	0.4991
3rd Necropsy	0.884	3	0.8293	WBC k/uL	Dose	-0.074	0.7035

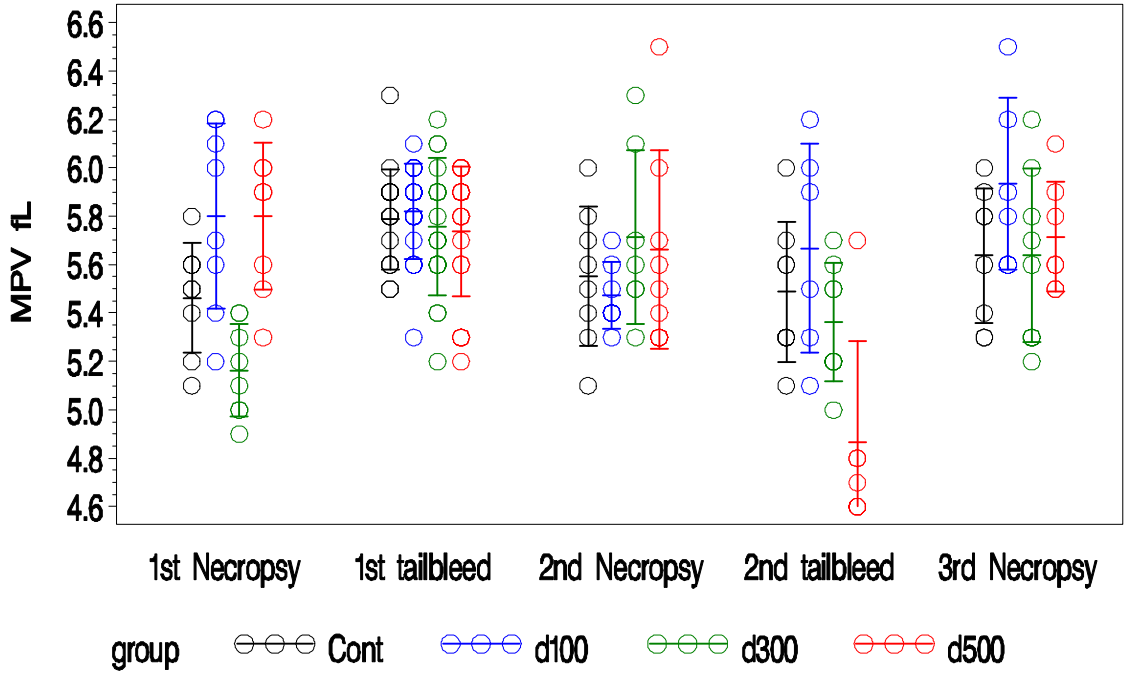


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Thrombocytes'; Measurement='MPV fL'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	15.760	3	**0.0013**	MPV fL	Dose	0.085	0.6434
1st tail-bleed	0.804	3	0.8486	MPV fL	Dose	-0.023	0.8593
2nd Necropsy	1.871	3	0.5995	MPV fL	Dose	0.112	0.5550
2nd tail-bleed	9.151	3	0.0274	MPV fL	Dose	-0.487	**0.0085**
3rd Necropsy	2.757	3	0.4306	MPV fL	Dose	-0.016	0.9326

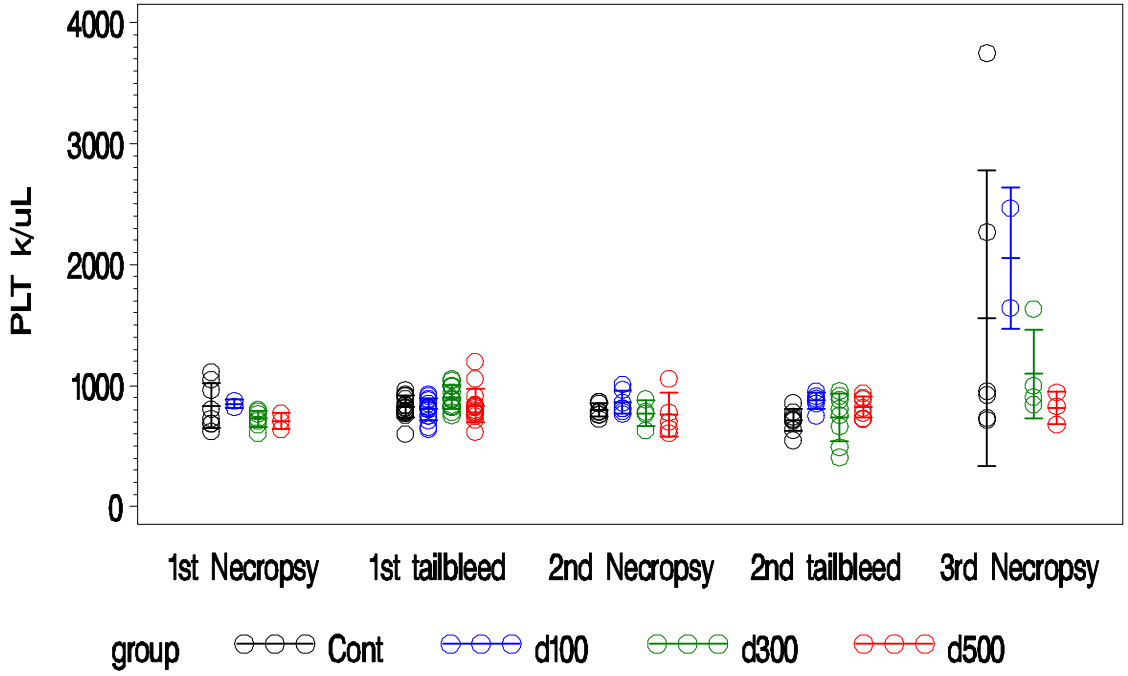


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.2, Hemavet Summary: All 3 Necropsies and Both Tail-bleeds
Analysis='Hemavet:Thrombocytes'; Measurement='PLT k/uL'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	4.093	3	0.2516	PLT k/uL	Dose	-0.328	0.1578
1st tail-bleed	7.442	3	0.0591	PLT k/uL	Dose	0.041	0.7568
2nd Necropsy	4.024	3	0.2589	PLT k/uL	Dose	-0.208	0.3287
2nd tail-bleed	7.282	3	0.0634	PLT k/uL	Dose	0.264	0.1656
3rd Necropsy	4.525	3	0.2101	PLT k/uL	Dose	-0.283	0.3076

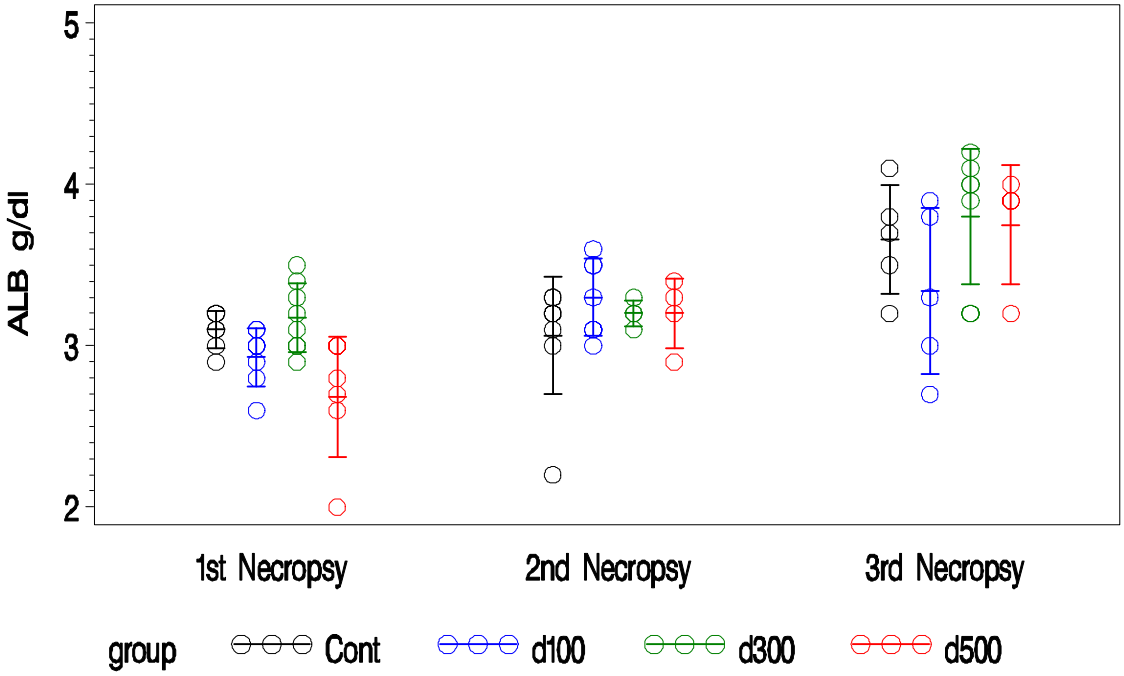


Procedure	1st Necropsy	1st tailbleed	2nd Necropsy	2nd tailbleed	3rd Necropsy
Date	06/09/2008	07/02/2008	07/21/2008	09/23/2008	10/13/2008

Scatterplot: circles=values; dashes(error bars)=means(SDs)

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='ALB g/dl'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

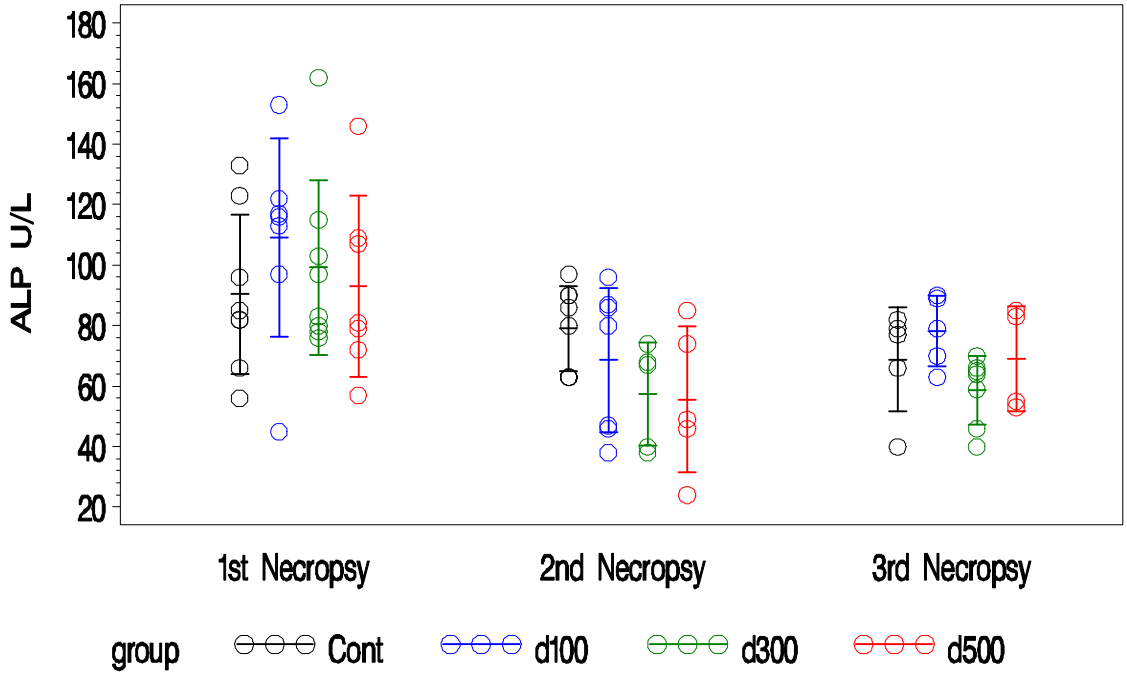
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	11.784	3	**0.0082**	ALB g/dl	Dose	-0.333	0.0837
2nd Necropsy	1.585	3	0.6629	ALB g/dl	Dose	0.146	0.5068
3rd Necropsy	4.038	3	0.2573	ALB g/dl	Dose	0.257	0.2610



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='ALP U/L'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

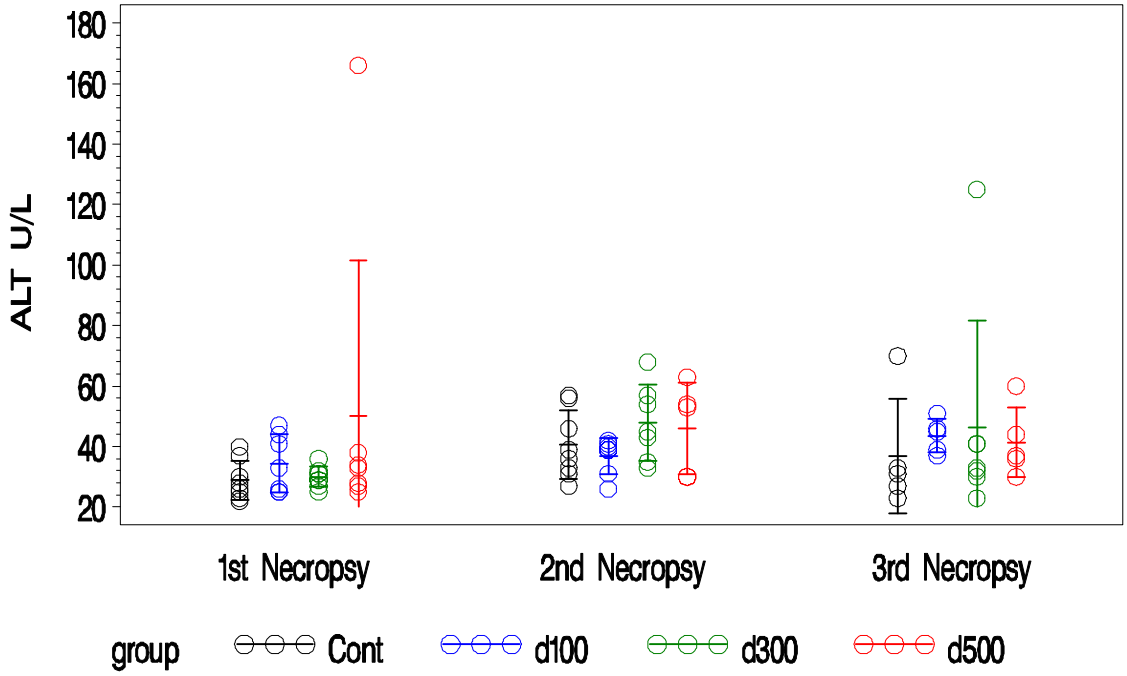
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	2.498	3	0.4756	ALP U/L	Dose	-0.071	0.7106
2nd Necropsy	4.977	3	0.1735	ALP U/L	Dose	-0.441	0.0274
3rd Necropsy	4.999	3	0.1719	ALP U/L	Dose	-0.207	0.3683



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='ALT U/L'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

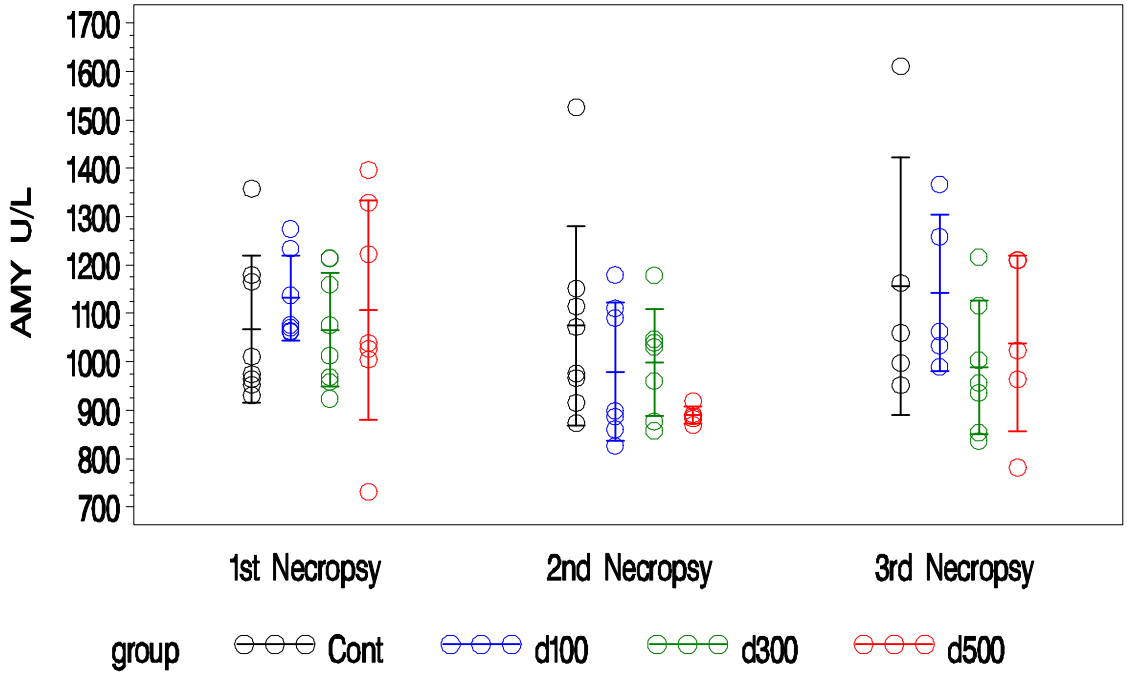
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	2.300	3	0.5125	ALT U/L	Dose	0.209	0.2675
2nd Necropsy	2.956	3	0.3984	ALT U/L	Dose	0.195	0.3305
3rd Necropsy	3.868	3	0.2761	ALT U/L	Dose	0.109	0.6280



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='AMY U/L'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

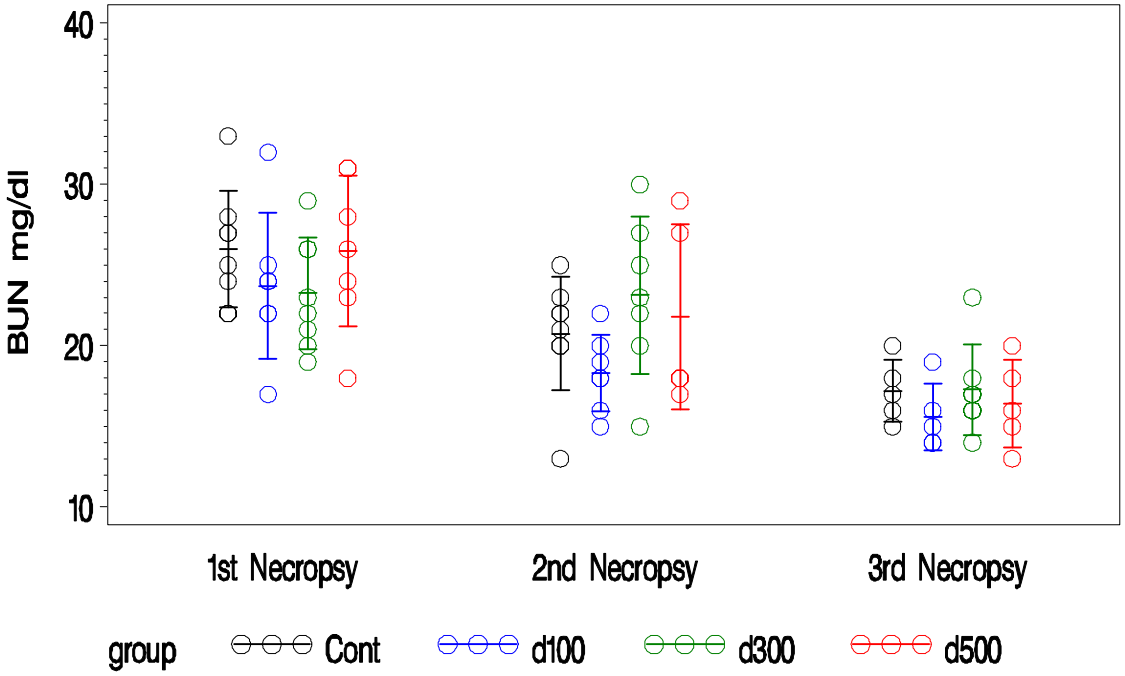
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	2.535	3	0.4690	AMY U/L	Dose	0.086	0.6508
2nd Necropsy	4.202	3	0.2405	AMY U/L	Dose	-0.351	0.0730
3rd Necropsy	3.344	3	0.3416	AMY U/L	Dose	-0.244	0.2747



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='BUN mg/dl'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

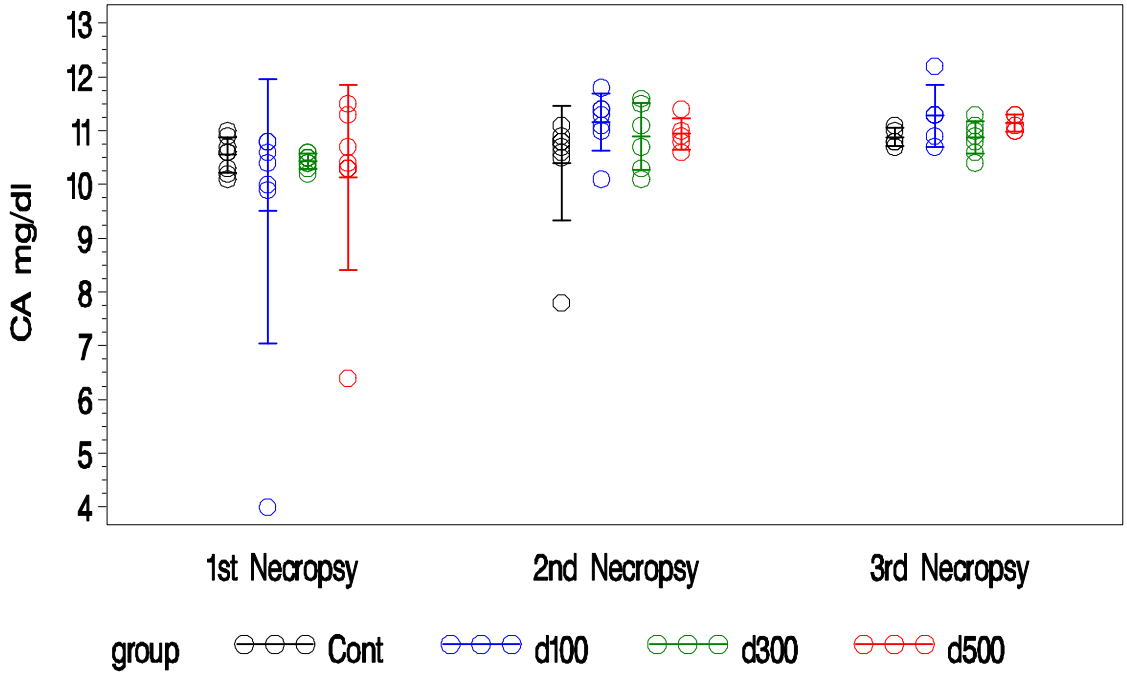
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	3.188	3	0.3635	BUN mg/dl	Dose	-0.042	0.8275
2nd Necropsy	5.079	3	0.1661	BUN mg/dl	Dose	0.100	0.6208
3rd Necropsy	2.159	3	0.5400	BUN mg/dl	Dose	-0.034	0.8798



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='CA mg/dl'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

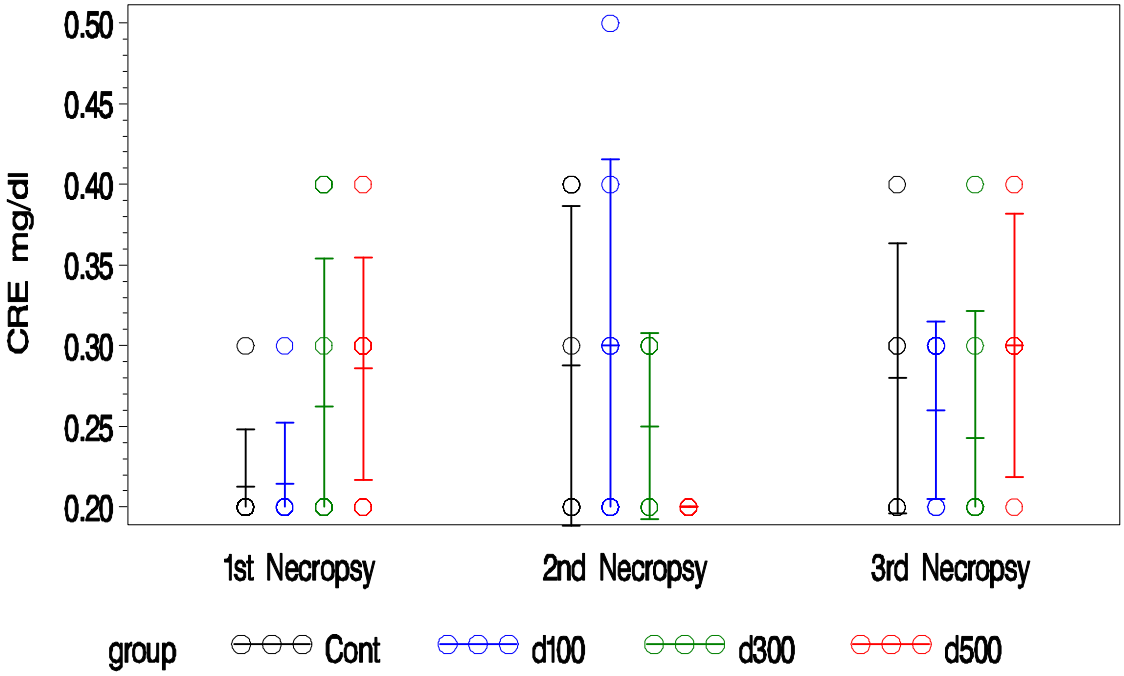
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	1.107	3	0.7755	CA mg/dl	Dose	-0.023	0.9060
2nd Necropsy	4.856	3	0.1827	CA mg/dl	Dose	0.191	0.3487
3rd Necropsy	4.908	3	0.1786	CA mg/dl	Dose	0.230	0.3035



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='CRE mg/dl'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

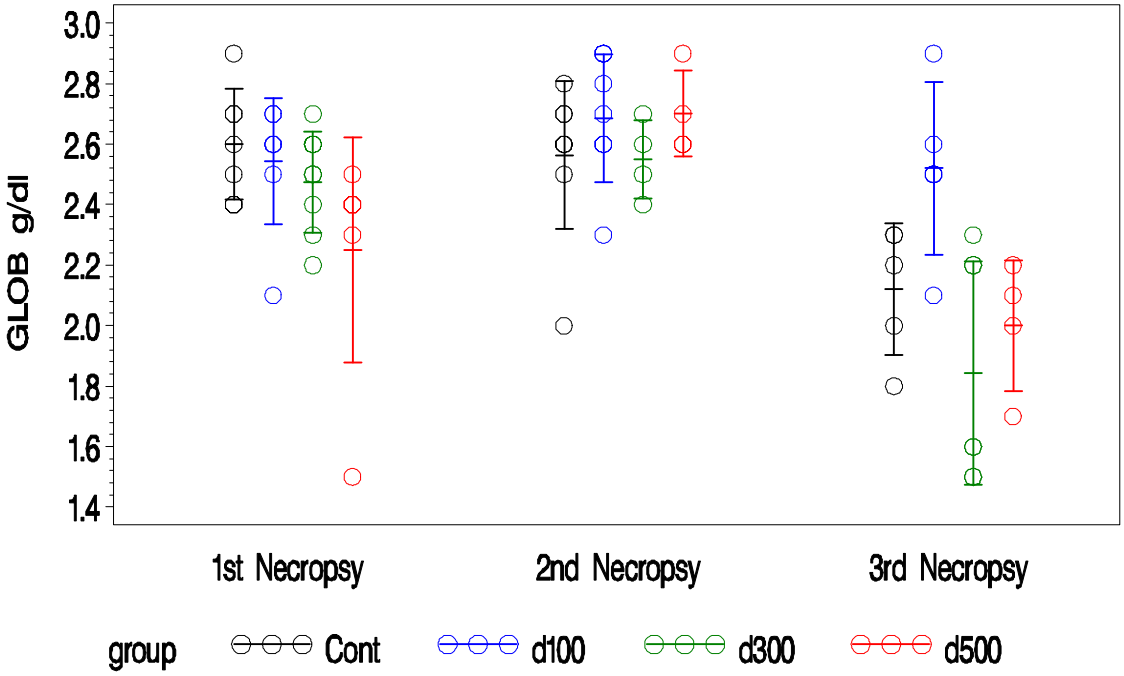
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	6.883	3	0.0757	CRE mg/dl	Dose	0.464	**0.0097**
2nd Necropsy	3.633	3	0.3039	CRE mg/dl	Dose	-0.312	0.1467
3rd Necropsy	2.013	3	0.5698	CRE mg/dl	Dose	0.008	0.9709



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='GLOB g/dl'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

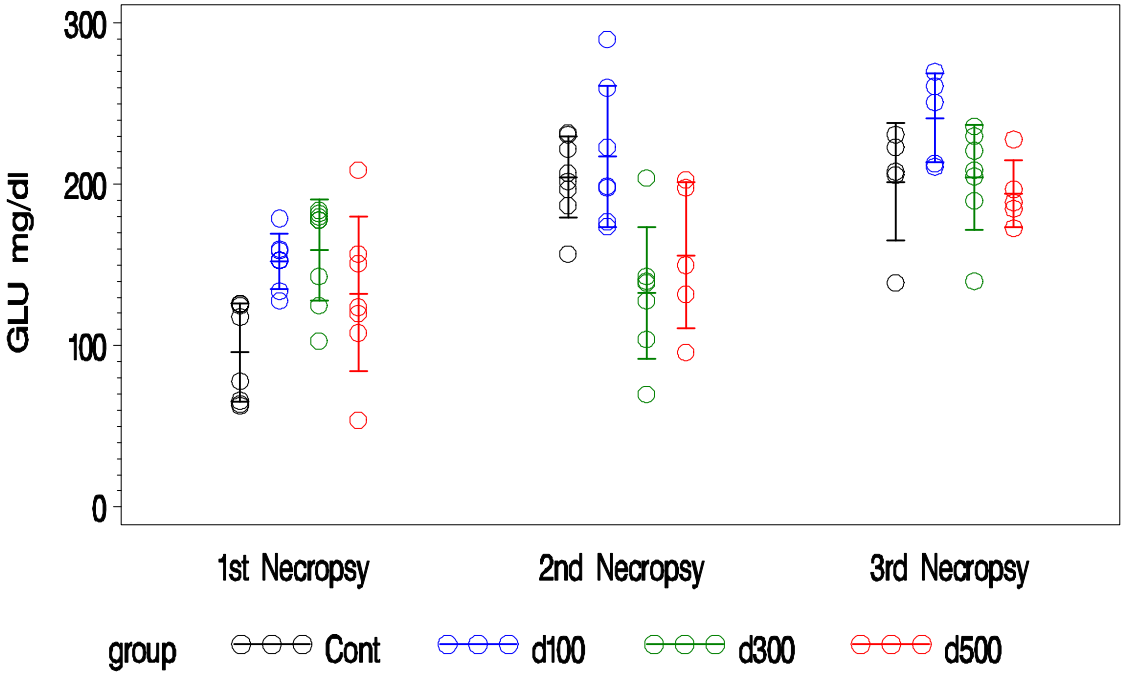
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	7.342	3	0.0618	GLOB g/dl	Dose	-0.493	**0.0077**
2nd Necropsy	2.970	3	0.3963	GLOB g/dl	Dose	0.087	0.6945
3rd Necropsy	8.736	3	0.0330	GLOB g/dl	Dose	-0.380	0.0897



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='GLU mg/dl'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

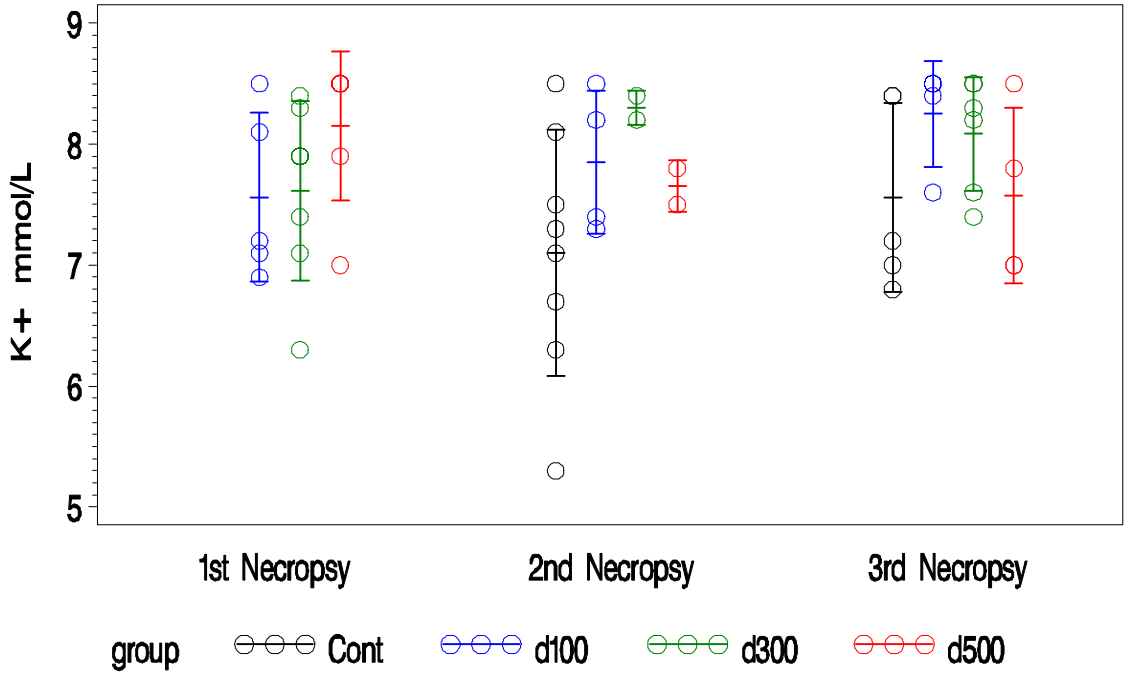
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	11.801	3	**0.0081**	GLU mg/dl	Dose	0.283	0.1301
2nd Necropsy	11.630	3	**0.0088**	GLU mg/dl	Dose	-0.543	**0.0034**
3rd Necropsy	6.951	3	0.0735	GLU mg/dl	Dose	-0.314	0.1552



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='K+ mmol/L'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

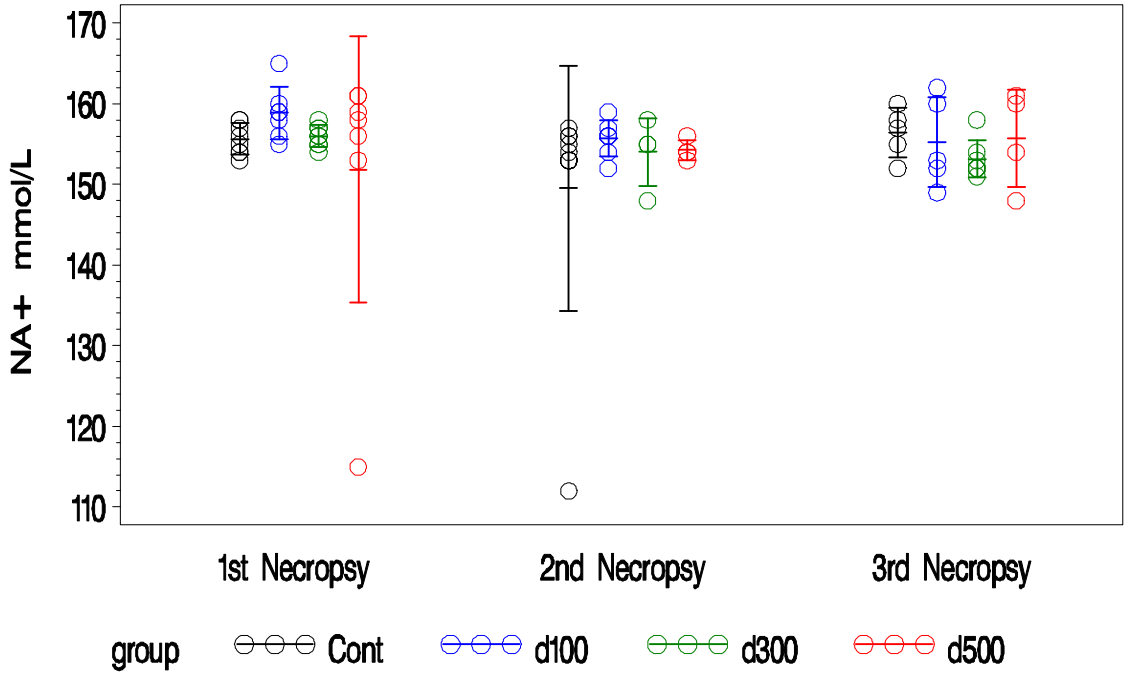
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	3.251	2	0.1969	K+ mmol/L	Dose	0.381	0.1187
2nd Necropsy	4.220	3	0.2387	K+ mmol/L	Dose	0.449	0.0812
3rd Necropsy	4.070	3	0.2540	K+ mmol/L	Dose	0.055	0.8225



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='NA+ mmol/L'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

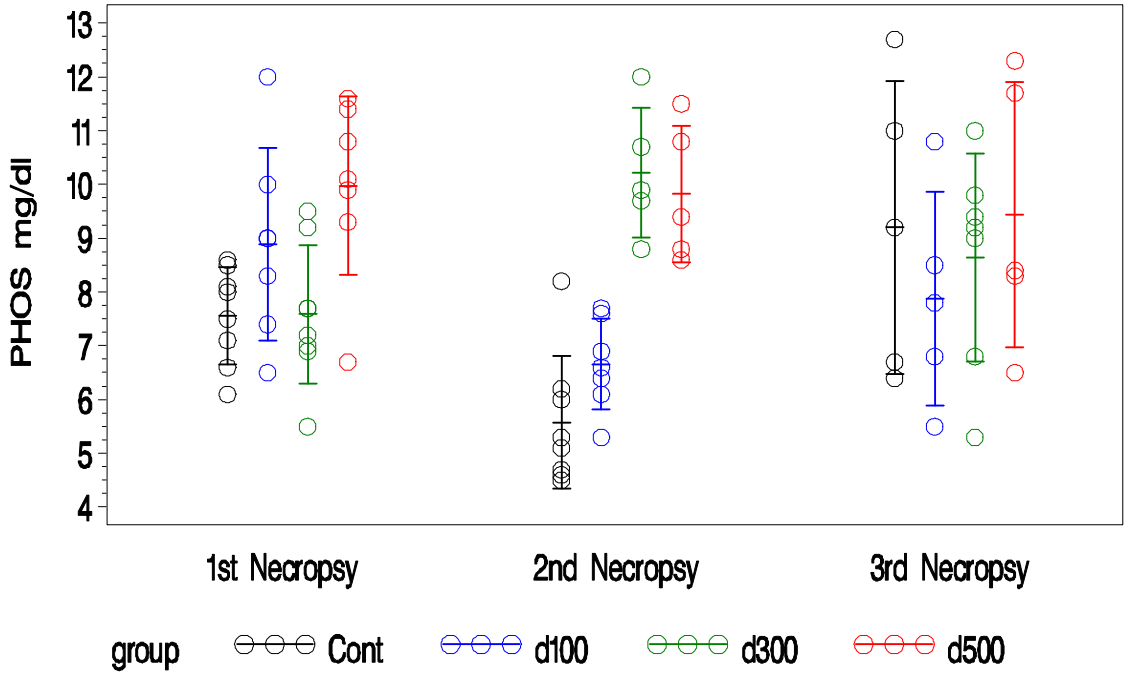
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	5.664	3	0.1292	NA+ mmol/L	Dose	0.111	0.5590
2nd Necropsy	2.183	3	0.5354	NA+ mmol/L	Dose	-0.008	0.9708
3rd Necropsy	2.427	3	0.4887	NA+ mmol/L	Dose	-0.135	0.5583



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='PHOS mg/dl'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

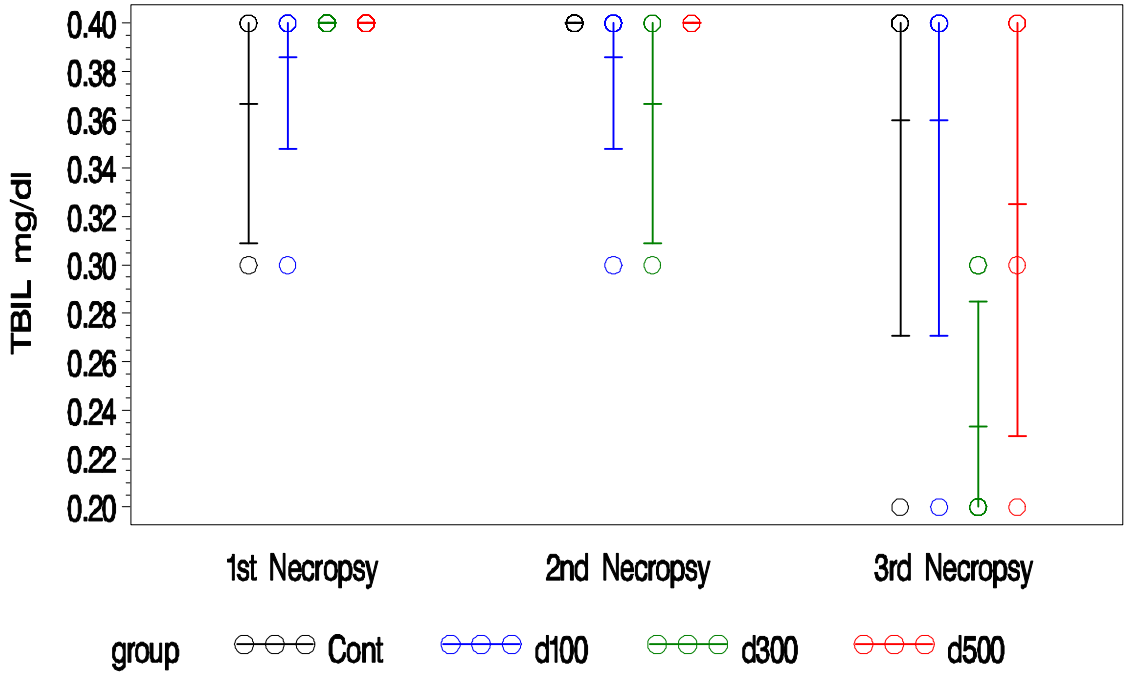
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	9.100	3	0.0280	PHOS mg/dl	Dose	0.372	0.0428
2nd Necropsy	18.903	3	**0.0003**	PHOS mg/dl	Dose	0.841	**<.0001**
3rd Necropsy	1.113	3	0.7738	PHOS mg/dl	Dose	0.079	0.7270



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='TBIL mg/dl'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

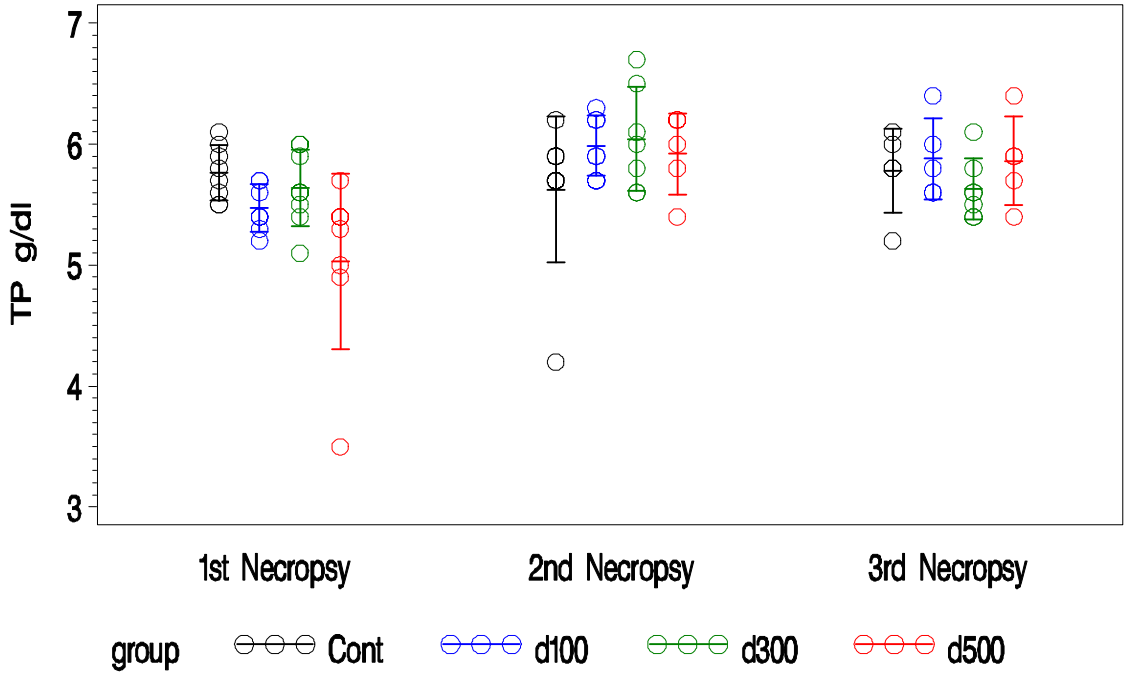
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	3.642	3	0.3028	TBIL mg/dl	Dose	0.363	0.0889
2nd Necropsy	2.915	3	0.4049	TBIL mg/dl	Dose	-0.245	0.2979
3rd Necropsy	7.362	3	0.0612	TBIL mg/dl	Dose	-0.351	0.1289



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='TP g/dl'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	10.889	3	0.0123	TP g/dl	Dose	-0.482	**0.0070**
2nd Necropsy	2.657	3	0.4476	TP g/dl	Dose	0.230	0.2489
3rd Necropsy	2.587	3	0.4597	TP g/dl	Dose	-0.078	0.7291



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='QC'
analysis of "normal" vs "abnormal"

Table 1 of flag by group					
Controlling for procedure=1st Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
normal	8	8	8	8	32
Total	8	8	8	8	32

Table 2 of flag by group					
Controlling for procedure=2nd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
normal	8	7	7	8	30
Total	8	7	7	8	30

Table 3 of flag by group					
Controlling for procedure=3rd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
normal	8	6	8	7	29
Total	8	6	8	7	29

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='HEM'
analysis of "normal" vs "abnormal"

Table 1 of flag by group					
Controlling for procedure=1st Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
normal	8	8	8	8	32
Total	8	8	8	8	32

Table 2 of flag by group					
Controlling for procedure=2nd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
normal	8	7	7	8	30
Total	8	7	7	8	30

Table 3 of flag by group					
Controlling for procedure=3rd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
normal	8	6	8	7	29
Total	8	6	8	7	29

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='ICT'
analysis of "normal" vs "abnormal"

Table 1 of flag by group					
Controlling for procedure=1st Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
normal	8	8	8	8	32
Total	8	8	8	8	32

Table 2 of flag by group					
Controlling for procedure=2nd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
normal	8	7	7	8	30
Total	8	7	7	8	30

Table 3 of flag by group					
Controlling for procedure=3rd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
normal	8	6	8	7	29
Total	8	6	8	7	29

APPENDIX #11.3.3, Vetscan Summary of Blood Chemistry: All 3 Necropsies
Analysis='Vetscan'; Measurement='LIP'
analysis of "normal" vs "abnormal"

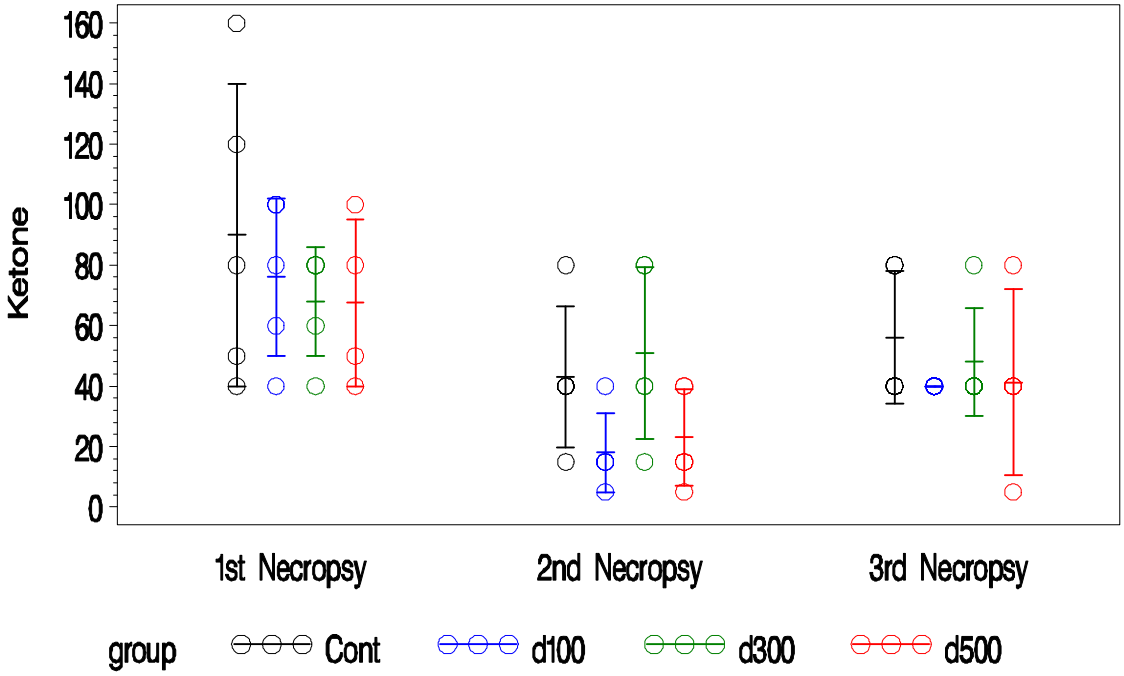
Table 1 of flag by group					
Controlling for procedure=1st Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
normal	8	8	8	8	32
Total	8	8	8	8	32

Table 2 of flag by group					
Controlling for procedure=2nd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
normal	8	7	7	8	30
Total	8	7	7	8	30

Table 3 of flag by group					
Controlling for procedure=3rd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
normal	8	6	8	7	29
Total	8	6	8	7	29

APPENDIX #11.3.4, Summary of Urinalysis Results: All 3 Necropsies
Analysis='Urinalysis'; Measurement='Ketone'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

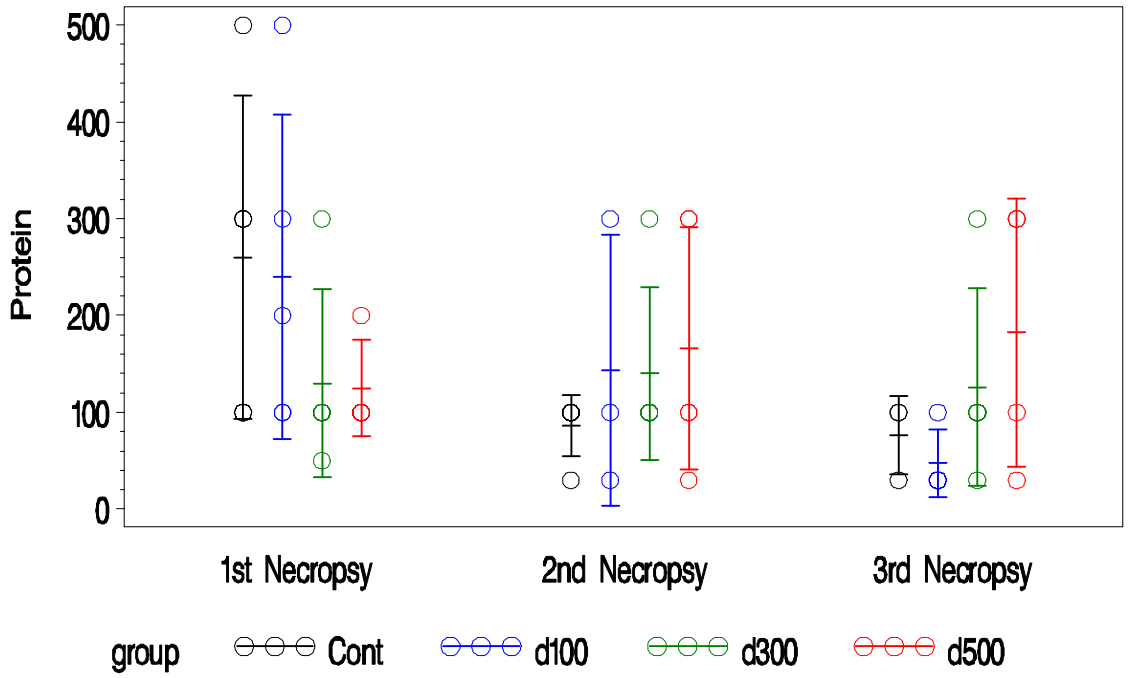
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	0.728	3	0.8667	Ketone	Dose	-0.190	0.4350
2nd Necropsy	6.641	3	0.0843	Ketone	Dose	-0.152	0.5223
3rd Necropsy	1.846	3	0.6049	Ketone	Dose	-0.214	0.3935



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.4, Summary of Urinalysis Results: All 3 Necropsies
Analysis='Urinalysis'; Measurement='Protein'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

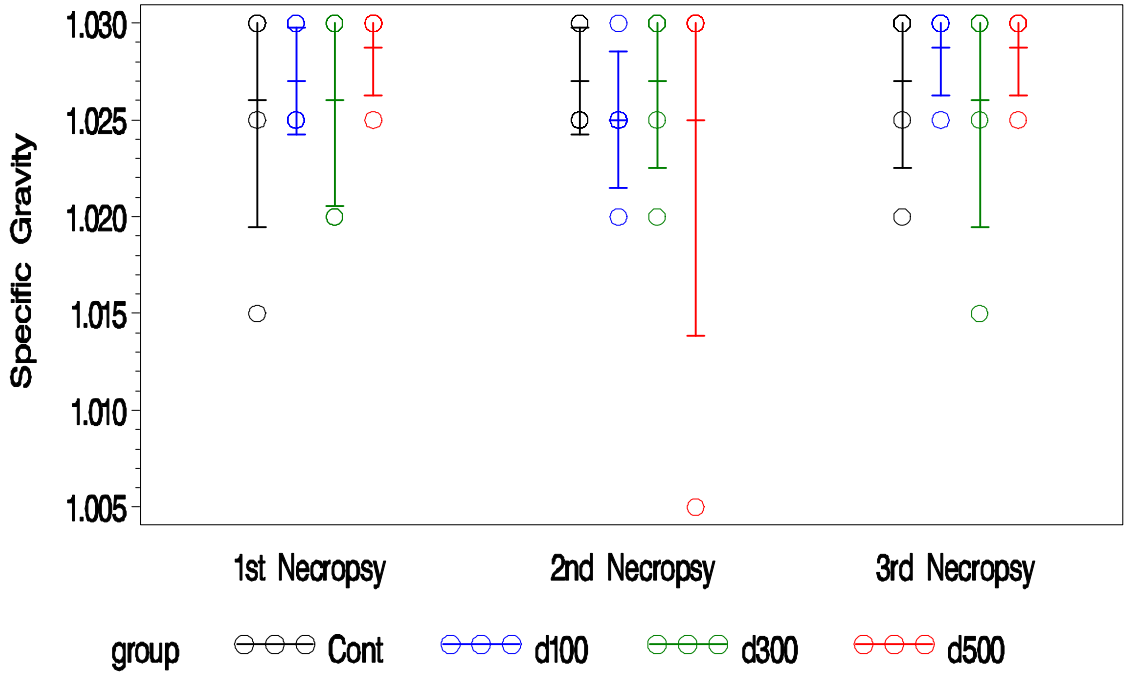
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	3.907	3	0.2717	Protein	Dose	-0.411	0.0806
2nd Necropsy	1.362	3	0.7146	Protein	Dose	0.264	0.2905
3rd Necropsy	3.931	3	0.2690	Protein	Dose	0.392	0.1334



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.4, Summary of Urinalysis Results: All 3 Necropsies
Analysis='Urinalysis'; Measurement='Specific Gravity'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

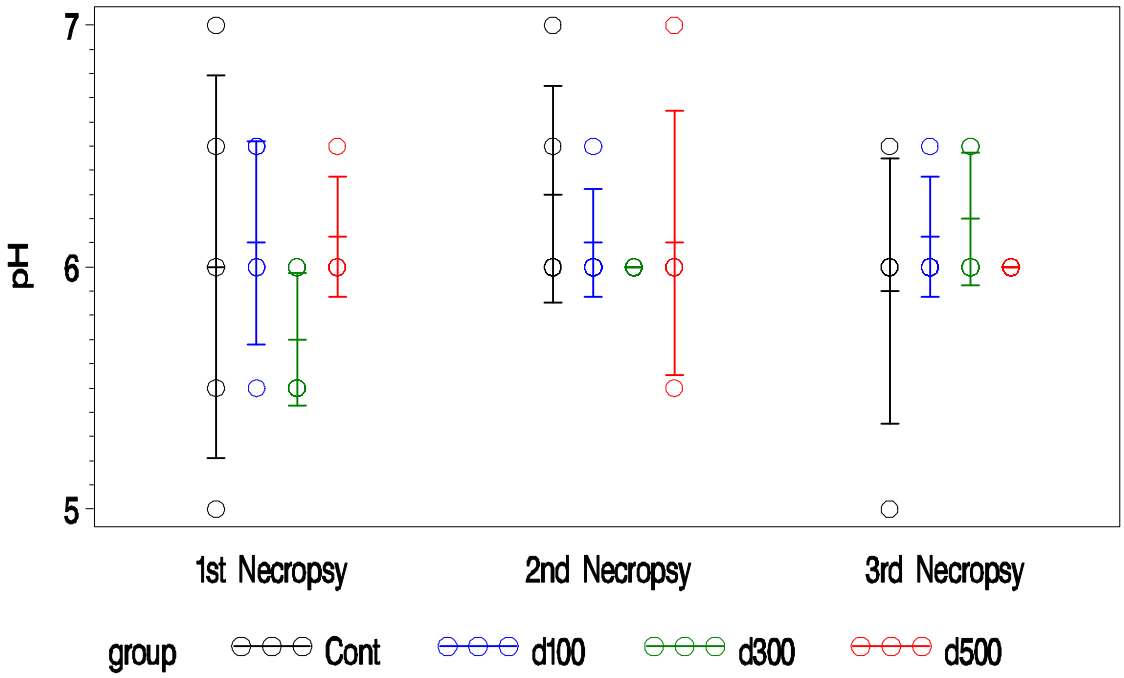
procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	0.849	3	0.8377	Specific Gravity	Dose	0.135	0.5828
2nd Necropsy	2.092	3	0.5535	Specific Gravity	Dose	0.212	0.3686
3rd Necropsy	0.733	3	0.8655	Specific Gravity	Dose	0.081	0.7507



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.4, Summary of Urinalysis Results: All 3 Necropsies
Analysis='Urinalysis'; Measurement='pH'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

procedure	Kruskal-Wallis Chi-Square	K-W DF	K-W Pvalue	Correl. OF	Correl. WITH	Spearman Correl.	Spearman Pvalue
1st Necropsy	3.046	3	0.3846	pH	Dose	-0.027	0.9135
2nd Necropsy	2.123	3	0.5473	pH	Dose	-0.296	0.2051
3rd Necropsy	2.046	3	0.5628	pH	Dose	0.043	0.8660



Procedure	1st Necropsy	2nd Necropsy	3rd Necropsy
Date	06/09/2008	07/21/2008	10/13/2008

APPENDIX #11.3.4, Summary of Urinalysis Results: All 3 Necropsies
Analysis='Urinalysis'; Measurement='Bilirubin'
analysis of "normal" vs "abnormal"

Table 1 of flag by group					
Controlling for procedure=1st Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Abnorm	5	5	5	4	19
Normal	0	0	0	0	0
Total	5	5	5	4	19

Row or column sum zero. No statistics computed for this table.

Table 2 of flag by group					
Controlling for procedure=2nd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Abnorm	3	3	5	3	14
Normal	2	2	0	2	6
Total	5	5	5	5	20

Fisher's Exact Test	
Table Probability (P)	0.0258
Pr <= P	0.4840

Table 3 of flag by group					
Controlling for procedure=3rd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Abnorm	5	4	3	4	16
Normal	0	0	2	0	2
Total	5	4	5	4	18

Fisher's Exact Test	
Table Probability (P)	0.0654
Pr <= P	0.2092

APPENDIX #11.3.4, Summary of Urinalysis Results: All 3 Necropsies
Analysis='Urinalysis'; Measurement='Blood'
analysis of "normal" vs "abnormal"

Table 1 of flag by group					
Controlling for procedure=1st Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Abnorm	1	0	0	2	3
Normal	4	5	5	2	16
Total	5	5	5	4	19

Fisher's Exact Test	
Table Probability (P)	0.0310
Pr <= P	0.1280

Table 2 of flag by group					
Controlling for procedure=2nd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Abnorm	0	0	0	1	1
Normal	5	5	5	4	19
Total	5	5	5	5	20

Fisher's Exact Test	
Table Probability (P)	0.2500
Pr <= P	1.0000

Table 3 of flag by group					
Controlling for procedure=3rd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Abnorm	0	0	0	0	0
Normal	5	4	5	4	18
Total	5	4	5	4	18

Row or column sum zero. No statistics computed for this table.

APPENDIX #11.3.4, Summary of Urinalysis Results: All 3 Necropsies
Analysis='Urinalysis'; Measurement='Glucose'
analysis of "normal" vs "abnormal"

Table 1 of flag by group					
Controlling for procedure=1st Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Normal	5	5	5	4	19
Total	5	5	5	4	19

Table 2 of flag by group					
Controlling for procedure=2nd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Normal	5	5	5	5	20
Total	5	5	5	5	20

Table 3 of flag by group					
Controlling for procedure=3rd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Normal	5	4	5	4	18
Total	5	4	5	4	18

APPENDIX #11.3.4, Summary of Urinalysis Results: All 3 Necropsies
Analysis='Urinalysis'; Measurement='Leukocytes'
analysis of "normal" vs "abnormal"

Table 1 of flag by group					
Controlling for procedure=1st Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Abnorm	1	3	0	0	4
Normal	4	2	5	4	15
Total	5	5	5	4	19

Fisher's Exact Test	
Table Probability (P)	0.0129
Pr <= P	0.1280

Table 2 of flag by group					
Controlling for procedure=2nd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Abnorm	0	1	1	1	3
Normal	5	4	4	4	17
Total	5	5	5	5	20

Fisher's Exact Test	
Table Probability (P)	0.1096
Pr <= P	1.0000

Table 3 of flag by group					
Controlling for procedure=3rd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Abnorm	0	0	4	0	4
Normal	5	4	1	4	14
Total	5	4	5	4	18

Fisher's Exact Test	
Table Probability (P)	0.0016
Pr <= P	**0.0039**

APPENDIX #11.3.4, Summary of Urinalysis Results: All 3 Necropsies
Analysis='Urinalysis'; Measurement='Nitrite'
analysis of "normal" vs "abnormal"

Table 1 of flag by group					
Controlling for procedure=1st Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Abnorm	0	0	0	0	0
Normal	5	5	5	4	19
Total	5	5	5	4	19

Row or column sum zero. No statistics computed for this table.

Table 2 of flag by group					
Controlling for procedure=2nd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Abnorm	0	0	0	1	1
Normal	5	5	5	4	19
Total	5	5	5	5	20

Fisher's Exact Test	
Table Probability (P)	0.2500
Pr <= P	1.0000

Table 3 of flag by group					
Controlling for procedure=3rd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Abnorm	2	1	0	0	3
Normal	3	3	5	4	15
Total	5	4	5	4	18

Fisher's Exact Test	
Table Probability (P)	0.0490
Pr <= P	0.4363

APPENDIX #11.3.4, Summary of Urinalysis Results: All 3 Necropsies
Analysis='Urinalysis'; Measurement='Urobilinogen'
analysis of "normal" vs "abnormal"

Table 1 of flag by group					
Controlling for procedure=1st Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Normal	5	5	5	4	19
Total	5	5	5	4	19

Table 2 of flag by group					
Controlling for procedure=2nd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Normal	5	5	5	5	20
Total	5	5	5	5	20

Table 3 of flag by group					
Controlling for procedure=3rd Necropsy					
flag(measure)	group				
Frequency	Cont	d100	d300	d500	Total
Normal	5	4	5	4	18
Total	5	4	5	4	18

Appendix 11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=acute fibrin thrombosis, artery

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	5	3	8
Con-	7	1	8
Total	12	4	16

Fisher's Exact Test	
Two-sided Pr <= P	0.5692

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	4	3	7
Con-	8	0	8
Total	12	3	15

Fisher's Exact Test	
Two-sided Pr <= P	0.0769

Appendix 11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=acute fibrin thrombosis, artery

Table 1 of dosegroup by score			
Controlling for necropsy=necropsy1			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by score			
Controlling for necropsy=necropsy2			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	5	3	8
Con-	7	1	8
Total	12	4	16

Table 3 of dosegroup by score			
Controlling for necropsy=necropsy3			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	4	3	7
Con-	8	0	8
Total	12	3	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	4.6944	0.0303

Appendix11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=acute hemorrhage

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	5	3	8
Con-	5	3	8
Total	10	6	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	4	4	8
Con-	6	2	8
Total	10	6	16

Fisher's Exact Test	
Two-sided Pr <= P	0.6084

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Appendix 11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=acute hemorrhage

Table 1 of dosegroup by score				
Controlling for necropsy=necropsy1				
dosegroup	score(value of Score)			
Frequency	0	1	2	Total
500-	5	3	0	8
Con-	5	2	1	8
Total	10	5	1	16

Table 2 of dosegroup by score				
Controlling for necropsy=necropsy2				
dosegroup	score(value of Score)			
Frequency	0	1	2	Total
500-	4	2	2	8
Con-	6	2	0	8
Total	10	4	2	16

Table 3 of dosegroup by score				
Controlling for necropsy=necropsy3				
dosegroup	score(value of Score)			
Frequency	0	1	2	Total
500-	7	0	0	7
Con-	8	0	0	8
Total	15	0	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.6054	0.4365

Appendix 11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=acute hemorrhage, peritumoral

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	0	7
Con-	7	1	8
Total	14	1	15

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=acute hemorrhage, peritumoral

Table 1 of dosegroup by score			
Controlling for necropsy=necropsy1			
dosegroup	score(value of Score)		
Frequency	0	2	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by score			
Controlling for necropsy=necropsy2			
dosegroup	score(value of Score)		
Frequency	0	2	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 3 of dosegroup by score			
Controlling for necropsy=necropsy3			
dosegroup	score(value of Score)		
Frequency	0	2	Total
500-	7	0	7
Con-	7	1	8
Total	14	1	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.8750	0.3496

Appendix 11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=alveolar histiocytosis

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	6	2	8
Con-	8	0	8
Total	14	2	16

Fisher's Exact Test	
Two-sided Pr <= P	0.4667

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	0	7
Con-	6	2	8
Total	13	2	15

Fisher's Exact Test	
Two-sided Pr <= P	0.4667

Appendix 11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=alveolar histiocytosis

Table 1 of dosegroup by score			
Controlling for necropsy=necropsy1			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by score			
Controlling for necropsy=necropsy2			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	6	2	8
Con-	8	0	8
Total	14	2	16

Table 3 of dosegroup by score			
Controlling for necropsy=necropsy3			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	0	7
Con-	6	2	8
Total	13	2	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.0048	0.9449

Appendix 11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=alveolar septal edema

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	1	8
Con-	8	0	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	3	5	8
Con-	8	0	8
Total	11	5	16

Fisher's Exact Test	
Two-sided Pr <= P	0.0256

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Appendix 11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=alveolar septal edema

Table 1 of dosegroup by score			
Controlling for necropsy=necropsy1			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	1	8
Con-	8	0	8
Total	15	1	16

Table 2 of dosegroup by score			
Controlling for necropsy=necropsy2			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	3	5	8
Con-	8	0	8
Total	11	5	16

Table 3 of dosegroup by score			
Controlling for necropsy=necropsy3			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	7.7143	0.0055

Appendix 11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=hyperplasia, bronchial epithelium

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	1	8
Con-	8	0	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Appendix 11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=hyperplasia, bronchial epithelium

Table 1 of dosegroup by score			
Controlling for necropsy=necropsy1			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	1	8
Con-	8	0	8
Total	15	1	16

Table 2 of dosegroup by score			
Controlling for necropsy=necropsy2			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 3 of dosegroup by score			
Controlling for necropsy=necropsy3			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.0000	0.3173

Appendix 11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=lymphoid hyperplasia, peribronchiolar

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	1	8
Con-	8	0	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	6	1	7
Con-	7	1	8
Total	13	2	15

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.1 Lung: Analysis of Lung Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=lymphoid hyperplasia, peribronchiolar

Table 1 of dosegroup by score			
Controlling for necropsy=necropsy1			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by score			
Controlling for necropsy=necropsy2			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	1	8
Con-	8	0	8
Total	15	1	16

Table 3 of dosegroup by score			
Controlling for necropsy=necropsy3			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	6	1	7
Con-	7	1	8
Total	13	2	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.4509	0.5019

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=EMH

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	0	8	8
Con-	0	8	8
Total	0	16	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	4	4	8
Con-	4	4	8
Total	8	8	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	3	4	7
Con-	0	8	8
Total	3	12	15

Fisher's Exact Test	
Two-sided Pr <= P	0.0769

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=EMH

Table 1 of dosegroup by score					
Controlling for necropsy=necropsy1					
dosegroup	score(value of Score)				
Frequency	0	1	2	3	Total
500-	0	4	3	1	8
Con-	0	4	4	0	8
Total	0	8	7	1	16

Table 2 of dosegroup by score					
Controlling for necropsy=necropsy2					
dosegroup	score(value of Score)				
Frequency	0	1	2	3	Total
500-	4	4	0	0	8
Con-	4	4	0	0	8
Total	8	8	0	0	16

Table 3 of dosegroup by score					
Controlling for necropsy=necropsy3					
dosegroup	score(value of Score)				
Frequency	0	1	2	3	Total
500-	3	4	0	0	7
Con-	0	6	2	0	8
Total	3	10	2	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.0426	0.3072

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=acute hepatocellular necrosis

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	6	2	8
Con-	6	2	8
Total	12	4	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	5	3	8
Con-	4	4	8
Total	9	7	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	4	3	7
Con-	4	4	8
Total	8	7	15

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=acute hepatocellular necrosis

Table 1 of dosegroup by score				
Controlling for necropsy=necropsy1				
dosegroup	score(value of Score)			
Frequency	0	1	2	Total
500-	6	2	0	8
Con-	6	1	1	8
Total	12	3	1	16

Table 2 of dosegroup by score				
Controlling for necropsy=necropsy2				
dosegroup	score(value of Score)			
Frequency	0	1	2	Total
500-	5	1	2	8
Con-	4	3	1	8
Total	9	4	3	16

Table 3 of dosegroup by score				
Controlling for necropsy=necropsy3				
dosegroup	score(value of Score)			
Frequency	0	1	2	Total
500-	4	2	1	7
Con-	4	3	1	8
Total	8	5	2	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.0806	0.7764

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=chronic inflammation, lobular

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	4	4	8
Con-	3	5	8
Total	7	9	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	3	5	8
Con-	1	7	8
Total	4	12	16

Fisher's Exact Test	
Two-sided Pr <= P	0.5692

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	3	4	7
Con-	0	8	8
Total	3	12	15

Fisher's Exact Test	
Two-sided Pr <= P	0.0769

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=chronic inflammation, lobular

Table 1 of dosegroup by score				
Controlling for necropsy=necropsy1				
dosegroup	score(value of Score)			
Frequency	0	1	2	Total
500-	4	3	1	8
Con-	3	4	1	8
Total	7	7	2	16

Table 2 of dosegroup by score				
Controlling for necropsy=necropsy2				
dosegroup	score(value of Score)			
Frequency	0	1	2	Total
500-	3	5	0	8
Con-	1	7	0	8
Total	4	12	0	16

Table 3 of dosegroup by score				
Controlling for necropsy=necropsy3				
dosegroup	score(value of Score)			
Frequency	0	1	2	Total
500-	3	4	0	7
Con-	0	8	0	8
Total	3	12	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	2.8072	0.0938

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=chronic inflammation, periportal

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	6	2	8
Con-	6	2	8
Total	12	4	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	0	7
Con-	7	1	8
Total	14	1	15

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=chronic inflammation, periportal

Table 1 of dosegroup by score			
Controlling for necropsy=necropsy1			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by score			
Controlling for necropsy=necropsy2			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	6	2	8
Con-	6	2	8
Total	12	4	16

Table 3 of dosegroup by score			
Controlling for necropsy=necropsy3			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	0	7
Con-	7	1	8
Total	14	1	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.2076	0.6486

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=chronic inflammation, portal

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	1	8
Con-	8	0	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	7	1	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=chronic inflammation, portal

Table 1 of dosegroup by score			
Controlling for necropsy=necropsy1			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	1	8
Con-	8	0	8
Total	15	1	16

Table 2 of dosegroup by score			
Controlling for necropsy=necropsy2			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	7	1	8
Total	15	1	16

Table 3 of dosegroup by score			
Controlling for necropsy=necropsy3			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.0000	1.0000

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=chronic-active inflammation, periportal

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	1	8
Con-	8	0	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=chronic-active inflammation, periportal

Table 1 of dosegroup by score			
Controlling for necropsy=necropsy1			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by score			
Controlling for necropsy=necropsy2			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	1	8
Con-	8	0	8
Total	15	1	16

Table 3 of dosegroup by score			
Controlling for necropsy=necropsy3			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.0000	0.3173

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=chronic-active inflammation, portal

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	4	4	8
Con-	7	1	8
Total	11	5	16

Fisher's Exact Test	
Two-sided Pr <= P	0.2821

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=chronic-active inflammation, portal

Table 1 of dosegroup by score			
Controlling for necropsy=necropsy1			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	4	4	8
Con-	7	1	8
Total	11	5	16

Table 2 of dosegroup by score			
Controlling for necropsy=necropsy2			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 3 of dosegroup by score			
Controlling for necropsy=necropsy3			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	2.4545	0.1172

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=lymphoplasmacytic inflammation, periportal

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	0	7
Con-	7	1	8
Total	14	1	15

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=lymphoplasmacytic inflammation, periportal

Table 1 of dosegroup by score			
Controlling for necropsy=necropsy1			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by score			
Controlling for necropsy=necropsy2			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 3 of dosegroup by score			
Controlling for necropsy=necropsy3			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	0	7
Con-	7	1	8
Total	14	1	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.8750	0.3496

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=suppurative inflammation, lobular

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	7	1	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=suppurative inflammation, lobular

Table 1 of dosegroup by score			
Controlling for necropsy=necropsy1			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by score			
Controlling for necropsy=necropsy2			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	7	1	8
Total	15	1	16

Table 3 of dosegroup by score			
Controlling for necropsy=necropsy3			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.0000	0.3173

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Description=suppurative inflammation, periportal

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	8	0	8
Con-	7	1	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Appendix 11.5.2 Analysis of Liver Lesion Scores versus Dose group
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by score
Controlling for necropsy

Description=suppurative inflammation, periportal

Table 1 of dosegroup by score			
Controlling for necropsy=necropsy1			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	8	0	8
Total	16	0	16

Table 2 of dosegroup by score			
Controlling for necropsy=necropsy2			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	8	0	8
Con-	7	1	8
Total	15	1	16

Table 3 of dosegroup by score			
Controlling for necropsy=necropsy3			
dosegroup	score(value of Score)		
Frequency	0	1	Total
500-	7	0	7
Con-	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.0000	0.3173

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=adrenal

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	7	1	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	1	7
Con	8	0	8
Total	14	1	15
Frequency Missing = 1			

Fisher's Exact Test	
Two-sided Pr <= P	0.4667

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	5	1	6
Con	6	1	7
Total	11	2	13
Frequency Missing = 2			

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=adrenal

Table 1 of dosegroup by _score				
Controlling for necropsy=necropsy1				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	8	0	0	8
Con	7	1	0	8
Total	15	1	0	16

Table 2 of dosegroup by _score				
Controlling for necropsy=necropsy2				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	6	1	0	7
Con	8	0	0	8
Total	14	1	0	15
Frequency Missing = 1				

Table 3 of dosegroup by _score				
Controlling for necropsy=necropsy3				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	5	0	1	6
Con	6	1	0	7
Total	11	1	1	13
Frequency Missing = 2				

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.2537	0.6145

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=bone marrow

Table 1 of dosegroup by any_pos		
Controlling for necropsy=necropsy1		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by any_pos		
Controlling for necropsy=necropsy2		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	7	7
Con	8	8
Total	15	15
Frequency Missing = 1		

Table 3 of dosegroup by any_pos		
Controlling for necropsy=necropsy3		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	7	7
Con	8	8
Total	15	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=bone marrow

Table 1 of dosegroup by _score		
Controlling for necropsy=necropsy1		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by _score		
Controlling for necropsy=necropsy2		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	7	7
Con	8	8
Total	15	15
Frequency Missing = 1		

Table 3 of dosegroup by _score		
Controlling for necropsy=necropsy3		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	7	7
Con	8	8
Total	15	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=brain

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	1	8
Con	7	1	8
Total	14	2	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	1	8
Con	8	0	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=brain

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	2	Total
500	7	1	8
Con	7	1	8
Total	14	2	16

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	2	Total
500	7	1	8
Con	8	0	8
Total	15	1	16

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	2	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.3488	0.5548

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=cecum

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	1	8
Con	5	3	8
Total	12	4	16

Fisher's Exact Test	
Two-sided Pr <= P	0.5692

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	2	8
Con	3	5	8
Total	9	7	16

Fisher's Exact Test	
Two-sided Pr <= P	0.3147

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	1	7
Con	7	1	8
Total	13	2	15

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=cecum

Table 1 of dosegroup by _score				
Controlling for necropsy=necropsy1				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	7	1	0	8
Con	5	3	0	8
Total	12	4	0	16

Table 2 of dosegroup by _score				
Controlling for necropsy=necropsy2				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	6	2	0	8
Con	3	4	1	8
Total	9	6	1	16

Table 3 of dosegroup by _score				
Controlling for necropsy=necropsy3				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	6	1	0	7
Con	7	1	0	8
Total	13	2	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	3.0062	0.0829

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=cervix

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	8	0	8
Total	15	0	15
Frequency Missing = 1			

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	6	0	6
Total	14	0	14
Frequency Missing = 2			

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	0	6
Con	3	1	4
Total	9	1	10
Frequency Missing = 5			

Fisher's Exact Test	
Two-sided Pr <= P	0.4000

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=cervix

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	0	7
Con	8	0	8
Total	15	0	15
Frequency Missing = 1			

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	6	0	6
Total	14	0	14
Frequency Missing = 2			

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	6	0	6
Con	3	1	4
Total	9	1	10
Frequency Missing = 5			

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.5000	0.2207

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=colon

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	7	1	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	7	1	8
Total	14	1	15

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=colon

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	7	1	8
Total	15	1	16

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	0	7
Con	7	1	8
Total	14	1	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.8731	0.1711

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=duodenum

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	2	8
Con	8	0	8
Total	14	2	16

Fisher's Exact Test	
Two-sided Pr <= P	0.4667

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	2	8
Con	6	2	8
Total	12	4	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	4	3	7
Con	4	4	8
Total	8	7	15

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=duodenum

Table 1 of dosegroup by _score				
Controlling for necropsy=necropsy1				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	6	1	1	8
Con	8	0	0	8
Total	14	1	1	16

Table 2 of dosegroup by _score				
Controlling for necropsy=necropsy2				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	6	1	1	8
Con	6	2	0	8
Total	12	3	1	16

Table 3 of dosegroup by _score				
Controlling for necropsy=necropsy3				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	4	3	0	7
Con	4	3	1	8
Total	8	6	1	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.3855	0.5347

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=esophagus

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	5	2	7
Con	5	2	7
Total	10	4	14
Frequency Missing = 1			

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=esophagus

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	5	2	7
Con	5	2	7
Total	10	4	14
Frequency Missing = 1			

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.0000	1.0000

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity
in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=eye

Table 1 of dosegroup by any_pos		
Controlling for necropsy=necropsy1		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	7	7
Con	8	8
Total	15	15
Frequency Missing = 1		

Table 2 of dosegroup by any_pos		
Controlling for necropsy=necropsy2		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	8	8
Total	16	16

Table 3 of dosegroup by any_pos		
Controlling for necropsy=necropsy3		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	7	7
Con	8	8
Total	15	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=eye

Table 1 of dosegroup by _score		
Controlling for necropsy=necropsy1		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	7	7
Con	8	8
Total	15	15
Frequency Missing = 1		

Table 2 of dosegroup by _score		
Controlling for necropsy=necropsy2		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	8	8
Total	16	16

Table 3 of dosegroup by _score		
Controlling for necropsy=necropsy3		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	7	7
Con	8	8
Total	15	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=femur

Table 1 of dosegroup by any_pos		
Controlling for necropsy=necropsy1		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by any_pos		
Controlling for necropsy=necropsy2		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	7	7
Con	8	8
Total	15	15
Frequency Missing = 1		

Table 3 of dosegroup by any_pos		
Controlling for necropsy=necropsy3		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	7	7
Con	8	8
Total	15	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=femur

Table 1 of dosegroup by _score		
Controlling for necropsy=necropsy1		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by _score		
Controlling for necropsy=necropsy2		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	7	7
Con	8	8
Total	15	15
Frequency Missing = 1		

Table 3 of dosegroup by _score		
Controlling for necropsy=necropsy3		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	7	7
Con	8	8
Total	15	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=heart

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	6	2	8
Total	14	2	16

Fisher's Exact Test	
Two-sided Pr <= P	0.4667

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=heart

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	6	2	8
Total	14	2	16

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	2.1429	0.1432

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=ileum

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	1	8
Con	8	0	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	1	8
Con	8	0	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	1	7
Con	7	0	7
Total	13	1	14
Frequency Missing = 1			

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=ileum

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	1	8
Con	8	0	8
Total	15	1	16

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	1	8
Con	8	0	8
Total	15	1	16

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	6	1	7
Con	7	0	7
Total	13	1	14
Frequency Missing = 1			

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	3.0000	0.0833

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=jejunum

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	1	8
Con	8	0	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	2	8
Con	4	4	8
Total	10	6	16

Fisher's Exact Test	
Two-sided Pr <= P	0.6084

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	7	1	8
Total	14	1	15

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=jejunum

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	1	8
Con	8	0	8
Total	15	1	16

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	6	2	8
Con	4	4	8
Total	10	6	16

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	0	7
Con	7	1	8
Total	14	1	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.6234	0.4298

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=kidney

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	1	8
Con	7	1	8
Total	14	2	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	2	6	8
Con	5	3	8
Total	7	9	16

Fisher's Exact Test	
Two-sided Pr <= P	0.3147

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	1	7
Con	8	0	8
Total	14	1	15

Fisher's Exact Test	
Two-sided Pr <= P	0.4667

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=kidney

Table 1 of dosegroup by _score					
Controlling for necropsy=necropsy1					
dosegroup	_score(value of Score)				
Frequency	0	1	2	3	Total
500	7	1	0	0	8
Con	7	1	0	0	8
Total	14	2	0	0	16

Table 2 of dosegroup by _score					
Controlling for necropsy=necropsy2					
dosegroup	_score(value of Score)				
Frequency	0	1	2	3	Total
500	2	5	0	1	8
Con	5	3	0	0	8
Total	7	8	0	1	16

Table 3 of dosegroup by _score					
Controlling for necropsy=necropsy3					
dosegroup	_score(value of Score)				
Frequency	0	1	2	3	Total
500	6	0	1	0	7
Con	8	0	0	0	8
Total	14	0	1	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	3.1972	0.0738

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=lacrimal gland

Table 1 of dosegroup by any_pos		
Controlling for necropsy=necropsy1		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	2	2
Con	6	6
Total	8	8
Frequency Missing = 8		

Table 2 of dosegroup by any_pos		
Controlling for necropsy=necropsy2		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	5	5
Con	5	5
Total	10	10
Frequency Missing = 6		

Table 3 of dosegroup by any_pos		
Controlling for necropsy=necropsy3		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	6	6
Con	7	7
Total	13	13
Frequency Missing = 2		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=lacrimal gland

Table 1 of dosegroup by _score		
Controlling for necropsy=necropsy1		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	2	2
Con	6	6
Total	8	8
Frequency Missing = 8		

Table 2 of dosegroup by _score		
Controlling for necropsy=necropsy2		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	5	5
Con	5	5
Total	10	10
Frequency Missing = 6		

Table 3 of dosegroup by _score		
Controlling for necropsy=necropsy3		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	6	6
Con	7	7
Total	13	13
Frequency Missing = 2		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=mammary gland

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	5	2	7
Con	8	0	8
Total	13	2	15
Frequency Missing = 1			

Fisher's Exact Test	
Two-sided Pr <= P	0.2000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	7	0	7
Total	14	0	14
Frequency Missing = 2			

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=mammary gland

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	5	2	7
Con	8	0	8
Total	13	2	15
Frequency Missing = 1			

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	0	7
Con	7	0	7
Total	14	0	14
Frequency Missing = 2			

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	2.4615	0.1167

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=mandibular lymph node

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	1	8
Con	6	2	8
Total	13	3	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	5	1	6
Con	8	0	8
Total	13	1	14
Frequency Missing = 1			

Fisher's Exact Test	
Two-sided Pr <= P	0.4286

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=mandibular lymph node

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	1	8
Con	6	2	8
Total	13	3	16

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	5	1	6
Con	8	0	8
Total	13	1	14
Frequency Missing = 1			

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.0057	0.9398

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=mesenteric lymph node

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	0	6
Con	5	2	7
Total	11	2	13
Frequency Missing = 3			

Fisher's Exact Test	
Two-sided Pr <= P	0.4615

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=mesenteric lymph node

Table 1 of dosegroup by _score				
Controlling for necropsy=necropsy1				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	6	0	0	6
Con	5	1	1	7
Total	11	1	1	13
Frequency Missing = 3				

Table 2 of dosegroup by _score				
Controlling for necropsy=necropsy2				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	8	0	0	8
Con	8	0	0	8
Total	16	0	0	16

Table 3 of dosegroup by _score				
Controlling for necropsy=necropsy3				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	7	0	0	7
Con	8	0	0	8
Total	15	0	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.6531	0.1985

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=optic nerve

Table 1 of dosegroup by any_pos		
Controlling for necropsy=necropsy1		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	7	7
Con	3	3
Total	10	10
Frequency Missing = 6		

Table 2 of dosegroup by any_pos		
Controlling for necropsy=necropsy2		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	1	1
Con	4	4
Total	5	5
Frequency Missing = 11		

Table 3 of dosegroup by any_pos		
Controlling for necropsy=necropsy3		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	3	3
Con	1	1
Total	4	4
Frequency Missing = 11		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=optic nerve

Table 1 of dosegroup by _score		
Controlling for necropsy=necropsy1		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	7	7
Con	3	3
Total	10	10
Frequency Missing = 6		

Table 2 of dosegroup by _score		
Controlling for necropsy=necropsy2		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	1	1
Con	4	4
Total	5	5
Frequency Missing = 11		

Table 3 of dosegroup by _score		
Controlling for necropsy=necropsy3		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	3	3
Con	1	1
Total	4	4
Frequency Missing = 11		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=ovary

Table 1 of dosegroup by any_pos		
Controlling for necropsy=necropsy1		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by any_pos		
Controlling for necropsy=necropsy2		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	6	6
Total	14	14
Frequency Missing = 2		

Table 3 of dosegroup by any_pos		
Controlling for necropsy=necropsy3		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	5	5
Con	8	8
Total	13	13
Frequency Missing = 2		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=ovary

Table 1 of dosegroup by _score		
Controlling for necropsy=necropsy1		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by _score		
Controlling for necropsy=necropsy2		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	6	6
Total	14	14
Frequency Missing = 2		

Table 3 of dosegroup by _score		
Controlling for necropsy=necropsy3		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	5	5
Con	8	8
Total	13	13
Frequency Missing = 2		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=oviduct

Table 1 of dosegroup by any_pos		
Controlling for necropsy=necropsy1		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by any_pos		
Controlling for necropsy=necropsy2		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	5	5
Total	13	13
Frequency Missing = 3		

Table 3 of dosegroup by any_pos		
Controlling for necropsy=necropsy3		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	6	6
Con	7	7
Total	13	13
Frequency Missing = 2		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=oviduct

Table 1 of dosegroup by _score		
Controlling for necropsy=necropsy1		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by _score		
Controlling for necropsy=necropsy2		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	5	5
Total	13	13
Frequency Missing = 3		

Table 3 of dosegroup by _score		
Controlling for necropsy=necropsy3		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	6	6
Con	7	7
Total	13	13
Frequency Missing = 2		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=pancreas

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	7	1	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=pancreas

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	7	1	8
Total	15	1	16

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.0000	0.3173

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=parathyroid

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	2	0	2
Con	2	0	2
Total	4	0	4
Frequency Missing = 12			

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	2	0	2
Con	7	0	7
Total	9	0	9
Frequency Missing = 7			

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	1	1	2
Con	3	0	3
Total	4	1	5
Frequency Missing = 10			

Fisher's Exact Test	
Two-sided Pr <= P	0.4000

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=parathyroid

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	2	0	2
Con	2	0	2
Total	4	0	4
Frequency Missing = 12			

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	2	0	2
Con	7	0	7
Total	9	0	9
Frequency Missing = 7			

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	1	1	2
Con	3	0	3
Total	4	1	5
Frequency Missing = 10			

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.5000	0.2207

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=pituitary

Table 1 of dosegroup by any_pos		
Controlling for necropsy=necropsy1		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	6	6
Con	8	8
Total	14	14
Frequency Missing = 2		

Table 2 of dosegroup by any_pos		
Controlling for necropsy=necropsy2		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	5	5
Con	8	8
Total	13	13
Frequency Missing = 3		

Table 3 of dosegroup by any_pos		
Controlling for necropsy=necropsy3		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	6	6
Con	6	6
Total	12	12
Frequency Missing = 3		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=pituitary

Table 1 of dosegroup by _score		
Controlling for necropsy=necropsy1		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	6	6
Con	8	8
Total	14	14
Frequency Missing = 2		

Table 2 of dosegroup by _score		
Controlling for necropsy=necropsy2		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	5	5
Con	8	8
Total	13	13
Frequency Missing = 3		

Table 3 of dosegroup by _score		
Controlling for necropsy=necropsy3		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	6	6
Con	6	6
Total	12	12
Frequency Missing = 3		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=rectum

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	5	2	7
Con	7	1	8
Total	12	3	15
Frequency Missing = 1			

Fisher's Exact Test	
Two-sided Pr <= P	0.5692

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	1	7
Con	5	3	8
Total	11	4	15

Fisher's Exact Test	
Two-sided Pr <= P	0.5692

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=rectum

Table 1 of dosegroup by _score				
Controlling for necropsy=necropsy1				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	5	1	1	7
Con	7	1	0	8
Total	12	2	1	15
Frequency Missing = 1				

Table 2 of dosegroup by _score				
Controlling for necropsy=necropsy2				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	8	0	0	8
Con	8	0	0	8
Total	16	0	0	16

Table 3 of dosegroup by _score				
Controlling for necropsy=necropsy3				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	6	1	0	7
Con	5	3	0	8
Total	11	4	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.0339	0.8539

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=salivary

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	2	8
Con	6	2	8
Total	12	4	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	5	3	8
Con	3	5	8
Total	8	8	16

Fisher's Exact Test	
Two-sided Pr <= P	0.6193

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	2	5	7
Con	3	5	8
Total	5	10	15

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=salivary

Table 1 of dosegroup by _score				
Controlling for necropsy=necropsy1				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	6	2	0	8
Con	6	1	1	8
Total	12	3	1	16

Table 2 of dosegroup by _score				
Controlling for necropsy=necropsy2				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	5	3	0	8
Con	3	5	0	8
Total	8	8	0	16

Table 3 of dosegroup by _score				
Controlling for necropsy=necropsy3				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	2	5	0	7
Con	3	5	0	8
Total	5	10	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.3997	0.5273

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=sciatic nerve

Table 1 of dosegroup by any_pos		
Controlling for necropsy=necropsy1		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	7	7
Con	7	7
Total	14	14
Frequency Missing = 2		

Table 2 of dosegroup by any_pos		
Controlling for necropsy=necropsy2		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	7	7
Total	15	15
Frequency Missing = 1		

Table 3 of dosegroup by any_pos		
Controlling for necropsy=necropsy3		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	4	4
Con	7	7
Total	11	11
Frequency Missing = 4		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=sciatic nerve

Table 1 of dosegroup by _score		
Controlling for necropsy=necropsy1		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	7	7
Con	7	7
Total	14	14
Frequency Missing = 2		

Table 2 of dosegroup by _score		
Controlling for necropsy=necropsy2		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	7	7
Total	15	15
Frequency Missing = 1		

Table 3 of dosegroup by _score		
Controlling for necropsy=necropsy3		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	4	4
Con	7	7
Total	11	11
Frequency Missing = 4		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=skeletal muscle

Table 1 of dosegroup by any_pos		
Controlling for necropsy=necropsy1		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by any_pos		
Controlling for necropsy=necropsy2		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	8	8
Total	16	16

Table 3 of dosegroup by any_pos		
Controlling for necropsy=necropsy3		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	7	7
Con	8	8
Total	15	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=skeletal muscle

Table 1 of dosegroup by _score		
Controlling for necropsy=necropsy1		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by _score		
Controlling for necropsy=necropsy2		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	8	8
Total	16	16

Table 3 of dosegroup by _score		
Controlling for necropsy=necropsy3		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	7	7
Con	8	8
Total	15	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=skin dorsal

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	5	3	8
Con	8	0	8
Total	13	3	16

Fisher's Exact Test	
Two-sided Pr <= P	0.2000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	1	7
Con	6	2	8
Total	12	3	15

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=skin dorsal

Table 1 of dosegroup by _score				
Controlling for necropsy=necropsy1				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	5	1	2	8
Con	8	0	0	8
Total	13	1	2	16

Table 2 of dosegroup by _score				
Controlling for necropsy=necropsy2				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	8	0	0	8
Con	8	0	0	8
Total	16	0	0	16

Table 3 of dosegroup by _score				
Controlling for necropsy=necropsy3				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	6	1	0	7
Con	6	2	0	8
Total	12	3	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.6811	0.1948

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=skin ventral

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	5	3	8
Con	4	4	8
Total	9	7	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	2	8
Con	7	1	8
Total	13	3	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	4	3	7
Con	8	0	8
Total	12	3	15

Fisher's Exact Test	
Two-sided Pr <= P	0.0769

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=skin ventral

Table 1 of dosegroup by _score				
Controlling for necropsy=necropsy1				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	5	2	1	8
Con	4	2	2	8
Total	9	4	3	16

Table 2 of dosegroup by _score				
Controlling for necropsy=necropsy2				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	6	1	1	8
Con	7	1	0	8
Total	13	2	1	16

Table 3 of dosegroup by _score				
Controlling for necropsy=necropsy3				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	4	2	1	7
Con	8	0	0	8
Total	12	2	1	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.8671	0.3518

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=spinal cord

Table 1 of dosegroup by any_pos		
Controlling for necropsy=necropsy1		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by any_pos		
Controlling for necropsy=necropsy2		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	8	8
Total	16	16

Table 3 of dosegroup by any_pos		
Controlling for necropsy=necropsy3		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	7	7
Con	8	8
Total	15	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=spinal cord

Table 1 of dosegroup by _score		
Controlling for necropsy=necropsy1		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by _score		
Controlling for necropsy=necropsy2		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	8	8
Total	16	16

Table 3 of dosegroup by _score		
Controlling for necropsy=necropsy3		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	7	7
Con	8	8
Total	15	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=spleen

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	7	1	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=spleen

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	2	Total
500	8	0	8
Con	7	1	8
Total	15	1	16

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	2	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	2	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.0000	0.3173

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=stomach

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	5	2	7
Con	7	1	8
Total	12	3	15
Frequency Missing = 1			

Fisher's Exact Test	
Two-sided Pr <= P	0.5692

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	2	8
Con	8	0	8
Total	14	2	16

Fisher's Exact Test	
Two-sided Pr <= P	0.4667

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	1	7
Con	7	1	8
Total	13	2	15

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=stomach

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	5	2	7
Con	7	1	8
Total	12	3	15
Frequency Missing = 1			

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	6	2	8
Con	8	0	8
Total	14	2	16

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	6	1	7
Con	7	1	8
Total	13	2	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.7705	0.1833

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=thymus

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	8	0	8
Total	15	0	15
Frequency Missing = 1			

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	6	1	7
Total	14	1	15
Frequency Missing = 1			

Fisher's Exact Test	
Two-sided Pr <= P	0.4667

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	7	0	7
Total	14	0	14
Frequency Missing = 1			

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=thymus

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	0	7
Con	8	0	8
Total	15	0	15
Frequency Missing = 1			

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	6	1	7
Total	14	1	15
Frequency Missing = 1			

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	0	7
Con	7	0	7
Total	14	0	14
Frequency Missing = 1			

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.1429	0.2850

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=thyroid

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	4	1	5
Con	8	0	8
Total	12	1	13
Frequency Missing = 3			

Fisher's Exact Test	
Two-sided Pr <= P	0.3846

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=thyroid

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	4	1	5
Con	8	0	8
Total	12	1	13
Frequency Missing = 3			

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.6000	0.2059

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=tongue

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	8	0	8
Con	7	1	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=tongue

Table 1 of dosegroup by _score			
Controlling for necropsy=necropsy1			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	8	0	8
Total	16	0	16

Table 2 of dosegroup by _score			
Controlling for necropsy=necropsy2			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	8	0	8
Con	7	1	8
Total	15	1	16

Table 3 of dosegroup by _score			
Controlling for necropsy=necropsy3			
dosegroup	_score(value of Score)		
Frequency	0	1	Total
500	7	0	7
Con	8	0	8
Total	15	0	15

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.0000	0.3173

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=trachea

Table 1 of dosegroup by any_pos		
Controlling for necropsy=necropsy1		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by any_pos		
Controlling for necropsy=necropsy2		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	8	8
Con	8	8
Total	16	16

Table 3 of dosegroup by any_pos		
Controlling for necropsy=necropsy3		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	6	6
Con	8	8
Total	14	14
Frequency Missing = 1		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=trachea

Table 1 of dosegroup by _score		
Controlling for necropsy=necropsy1		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	8	8
Total	16	16

Table 2 of dosegroup by _score		
Controlling for necropsy=necropsy2		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	8	8
Con	8	8
Total	16	16

Table 3 of dosegroup by _score		
Controlling for necropsy=necropsy3		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	6	6
Con	8	8
Total	14	14
Frequency Missing = 1		

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=urinary bladder

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	7	1	8
Con	8	0	8
Total	15	1	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	2	8
Con	6	1	7
Total	12	3	15
Frequency Missing = 1			

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	4	3	7
Con	6	0	6
Total	10	3	13
Frequency Missing = 2			

Fisher's Exact Test	
Two-sided Pr <= P	0.1923

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=urinary bladder

Table 1 of dosegroup by _score				
Controlling for necropsy=necropsy1				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	7	1	0	8
Con	8	0	0	8
Total	15	1	0	16

Table 2 of dosegroup by _score				
Controlling for necropsy=necropsy2				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	6	1	1	8
Con	6	1	0	7
Total	12	2	1	15
Frequency Missing = 1				

Table 3 of dosegroup by _score				
Controlling for necropsy=necropsy3				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	4	3	0	7
Con	6	0	0	6
Total	10	3	0	13
Frequency Missing = 2				

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	3.4614	0.0628

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=uterus

Table 1 of dosegroup by any_pos			
Controlling for necropsy=necropsy1			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	5	3	8
Con	5	3	8
Total	10	6	16

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 2 of dosegroup by any_pos			
Controlling for necropsy=necropsy2			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	6	2	8
Con	5	2	7
Total	11	4	15
Frequency Missing = 1			

Fisher's Exact Test	
Two-sided Pr <= P	1.0000

Table 3 of dosegroup by any_pos			
Controlling for necropsy=necropsy3			
dosegroup	any_pos(Any Positive Score)		
Frequency	neg	POS	Total
500	5	1	6
Con	8	0	8
Total	13	1	14
Frequency Missing = 1			

Fisher's Exact Test	
Two-sided Pr <= P	0.4286

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

Summary Statistics for dosegroup by _score
Controlling for necropsy

organ/site=uterus

Table 1 of dosegroup by _score				
Controlling for necropsy=necropsy1				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	5	1	2	8
Con	5	0	3	8
Total	10	1	5	16

Table 2 of dosegroup by _score				
Controlling for necropsy=necropsy2				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	6	1	1	8
Con	5	1	1	7
Total	11	2	2	15
Frequency Missing = 1				

Table 3 of dosegroup by _score				
Controlling for necropsy=necropsy3				
dosegroup	_score(value of Score)			
Frequency	0	1	2	Total
500	5	1	0	6
Con	8	0	0	8
Total	13	1	0	14
Frequency Missing = 1				

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)				
Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.0028	0.9576

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=vagina

Table 1 of dosegroup by any_pos		
Controlling for necropsy=necropsy1		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	7	7
Con	7	7
Total	14	14
Frequency Missing = 2		

Table 2 of dosegroup by any_pos		
Controlling for necropsy=necropsy2		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	7	7
Con	7	7
Total	14	14
Frequency Missing = 2		

Table 3 of dosegroup by any_pos		
Controlling for necropsy=necropsy3		
dosegroup	any_pos(Any Positive Score)	
Frequency	neg	Total
500	7	7
Con	8	8
Total	15	15

Appendix 11.5.3 Other Organs: Evaluation of Lesion Number and Severity in Organs and Sites Other than Liver or Lung
Fisher exact test on individual necropsies
Stratified CMH correlation test for overall effect

organ/site=vagina

Table 1 of dosegroup by _score		
Controlling for necropsy=necropsy1		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	7	7
Con	7	7
Total	14	14
Frequency Missing = 2		

Table 2 of dosegroup by _score		
Controlling for necropsy=necropsy2		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	7	7
Con	7	7
Total	14	14
Frequency Missing = 2		

Table 3 of dosegroup by _score		
Controlling for necropsy=necropsy3		
dosegroup	_score(value of Score)	
Frequency	0	Total
500	7	7
Con	8	8
Total	15	15

Appendix 11.6.1 Weekly Mouse Weights, 03-25-09 (data QA-approved 03-19-09): Mouse Weights and Weight Changes Over the GLP Study Period Descriptive Statistics by Study Day and Dose Group

Day=1 datevalue=05/27/2008 Event=Weighing01

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	24	mousewt	18.434	0.768	18.430	16.140	19.824
		wtchange	.	.	.	.	.
dose100	24	mousewt	18.882	1.592	18.603	16.792	25.156
		wtchange	.	.	.	.	.
dose300	24	mousewt	18.249	0.482	18.330	17.252	19.128
		wtchange	.	.	.	.	.
dose500	24	mousewt	18.285	0.754	18.161	16.163	19.846
		wtchange	.	.	.	.	.

Day=9 datevalue=06/04/2008 Event=Weighing02

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	24	mousewt	19.270	0.659	19.245	18.160	20.365
		wtchange	0.836	0.479	0.719	0.036	2.020
dose100	24	mousewt	19.003	0.667	19.001	17.734	20.405
		wtchange	0.121	1.436	0.364	-6.051	1.480
dose300	24	mousewt	18.961	0.666	19.038	17.131	20.166
		wtchange	0.712	0.504	0.632	-0.438	1.666
dose500	24	mousewt	18.869	0.918	19.151	16.473	19.967
		wtchange	0.584	0.783	0.789	-1.816	1.472

Day=14 datevalue=06/09/2008 Event=Necropsy#1

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	19.310	0.445	19.308	18.697	20.062
		wtchange	0.037	0.773	0.085	-0.961	1.620
dose100	8	mousewt	19.153	0.891	19.216	17.412	20.409
		wtchange	0.298	1.051	0.687	-2.068	1.127
dose300	8	mousewt	19.068	0.596	18.978	18.407	20.314
		wtchange	0.293	0.611	0.260	-0.584	1.468
dose500	8	mousewt	18.591	1.002	18.667	17.094	19.628
		wtchange	-0.092	0.384	-0.135	-0.576	0.489

Appendix 11.6.1 Weekly Mouse Weights, 03-25-09 (data QA-approved 03-19-09): Mouse Weights and Weight Changes Over the GLP Study Period Descriptive Statistics by Study Day and Dose Group

Day=16 datevalue=06/11/2008 Event=Weighing03

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	16	mousewt	19.434	0.935	19.292	17.509	21.104
		wtchange	0.165	0.614	0.168	-1.246	1.302
dose100	16	mousewt	19.691	0.709	19.766	18.365	21.109
		wtchange	0.614	0.726	0.697	-0.644	2.273
dose300	16	mousewt	19.884	0.508	19.811	18.732	20.827
		wtchange	0.830	0.703	0.742	-0.552	2.515
dose500	16	mousewt	19.572	0.731	19.552	18.153	20.684
		wtchange	0.610	0.699	0.483	-0.043	2.959

Day=23 datevalue=06/18/2008 Event=Weighing04

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	16	mousewt	19.615	0.793	19.674	18.010	21.049
		wtchange	0.181	0.532	0.292	-1.078	0.928
dose100	16	mousewt	19.841	0.669	19.801	19.071	21.721
		wtchange	0.150	0.612	0.158	-1.379	1.130
dose300	16	mousewt	20.054	0.612	19.915	18.782	20.995
		wtchange	0.170	0.536	0.084	-0.816	1.267
dose500	16	mousewt	20.005	0.784	20.044	18.327	21.191
		wtchange	0.433	0.539	0.502	-0.567	1.294

Day=29 datevalue=06/24/2008 Event=Weighing05

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	16	mousewt	19.787	0.734	19.655	18.774	21.363
		wtchange	0.173	0.517	0.250	-1.277	1.077
dose100	16	mousewt	20.019	0.598	19.980	18.964	21.210
		wtchange	0.178	0.464	0.225	-0.550	1.006
dose300	16	mousewt	20.210	0.721	20.365	18.547	21.161
		wtchange	0.156	0.612	0.080	-0.832	1.502
dose500	16	mousewt	20.032	0.708	20.173	18.544	21.109
		wtchange	0.027	0.544	0.003	-0.821	1.181

Appendix 11.6.1 Weekly Mouse Weights, 03-25-09 (data QA-approved 03-19-09): Mouse Weights and Weight Changes Over the GLP Study Period Descriptive Statistics by Study Day and Dose Group

Day=37 datevalue=07/02/2008 Event=Weighing06

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	16	mousewt	20.294	0.923	20.224	17.844	22.097
		wtchange	0.506	0.565	0.608	-0.930	1.572
dose100	16	mousewt	20.340	0.709	20.293	19.065	21.497
		wtchange	0.321	0.445	0.252	-0.596	1.160
dose300	16	mousewt	20.488	0.532	20.560	19.306	21.149
		wtchange	0.278	0.377	0.384	-0.513	0.868
dose500	16	mousewt	20.234	0.816	20.365	19.153	21.623
		wtchange	0.202	0.423	0.091	-0.503	1.003

Day=44 datevalue=07/09/2008 Event=Weighing07

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	16	mousewt	20.696	0.756	20.365	19.870	22.469
		wtchange	0.402	0.626	0.316	-0.679	2.050
dose100	15	mousewt	20.762	0.971	20.665	19.346	22.344
		wtchange	0.462	0.746	0.238	-0.582	1.621
dose300	16	mousewt	20.714	0.694	20.823	19.359	21.829
		wtchange	0.226	0.569	0.079	-0.835	1.300
dose500	16	mousewt	20.779	0.712	20.527	19.681	22.062
		wtchange	0.545	0.564	0.664	-1.082	1.197

Day=51 datevalue=07/16/2008 Event=Weighing08

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	16	mousewt	20.892	0.830	20.761	19.413	22.449
		wtchange	0.196	0.617	0.057	-0.693	1.425
dose100	15	mousewt	20.817	0.759	20.885	19.714	22.026
		wtchange	0.055	0.625	-0.017	-1.152	1.414
dose300	16	mousewt	20.847	0.686	20.890	19.449	21.902
		wtchange	0.133	0.836	0.106	-1.438	1.736
dose500	16	mousewt	20.807	0.742	20.878	19.494	21.822
		wtchange	0.028	0.573	-0.050	-0.766	1.015

Appendix 11.6.1 Weekly Mouse Weights, 03-25-09 (data QA-approved 03-19-09): Mouse Weights and Weight Changes Over the GLP Study Period Descriptive Statistics by Study Day and Dose Group

Day=56 datevalue=07/21/2008 Event=Necropsy#2

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	20.645	1.046	20.247	19.103	22.256
		wtchange	-0.538	1.007	-0.309	-2.104	0.810
dose100	7	mousewt	20.431	0.826	20.173	19.719	22.090
		wtchange	-0.472	0.841	-0.712	-1.532	0.686
dose300	8	mousewt	20.095	1.404	19.949	18.125	22.111
		wtchange	-0.787	0.890	-0.938	-1.932	0.552
dose500	8	mousewt	20.223	1.165	20.165	18.660	21.719
		wtchange	-0.955	1.375	-1.293	-2.674	0.835

Day=58 datevalue=07/23/2008 Event=Weighing09

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	20.921	1.165	20.627	19.284	22.601
		wtchange	0.320	0.546	0.430	-0.601	1.058
dose100	8	mousewt	21.341	1.032	21.245	19.394	22.750
		wtchange	0.598	0.499	0.706	-0.544	1.091
dose300	8	mousewt	21.240	0.342	21.351	20.630	21.580
		wtchange	0.429	0.793	0.611	-0.946	1.232
dose500	8	mousewt	20.559	0.498	20.635	19.851	21.130
		wtchange	0.122	0.487	0.191	-0.638	0.637

Day=65 datevalue=07/30/2008 Event=Weighing10

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	20.899	0.849	20.749	19.695	22.278
		wtchange	-0.022	0.434	0.042	-0.931	0.411
dose100	8	mousewt	21.501	0.914	21.456	20.045	22.715
		wtchange	0.161	0.401	0.052	-0.335	0.753
dose300	8	mousewt	21.180	0.619	21.205	20.165	21.842
		wtchange	-0.060	0.590	0.054	-1.340	0.474
dose500	8	mousewt	20.576	0.647	20.573	19.669	21.610
		wtchange	0.016	0.326	0.012	-0.422	0.511

Appendix 11.6.1 Weekly Mouse Weights, 03-25-09 (data QA-approved 03-19-09): Mouse Weights and Weight Changes Over the GLP Study Period Descriptive Statistics by Study Day and Dose Group

Day=75 datevalue=08/09/2008 Event=Weighing11

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	20.905	1.032	21.009	19.566	22.397
		wtchange	0.006	0.425	-0.138	-0.377	0.897
dose100	8	mousewt	21.390	1.500	21.101	19.610	23.602
		wtchange	-0.112	0.724	-0.356	-0.900	1.051
dose300	8	mousewt	21.377	0.469	21.462	20.437	21.998
		wtchange	0.198	0.438	0.232	-0.411	0.995
dose500	8	mousewt	20.961	0.765	21.152	19.843	21.820
		wtchange	0.385	0.483	0.391	-0.333	1.205

Day=79 datevalue=08/13/2008 Event=Weighing12

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	20.964	0.662	21.031	20.060	21.729
		wtchange	0.059	0.433	0.096	-0.668	0.637
dose100	8	mousewt	21.166	0.801	21.249	20.035	22.069
		wtchange	-0.224	0.882	0.135	-1.833	0.544
dose300	8	mousewt	21.296	0.385	21.254	20.668	21.891
		wtchange	-0.082	0.434	-0.047	-0.712	0.519
dose500	8	mousewt	21.256	1.243	20.833	20.015	23.162
		wtchange	0.295	0.889	0.299	-1.121	1.342

Day=85 datevalue=08/19/2008 Event=Weighing13

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	21.320	0.589	21.458	20.191	21.924
		wtchange	0.356	0.337	0.304	-0.059	0.873
dose100	8	mousewt	21.843	1.038	21.925	20.101	23.173
		wtchange	0.678	0.428	0.712	0.057	1.216
dose300	8	mousewt	21.699	0.429	21.792	20.986	22.185
		wtchange	0.403	0.199	0.319	0.223	0.787
dose500	8	mousewt	21.195	0.625	21.252	20.290	22.145
		wtchange	-0.061	0.748	0.165	-1.114	0.925

Appendix 11.6.1 Weekly Mouse Weights, 03-25-09 (data QA-approved 03-19-09): Mouse Weights and Weight Changes Over the GLP Study Period Descriptive Statistics by Study Day and Dose Group

Day=93 datevalue=08/27/2008 Event=Weighing14

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	21.858	1.011	21.997	19.933	22.918
		wtchange	0.537	0.479	0.541	-0.258	1.238
dose100	8	mousewt	20.938	1.379	21.011	18.883	22.834
		wtchange	-0.905	1.096	-0.778	-2.204	0.635
dose300	8	mousewt	22.208	0.629	22.353	21.127	23.114
		wtchange	0.509	0.443	0.386	-0.033	1.311
dose500	8	mousewt	21.518	0.656	21.655	20.496	22.342
		wtchange	0.323	0.511	0.201	-0.105	1.532

Day=99 datevalue=09/02/2008 Event=Weighing15

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	22.042	0.923	22.162	20.587	23.340
		wtchange	0.184	0.463	0.185	-0.691	0.691
dose100	8	mousewt	21.832	1.197	21.534	20.163	23.584
		wtchange	0.894	0.696	0.813	0.052	2.023
dose300	8	mousewt	22.482	0.820	22.496	20.931	23.334
		wtchange	0.274	0.531	0.318	-0.610	0.941
dose500	8	mousewt	21.808	1.048	21.635	20.592	23.517
		wtchange	0.291	0.761	0.205	-1.210	1.233

Day=106 datevalue=09/09/2008 Event=Weighing16

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	22.301	1.031	22.252	20.654	23.805
		wtchange	0.259	0.277	0.388	-0.215	0.515
dose100	8	mousewt	21.840	1.054	22.027	20.406	22.974
		wtchange	0.008	0.942	-0.550	-0.977	1.357
dose300	8	mousewt	22.821	0.475	22.858	22.124	23.572
		wtchange	0.339	0.642	0.439	-0.916	1.193
dose500	8	mousewt	22.286	0.779	22.478	20.881	23.178
		wtchange	0.478	0.540	0.535	-0.339	1.194

Appendix 11.6.1 Weekly Mouse Weights, 03-25-09 (data QA-approved 03-19-09): Mouse Weights and Weight Changes Over the GLP Study Period Descriptive Statistics by Study Day and Dose Group

Day=113 datevalue=09/16/2008 Event=Weighing17

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	23.108	2.762	22.050	21.435	29.589
		wtchange	0.807	3.331	-0.253	-1.019	8.935
dose100	8	mousewt	21.807	1.306	21.713	19.943	23.498
		wtchange	-0.033	0.807	-0.114	-1.013	0.965
dose300	8	mousewt	22.573	0.511	22.490	22.070	23.262
		wtchange	-0.248	0.560	-0.207	-1.382	0.551
dose500	8	mousewt	21.932	1.022	21.800	20.310	23.510
		wtchange	-0.353	0.562	-0.428	-1.030	0.629

Day=120 datevalue=09/23/2008 Event=Weighing18

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	22.356	1.051	21.990	20.951	24.510
		wtchange	-0.752	3.188	0.391	-8.638	0.542
dose100	6	mousewt	22.613	0.937	22.400	21.510	23.805
		wtchange	0.246	0.535	0.151	-0.273	1.251
dose300	8	mousewt	22.668	0.577	22.605	22.010	23.750
		wtchange	0.095	0.282	0.079	-0.280	0.510
dose500	8	mousewt	22.302	0.889	22.205	20.755	23.750
		wtchange	0.369	0.220	0.343	0.040	0.740

Day=128 datevalue=10/01/2008 Event=Weighing19

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	21.640	1.047	21.583	20.285	23.850
		wtchange	-0.716	0.244	-0.768	-0.961	-0.246
dose100	6	mousewt	22.661	0.900	22.569	21.610	23.921
		wtchange	0.048	0.823	0.063	-1.000	1.187
dose300	8	mousewt	22.244	0.545	22.460	21.129	22.750
		wtchange	-0.424	0.556	-0.353	-1.340	0.359
dose500	7	mousewt	22.216	0.540	22.103	21.471	22.979
		wtchange	-0.307	0.825	0.029	-1.800	0.551

Appendix 11.6.1 Weekly Mouse Weights, 03-25-09 (data QA-approved 03-19-09): Mouse Weights and Weight Changes Over the GLP Study Period Descriptive Statistics by Study Day and Dose Group

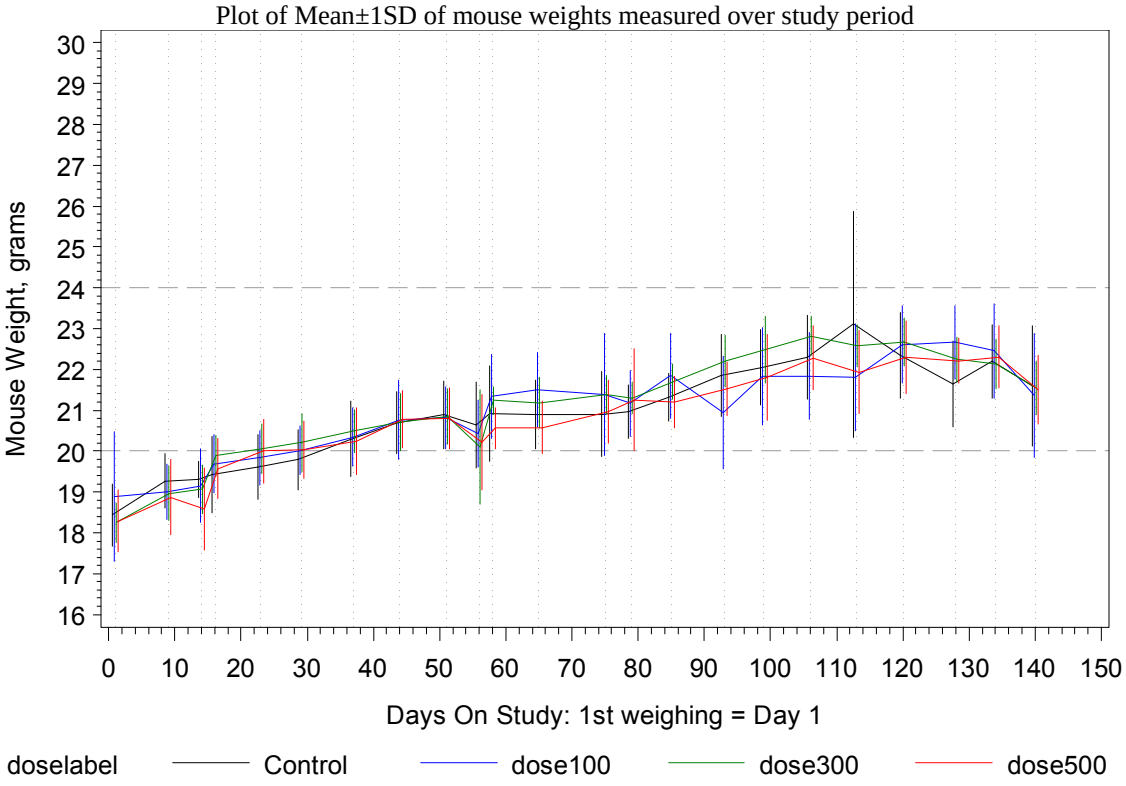
Day=134 datevalue=10/07/2008 Event=Weighing20

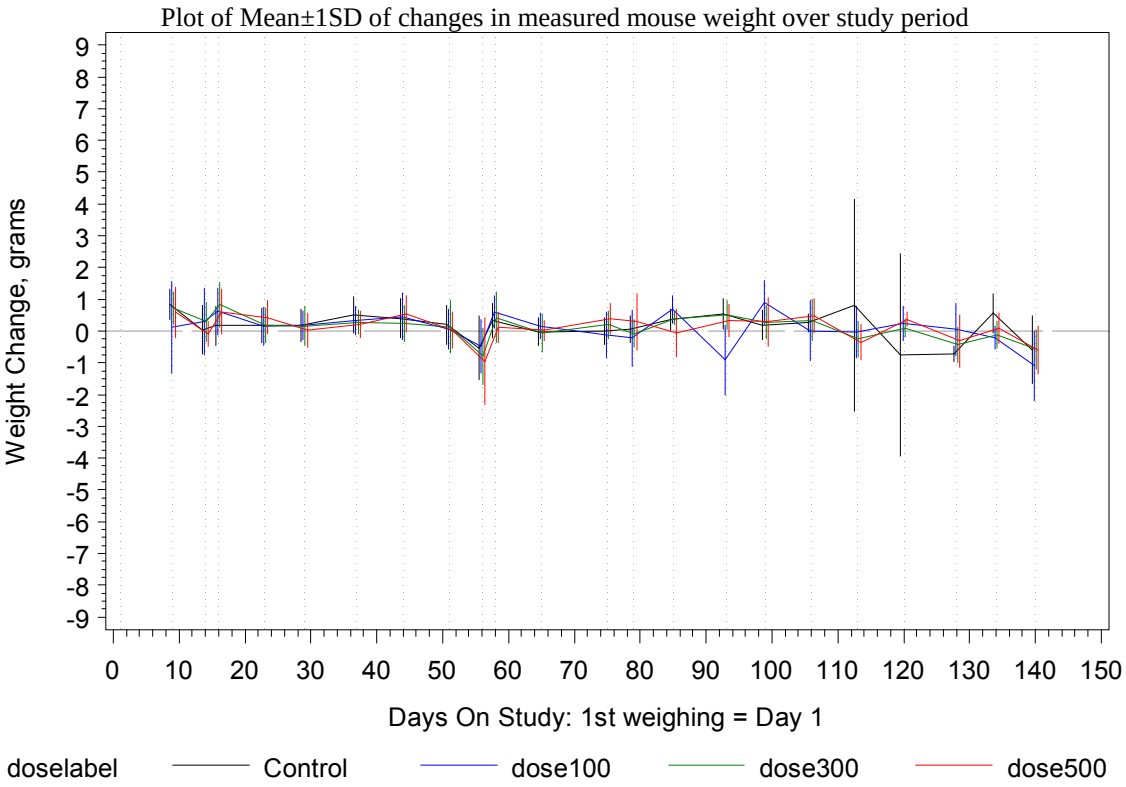
doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	22.197	0.903	22.471	20.650	23.587
		wtchange	0.557	0.622	0.743	-0.450	1.255
dose100	6	mousewt	22.453	1.156	22.250	21.106	24.414
		wtchange	-0.208	0.363	-0.319	-0.504	0.493
dose300	8	mousewt	22.133	0.614	22.239	20.990	22.836
		wtchange	-0.111	0.422	-0.044	-0.819	0.536
dose500	7	mousewt	22.307	0.767	22.666	20.781	23.033
		wtchange	0.091	0.482	-0.033	-0.690	0.762

Day=140 datevalue=10/13/2008 Event=Necropsy#3

doselabel	N Obs	Variable	Mean	Std Dev	Median	Minimum	Maximum
Control	8	mousewt	21.601	1.478	20.990	19.524	23.756
		wtchange	-0.596	1.069	-0.261	-2.049	0.697
dose100	6	mousewt	21.364	1.512	21.034	19.760	23.830
		wtchange	-1.090	1.098	-1.064	-2.805	0.460
dose300	8	mousewt	21.554	0.653	21.739	20.388	22.505
		wtchange	-0.580	0.621	-0.500	-1.496	0.282
dose500	8	mousewt	21.508	0.844	21.302	20.613	22.970
		wtchange	-0.605	0.758	-0.679	-1.484	0.362

Appendix 11.6.1 Weekly Mouse Weights, 03-25-09 (data QA-approved 03-19-09):
Mouse Weights and Weight Changes Over the GLP Study Period
Growth Curves and Weight-Change Plots





Error bars span Group Mean +/- One SD for given group on indicated study day

Appendix 11.6.1 Weekly Mouse Weights, 03-25-09 (data QA-approved 03-19-09): Mouse Weights and Weight Changes Over the GLP Study Period
Repeated Measures Analysis of Mouse Weights over Study Period

Model Information	
Data Set	WORK.UNIMOUSE
Dependent Variable	mousewt
Covariance Structure	Spatial Power
Subject Effect	mouse_id
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Between-Within

Number of Observations	
Number of Observations Read	1043
Number of Observations Used	1043
Number of Observations Not Used	0

Covariance Parameter Estimates					
Cov Parm	**Subject**	**Estimate**	**Standard Error**	**Z Value**	**Pr Z**
SP(POW)	mouse_id	0.9284	0.006123	151.62	<.0001
Residual		0.7931	0.05152	15.39	<.0001

Fit Statistics	
-2 Res Log Likelihood	2302.4
AIC (smaller is better)	2306.4
AICC (smaller is better)	2306.4
BIC (smaller is better)	2311.5

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
doselabel	3	92	1.03	0.3819
Day	22	859	38.00	<.0001
doselabel*Day	66	859	1.28	0.0722

Within-Subject Covariance Structure = 1st-order AutoRegressive, modelled using the Spatial Power model to adjust for the unequal spacing between successive measures

Appendix 11.6.1 Weekly Mouse Weights, 03-25-09 (data QA-approved 03-19-09): Mouse Weights and Weight Changes Over the GLP Study Period
Repeated Measures Analysis of Weight Changes over Study Period

Model Information	
Data Set	WORK.UNIMOUSE
Dependent Variable	wtchange
Covariance Structure	Spatial Power
Subject Effect	mouse_id
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Between-Within

Number of Observations	
Number of Observations Read	947
Number of Observations Used	947
Number of Observations Not Used	0

Covariance Parameter Estimates					
Cov Parm	**Subject**	**Estimate**	**Standard Error**	**Z Value**	**Pr Z**
SP(POW)	mouse_id	0.01953	4.5367	0.00	0.9966
Residual		0.6065	0.02927	20.72	<.0001

Fit Statistics	
-2 Res Log Likelihood	2210.5
AIC (smaller is better)	2214.5
AICC (smaller is better)	2214.6
BIC (smaller is better)	2219.7

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
doselabel	3	92	0.18	0.9106
Day	21	767	7.18	<.0001
doselabel*Day	63	767	1.41	0.0225

Within-Subject Covariance Structure = 1st-order AutoRegressive, modelled using the Spatial Power model to adjust for the unequal spacing between successive measures

Appendix 11.7.1 Organ Weights: GLP Mice at Necropsies 1,2,3 (data QA-approved 03-19-09)
Organ = 'Heart'
Descriptive statistics

Organ=Heart

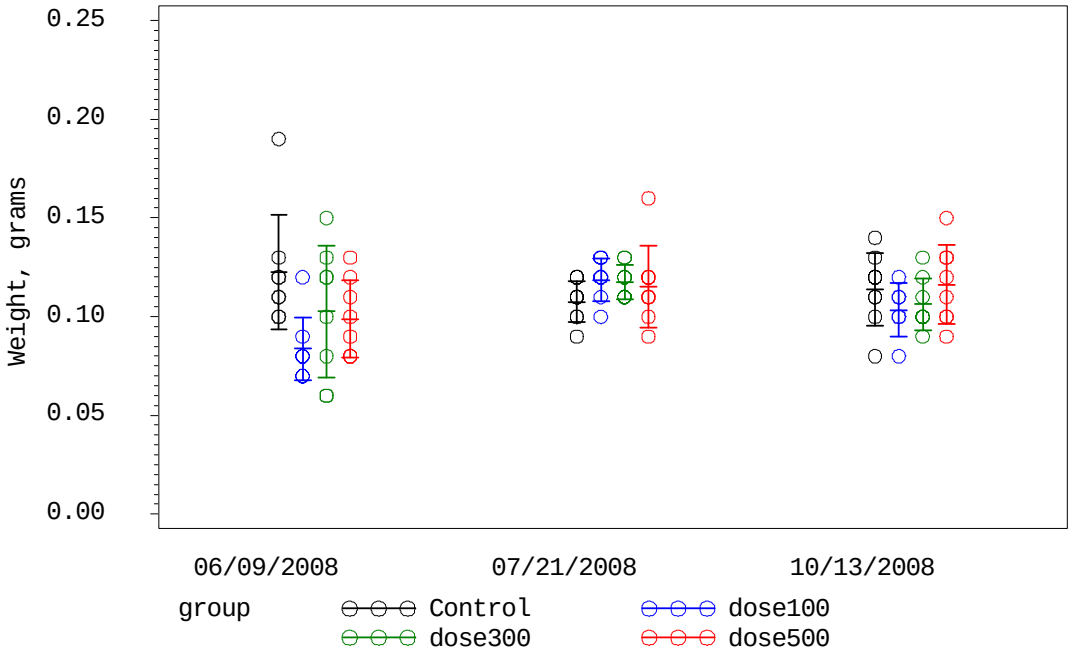
procedure	group	N Obs	Variable	Label	N	Mean	Std Dev	Median	Minimum	Maximum
1st Necropsy	Control	8	weight_g	weight(g)	8	0.1225	0.0292	0.1150	0.1000	0.1900
			wt_percent	weight(%)	8	0.6341	0.1492	0.5899	0.5168	0.9842
	dose100	8	weight_g	weight(g)	8	0.0838	0.0160	0.0800	0.0700	0.1200
			wt_percent	weight(%)	8	0.4370	0.0773	0.4225	0.3653	0.6057
	dose300	8	weight_g	weight(g)	8	0.1025	0.0333	0.1100	0.0600	0.1500
			wt_percent	weight(%)	8	0.5409	0.1840	0.5748	0.2954	0.8052
	dose500	8	weight_g	weight(g)	8	0.0988	0.0196	0.0950	0.0800	0.1300
			wt_percent	weight(%)	8	0.5287	0.0841	0.5031	0.4304	0.6631
2nd Necropsy	Control	8	weight_g	weight(g)	8	0.1075	0.0104	0.1100	0.0900	0.1200
			wt_percent	weight(%)	8	0.5226	0.0635	0.5399	0.4177	0.6282
	dose100	7	weight_g	weight(g)	7	0.1186	0.0107	0.1200	0.1000	0.1300
			wt_percent	weight(%)	7	0.5807	0.0526	0.5877	0.5067	0.6593
	dose300	8	weight_g	weight(g)	8	0.1175	0.0089	0.1150	0.1100	0.1300
			wt_percent	weight(%)	8	0.5870	0.0570	0.5835	0.5067	0.6688
	dose500	8	weight_g	weight(g)	8	0.1150	0.0207	0.1100	0.0900	0.1600
			wt_percent	weight(%)	8	0.5692	0.1010	0.5412	0.4592	0.7838
3rd Necropsy	Control	8	weight_g	weight(g)	8	0.1138	0.0185	0.1150	0.0800	0.1400
			wt_percent	weight(%)	8	0.5295	0.1013	0.5264	0.3859	0.7171
	dose100	6	weight_g	weight(g)	6	0.1033	0.0137	0.1050	0.0800	0.1200
			wt_percent	weight(%)	6	0.4873	0.0839	0.5081	0.3595	0.5920
	dose300	8	weight_g	weight(g)	8	0.1063	0.0130	0.1000	0.0900	0.1300
			wt_percent	weight(%)	8	0.4933	0.0617	0.4803	0.4147	0.5928
	dose500	8	weight_g	weight(g)	8	0.1163	0.0200	0.1150	0.0900	0.1500
			wt_percent	weight(%)	8	0.5407	0.0940	0.5199	0.4316	0.7227

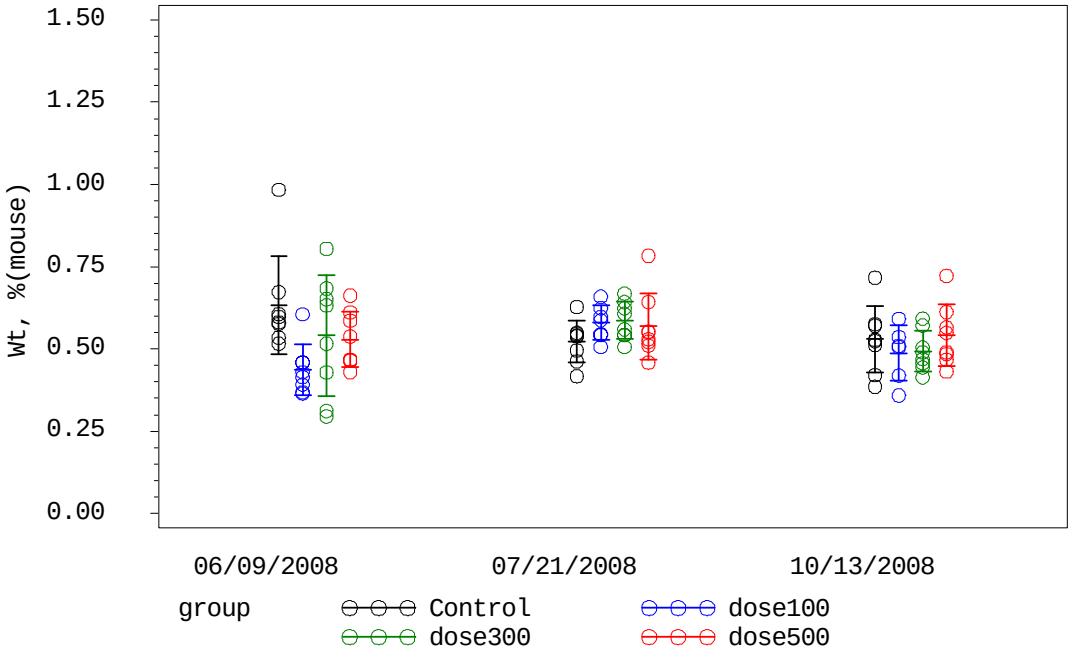
Appendix 11.7.1 Organ Weights: GLP Mice at Necropsies 1,2,3 (data QA-approved 03-19-09)
Organ = 'Heart'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

Organ	procedure	Variable	Kruskal-Wallis Chi-square	DF	Pr > Chi-Square
Heart	**1st Necropsy**	weight(g)	7.9366	3	0.0473
		weight(%)	8.7585	3	0.0327
Heart	**2nd Necropsy**	weight(g)	4.6193	3	0.2019
		weight(%)	4.1936	3	0.2413
Heart	**3rd Necropsy**	weight(g)	2.6230	3	0.4535
		weight(%)	1.5715	3	0.6659

Organ	procedure	Label	Group	Spearman corr.	P value
Heart	**1st Necropsy**	weight(g)	Dose Group	-0.15948	0.3833
		weight(%)	Dose Group	-0.07265	0.6927
Heart	**2nd Necropsy**	weight(g)	Dose Group	0.11599	0.5344
		weight(%)	Dose Group	0.16896	0.3636
Heart	**3rd Necropsy**	weight(g)	Dose Group	0.00728	0.9695
		weight(%)	Dose Group	0.00414	0.9827

Appendix 11.7.1 Organ Weights: GLP Mice at Necropsies 1,2,3 (data QA-approved 03-19-09)
Organ = 'Heart'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)





Scatterplot: circles=values; dashes(error bars)=means(SDs)

Appendix 11.7.1 Organ Weights: GLP Mice at Necropsies 1,2,3 (data QA-approved 03-19-09)
Organ = 'Kidney L/R'
Descriptive statistics

Organ=Kidney L/R

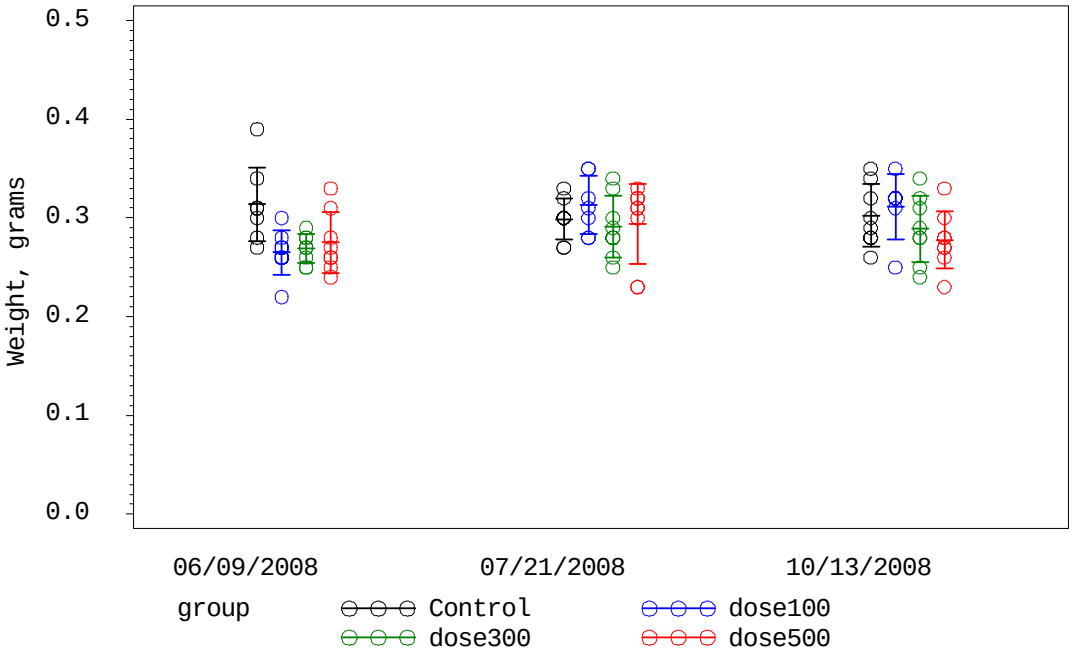
procedure	group	N Obs	Variable	Label	N	Mean	Std Dev	Median	Minimum	Maximum
1st Necropsy	Control	8	weight_g	weight(g)	8	0.3138	0.0374	0.3100	0.2700	0.3900
			wt_percent	weight(%)	8	1.6253	0.1942	1.5968	1.3954	2.0202
	dose100	8	weight_g	weight(g)	8	0.2650	0.0227	0.2650	0.2200	0.3000
			wt_percent	weight(%)	8	1.3840	0.1062	1.3750	1.1820	1.5507
	dose300	8	weight_g	weight(g)	8	0.2688	0.0146	0.2700	0.2500	0.2900
			wt_percent	weight(%)	8	1.4096	0.0677	1.3866	1.3406	1.5299
	dose500	8	weight_g	weight(g)	8	0.2750	0.0307	0.2650	0.2400	0.3300
			wt_percent	weight(%)	8	1.4787	0.1332	1.4825	1.2801	1.6832
2nd Necropsy	Control	8	weight_g	weight(g)	8	0.2988	0.0210	0.3000	0.2700	0.3300
			wt_percent	weight(%)	8	1.4470	0.0691	1.4783	1.3480	1.5315
	dose100	7	weight_g	weight(g)	7	0.3129	0.0293	0.3100	0.2800	0.3500
			wt_percent	weight(%)	7	1.5316	0.1330	1.5182	1.3451	1.7445
	dose300	8	weight_g	weight(g)	8	0.2913	0.0314	0.2850	0.2500	0.3400
			wt_percent	weight(%)	8	1.4500	0.1244	1.4675	1.2663	1.6334
	dose500	8	weight_g	weight(g)	8	0.2938	0.0403	0.3100	0.2300	0.3300
			wt_percent	weight(%)	8	1.4493	0.1578	1.4562	1.2194	1.6326
3rd Necropsy	Control	8	weight_g	weight(g)	8	0.3025	0.0315	0.2950	0.2600	0.3500
			wt_percent	weight(%)	8	1.3986	0.0787	1.3717	1.3287	1.5261
	dose100	6	weight_g	weight(g)	6	0.3117	0.0331	0.3200	0.2500	0.3500
			wt_percent	weight(%)	6	1.4641	0.1867	1.4608	1.2195	1.7713
	dose300	8	weight_g	weight(g)	8	0.2888	0.0340	0.2850	0.2400	0.3400
			wt_percent	weight(%)	8	1.3392	0.1456	1.3549	1.0972	1.5612
	dose500	8	weight_g	weight(g)	8	0.2775	0.0292	0.2750	0.2300	0.3300
			wt_percent	weight(%)	8	1.2888	0.1058	1.2813	1.1031	1.4367

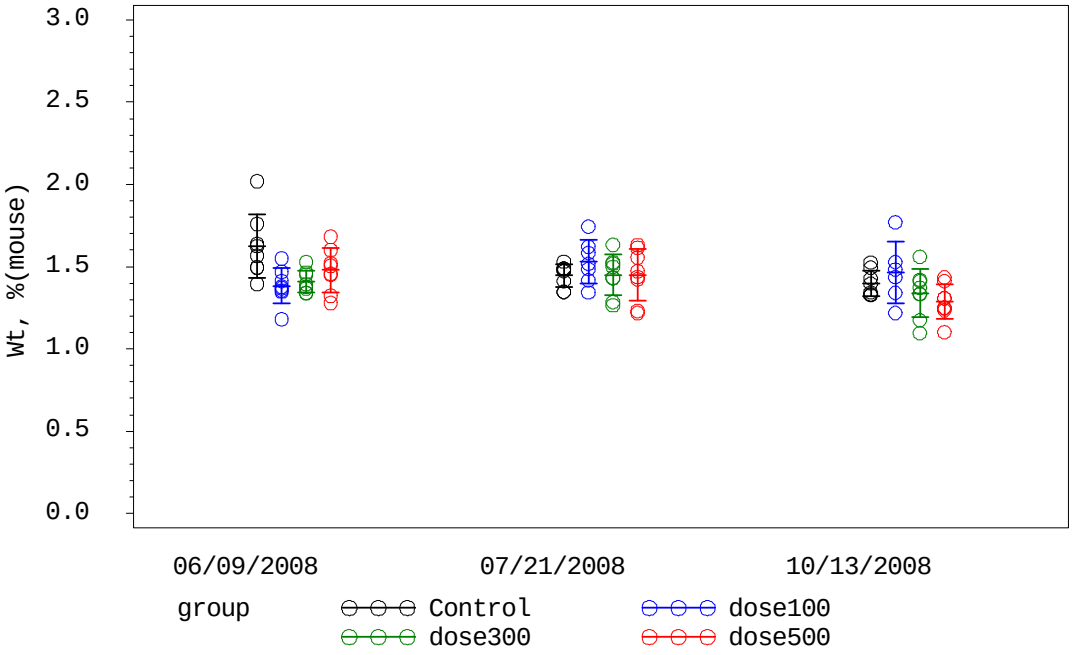
Appendix 11.7.1 Organ Weights: GLP Mice at Necropsies 1,2,3 (data QA-approved 03-19-09)
Organ = 'Kidney L/R'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

Organ	procedure	Variable	Kruskal-Wallis Chi-square	DF	Pr > Chi-Square
Kidney L/R	**1st Necropsy**	weight(g)	10.3143	3	0.0161
		weight(%)	10.6506	3	0.0138
Kidney L/R	**2nd Necropsy**	weight(g)	1.8904	3	0.5955
		weight(%)	1.5080	3	0.6804
Kidney L/R	**3rd Necropsy**	weight(g)	4.5562	3	0.2073
		weight(%)	6.1887	3	0.1028

Organ	procedure	Label	Group	Spearman corr.	P value
Kidney L/R	**1st Necropsy**	weight(g)	Dose Group	-0.39738	0.0243
		weight(%)	Dose Group	-0.26942	0.1359
Kidney L/R	**2nd Necropsy**	weight(g)	Dose Group	-0.02279	0.9032
		weight(%)	Dose Group	-0.00770	0.9672
Kidney L/R	**3rd Necropsy**	weight(g)	Dose Group	-0.33923	0.0667
		weight(%)	Dose Group	-0.38766	0.0343

Appendix 11.7.1 Organ Weights: GLP Mice at Necropsies 1,2,3 (data QA-approved 03-19-09)
Organ = 'Kidney L/R'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)





Scatterplot: circles=values; dashes(error bars)=means(SDs)

Appendix 11.7.1 Organ Weights: GLP Mice at Necropsies 1,2,3 (data QA-approved 03-19-09)
Organ = 'Liver'
Descriptive statistics

Organ=Liver

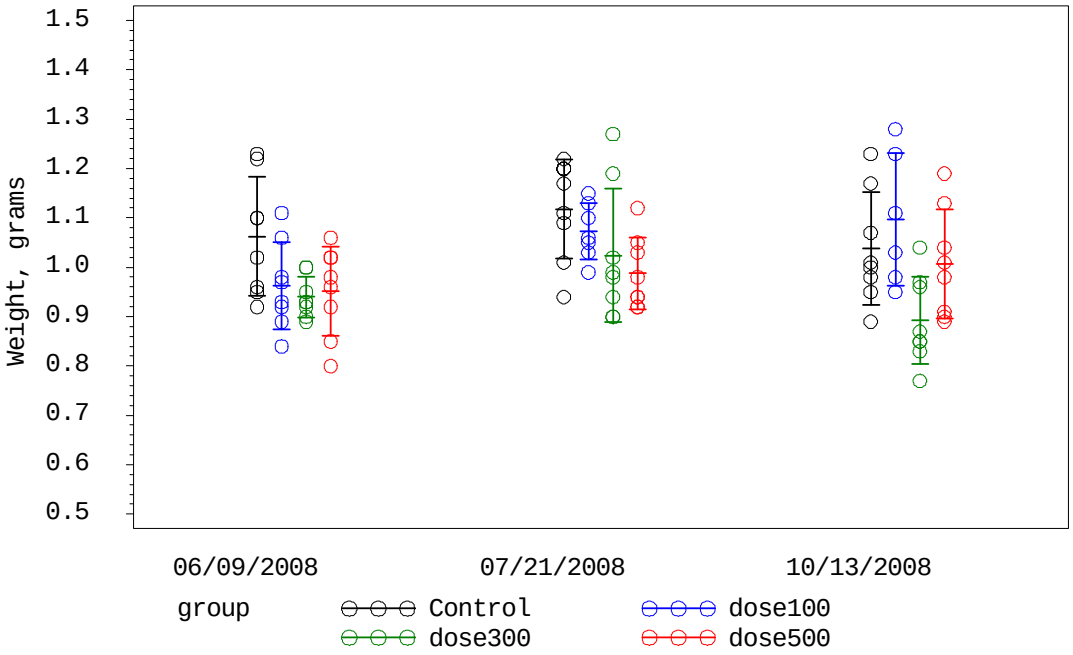
procedure	group	N Obs	Variable	Label	N	Mean	Std Dev	Median	Minimum	Maximum
1st Necropsy	Control	8	weight_g	weight(g)	8	1.0625	0.1201	1.0600	0.9200	1.2300
			wt_percent	weight(%)	8	5.5007	0.5888	5.4382	4.8266	6.3196
	dose100	8	weight_g	weight(g)	8	0.9625	0.0884	0.9500	0.8400	1.1100
			wt_percent	weight(%)	8	5.0296	0.4434	4.9816	4.2399	5.5709
	dose300	8	weight_g	weight(g)	8	0.9400	0.0414	0.9300	0.8900	1.0000
			wt_percent	weight(%)	8	4.9303	0.1808	4.8742	4.8045	5.3625
	dose500	8	weight_g	weight(g)	8	0.9513	0.0897	0.9700	0.8000	1.0600
			wt_percent	weight(%)	8	5.1269	0.5291	5.2245	4.3043	5.9496
2nd Necropsy	Control	8	weight_g	weight(g)	8	1.1175	0.1005	1.1400	0.9400	1.2200
			wt_percent	weight(%)	8	5.4066	0.3056	5.4500	4.9207	5.8983
	dose100	7	weight_g	weight(g)	7	1.0729	0.0568	1.0600	0.9900	1.1500
			wt_percent	weight(%)	7	5.2504	0.1575	5.2060	5.0162	5.4827
	dose300	8	weight_g	weight(g)	8	1.0238	0.1357	0.9850	0.9000	1.2700
			wt_percent	weight(%)	8	5.0890	0.4981	5.0380	4.5138	6.1011
	dose500	8	weight_g	weight(g)	8	0.9875	0.0727	0.9600	0.9200	1.1200
			wt_percent	weight(%)	8	4.8846	0.2476	4.8560	4.4622	5.1991
3rd Necropsy	Control	8	weight_g	weight(g)	8	1.0375	0.1137	1.0050	0.8900	1.2300
			wt_percent	weight(%)	8	4.7929	0.2278	4.7491	4.5585	5.2546
	dose100	6	weight_g	weight(g)	6	1.0967	0.1350	1.0700	0.9500	1.2800
			wt_percent	weight(%)	6	5.1453	0.6774	4.9113	4.6339	6.4777
	dose300	8	weight_g	weight(g)	8	0.8925	0.0889	0.8600	0.7700	1.0400
			wt_percent	weight(%)	8	4.1446	0.4384	4.0035	3.6693	4.7755
	dose500	8	weight_g	weight(g)	8	1.0063	0.1102	0.9950	0.8900	1.1900
			wt_percent	weight(%)	8	4.6778	0.4815	4.6202	4.2571	5.7336

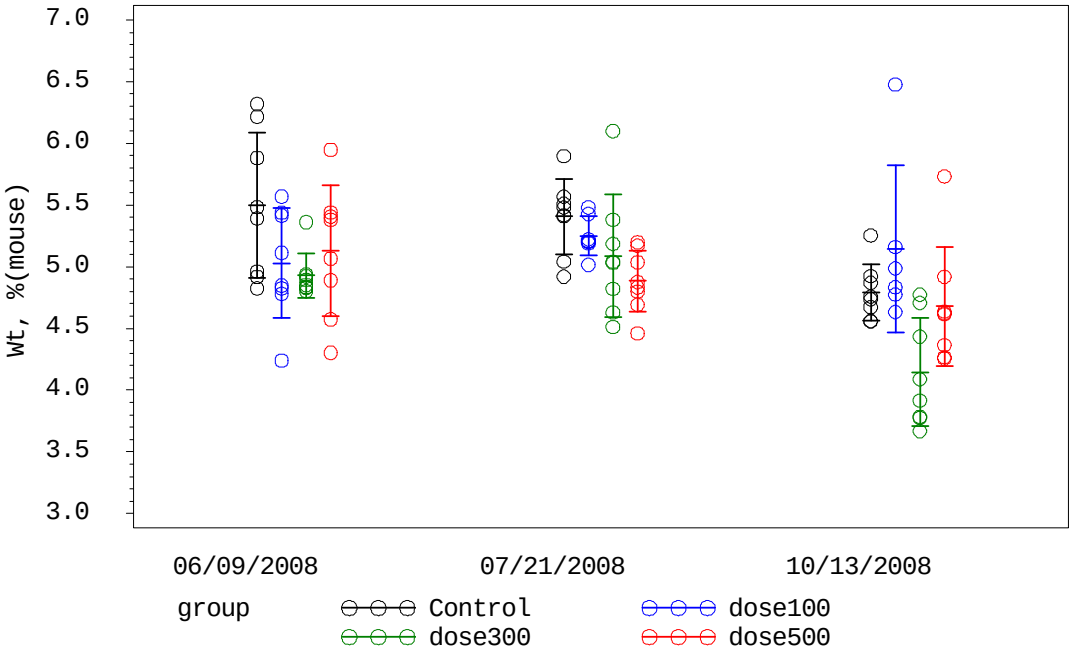
Appendix 11.7.1 Organ Weights: GLP Mice at Necropsies 1,2,3 (data QA-approved 03-19-09)
Organ = 'Liver'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

Organ	procedure	Variable	Kruskal-Wallis Chi-square	DF	Pr > Chi-Square
Liver	**1st Necropsy**	weight(g)	5.4889	3	0.1393
		weight(%)	5.2102	3	0.1570
Liver	**2nd Necropsy**	weight(g)	7.8096	3	0.0501
		weight(%)	11.7915	3	**0.0081**
Liver	**3rd Necropsy**	weight(g)	9.7359	3	0.0209
		weight(%)	12.4038	3	**0.0061**

Organ	procedure	Label	Group	Spearman corr.	P value
Liver	**1st Necropsy**	weight(g)	Dose Group	-0.30645	0.0880
		weight(%)	Dose Group	-0.23007	0.2052
Liver	**2nd Necropsy**	weight(g)	Dose Group	-0.49997	**0.0042**
		weight(%)	Dose Group	-0.62100	**0.0002**
Liver	**3rd Necropsy**	weight(g)	Dose Group	-0.22420	0.2336
		weight(%)	Dose Group	-0.35547	0.0539

Appendix 11.7.1 Organ Weights: GLP Mice at Necropsies 1,2,3 (data QA-approved 03-19-09)
Organ = 'Liver'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)





Scatterplot: circles=values; dashes(error bars)=means(SDs)

Appendix 11.7.1 Organ Weights: GLP Mice at Necropsies 1,2,3 (data QA-approved 03-19-09)
Organ = 'Spleen'
Descriptive statistics

Organ=Spleen

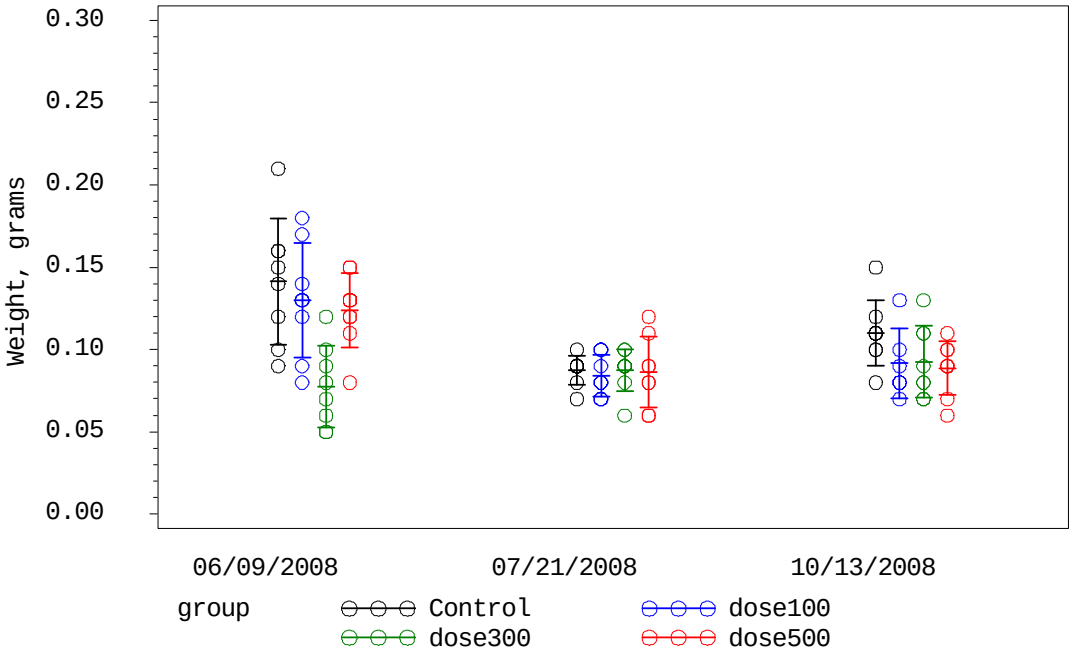
procedure	group	N Obs	Variable	Label	N	Mean	Std Dev	Median	Minimum	Maximum
1st Necropsy	Control	8	weight_g	weight(g)	8	0.1413	0.0383	0.1450	0.0900	0.2100
			wt_percent	weight(%)	8	0.7297	0.1919	0.7464	0.4759	1.0878
	dose100	8	weight_g	weight(g)	8	0.1300	0.0346	0.1300	0.0800	0.1800
			wt_percent	weight(%)	8	0.6761	0.1640	0.6765	0.4038	0.9197
	dose300	8	weight_g	weight(g)	8	0.0775	0.0249	0.0750	0.0500	0.1200
			wt_percent	weight(%)	8	0.4048	0.1224	0.3925	0.2681	0.5907
	dose500	8	weight_g	weight(g)	8	0.1238	0.0226	0.1250	0.0800	0.1500
			wt_percent	weight(%)	8	0.6665	0.1206	0.6967	0.4304	0.8072
2nd Necropsy	Control	8	weight_g	weight(g)	8	0.0875	0.0089	0.0900	0.0700	0.1000
			wt_percent	weight(%)	8	0.4240	0.0404	0.4296	0.3495	0.4711
	dose100	7	weight_g	weight(g)	7	0.0843	0.0127	0.0800	0.0700	0.1000
			wt_percent	weight(%)	7	0.4133	0.0661	0.4054	0.3363	0.4984
	dose300	8	weight_g	weight(g)	8	0.0875	0.0128	0.0900	0.0600	0.1000
			wt_percent	weight(%)	8	0.4363	0.0655	0.4428	0.3087	0.5359
	dose500	8	weight_g	weight(g)	8	0.0863	0.0213	0.0850	0.0600	0.1200
			wt_percent	weight(%)	8	0.4235	0.0865	0.4210	0.3061	0.5525
3rd Necropsy	Control	8	weight_g	weight(g)	8	0.1100	0.0200	0.1100	0.0800	0.1500
			wt_percent	weight(%)	8	0.5067	0.0655	0.4962	0.4098	0.6408
	dose100	6	weight_g	weight(g)	6	0.0917	0.0214	0.0850	0.0700	0.1300
			wt_percent	weight(%)	6	0.4353	0.1273	0.4038	0.2937	0.6579
	dose300	8	weight_g	weight(g)	8	0.0925	0.0219	0.0850	0.0700	0.1300
			wt_percent	weight(%)	8	0.4287	0.0978	0.3961	0.3226	0.5928
	dose500	8	weight_g	weight(g)	8	0.0888	0.0164	0.0900	0.0600	0.1100
			wt_percent	weight(%)	8	0.4127	0.0746	0.4288	0.2878	0.4818

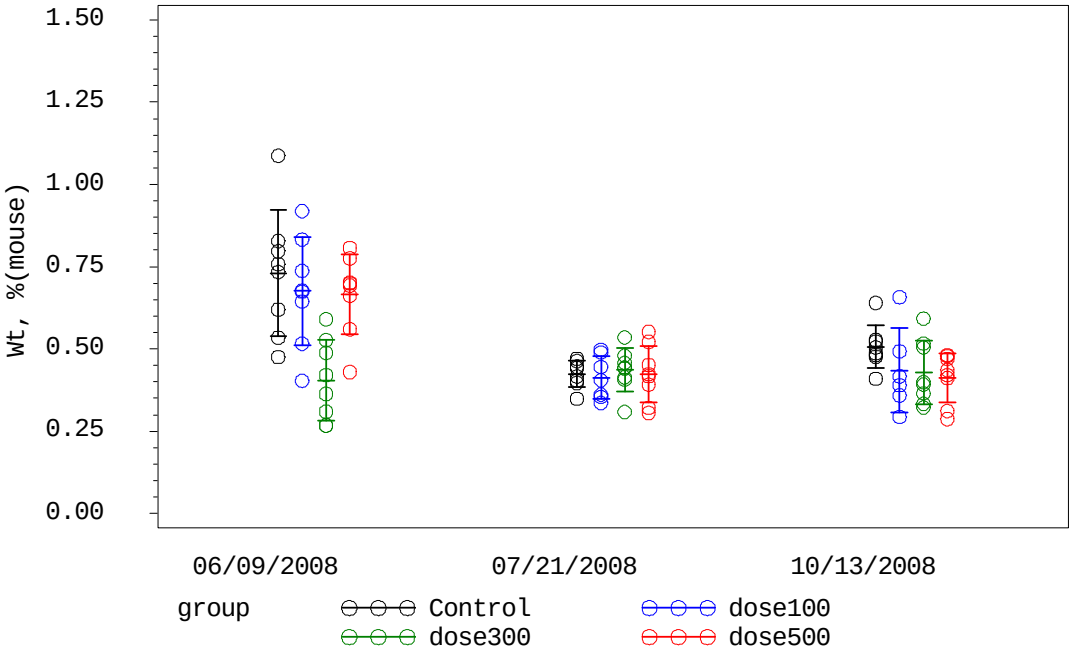
Appendix 11.7.1 Organ Weights: GLP Mice at Necropsies 1,2,3 (data QA-approved 03-19-09)
Organ = 'Spleen'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)

Organ	procedure	Variable	Kruskal-Wallis Chi-square	DF	Pr > Chi-Square
Spleen	**1st Necropsy**	weight(g)	12.7689	3	**0.0052**
		weight(%)	13.0256	3	**0.0046**
Spleen	**2nd Necropsy**	weight(g)	0.5583	3	0.9059
		weight(%)	0.4560	3	0.9284
Spleen	**3rd Necropsy**	weight(g)	5.1453	3	0.1615
		weight(%)	5.8677	3	0.1182

Organ	procedure	Label	Group	Spearman corr.	P value
Spleen	**1st Necropsy**	weight(g)	Dose Group	-0.29945	0.0959
		weight(%)	Dose Group	-0.23310	0.1992
Spleen	**2nd Necropsy**	weight(g)	Dose Group	-0.02904	0.8768
		weight(%)	Dose Group	0.03060	0.8702
Spleen	**3rd Necropsy**	weight(g)	Dose Group	-0.34206	0.0643
		weight(%)	Dose Group	-0.38536	0.0355

Appendix 11.7.1 Organ Weights: GLP Mice at Necropsies 1,2,3 (data QA-approved 03-19-09)
Organ = 'Spleen'
Kruskal-Wallis tests (for any difference between dose groups)
Spearman Correlations (for net trend with dose group)





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Appendices II Clinical

Protocol 1

In Support of Proposal Titled:

"Vaccination of High Risk Breast Cancer Patients"

Phase 1 Dose-Finding Study
of
a Carbohydrate Mimotope Based Vaccine with QS-21

Laura Hutchins, M.D., Principal Investigator
Thomas Kieber-Emmons, Ph.D., Subinvestigator
Issam Makhoul, M.D., Subinvestigator
Anne-Marie Maddox, M.D., Subinvestigator
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USEFUL FIGURES AND TABLES

1. PROTOCOL SUMMARY

Primary Objective – Safety: Determine the safety and tolerability of a peptide mimotope-based vaccine upon immunization of breast cancer patients

Secondary Objectives – Immune Response:

1) Determine whether immunization with the vaccine generates a humoral response against Tumor Associated Carbohydrate Antigens (TACAs– see **APPENDIX A** for abbreviations)
2) Determine the delayed-type hypersensitivity (DTH) response to the immunizing mimotope
3) Determine the effect of a late booster immunization on the humoral response against TACAs

Study Population: 6-12 research participants will be enrolled from the breast cancer clinics (Medical Oncology and Ladies Oncology Clinics) at the Winthrop P. Rockefeller Cancer Institute at the University of Arkansas for Medical Sciences (UAMS) campus.

Inclusion criteria: Females with histologically or cytologically confirmed stage IV breast cancer (newly diagnosed metastatic or relapsed after primary or adjunctive therapy, which has not required a treatment change for 2 months) will be invited to participate.

Exclusion Criteria: Women that are pregnant, breast-feeding, have autoimmune disease or are immunosuppressed or receiving systemic corticosteroids will be excluded from the study.

Investigational product: P10s-PADRE administered with the Stimulon® QS-21 adjuvant

Study Design: After signing IRB approved consent, two cohorts of 3-6 stage IV breast cancer patients will be enrolled. Initially, a single cohort will be administered 300 μg/mL P10s-PADRE formulated with 100 μg/mL QS-21 in saline by subcutaneous (SC) injection on 5 separate occasions (during Weeks 1, 2, 3, 7 and 19). The vaccine will be administered at rotating sites on the limbs or abdomen and by nurses in the Infusion Center at the Cancer Institute, using a dose volume of 0.5 mL per injection. Based on a series of criteria measuring tolerance and immune response in the first cohort on Week 9, the P10s-PADRE dose will either be increased to 500 μg/mL or decreased to 100 μg/mL for the second cohort of patients.

2. BACKGROUND

Anticipated anti-cancer impact of carbohydrate-targeted vaccines: The potential impact of vaccines that induce responses to tumor-associated carbohydrate antigens (TACAs) is demonstrated by clinical trials where patient survival significantly correlates with carbohydrate-reactive IgM levels (2). Such results suggest that TACA-targeting vaccines might have a beneficial effect on the course of malignant disease. TACA-induced responses could augment naturally occurring carbohydrate-reactive IgM antibodies that trigger apoptosis of tumor cells (3). TACAs are attractive targets because the majority of cell-surface proteins and lipids are glycosylated, and the glycosyl moiety is fundamental to the biological functions of these molecules in cancer cells (4,5). A unique advantage in targeting TACAs is that multiple proteins and lipids on the cancer cell can be modified with the same carbohydrate structure. Thus, targeting the carbohydrate antigen broadens the spectrum of antigens recognized by the immune

response, thereby lowering the risk of developing resistant tumors due to the loss of any one antigen (6). In addition, antibodies that recognize glycolipids are more apt to mediate complement-dependent cytotoxicity (CDC) and may, therefore, be more cytotoxic to tumor cells than antibodies that recognize protein antigens (7). Furthermore, preclinical studies support the hypothesis that vaccine-induced responses against TACAs might have their greatest impact in the adjuvant setting, as such responses inhibit tumor outgrowth in metastatic models (8,9).

Approaches to augment immune responses to TACA: A variety of approaches are being taken to generate responses to TACAs. Because TACAs are T cell-independent antigens and self-antigens, conjugation to immunologic carrier proteins is perceived to be essential to recruit T-cell help in antibody generation. Conjugation does not, however, assure an increase in immunogenicity because conjugation strategies do not uniformly enhance carbohydrate immunogenicity (10,11). Furthermore, even with conjugation, the lack of induction of cellular immune responses that would amplify TACA-reactive humoral responses necessitates constant boosting with vaccine. Representative examples of carbohydrate-based conjugate vaccines in clinical development include those directed toward gangliosides (12-14), polysialic acid (15), Globo-H (16), Lewis Y (LeY) (1), and the sialosyl-TN (STn) antigen (17).

An approach predicted to facilitate cellular responses exploits the molecular mimicry of TACAs by protein surrogates, as they are T-cell-dependent antigens. Clinical characterizations of anti-idiotypic antibodies that mimic the GD3 ganglioside antigen (18) and GD2 (19) have been described. Carbohydrate mimetic peptides (CMPs) are alternatives to anti-idiotypic antibodies. The characterization of CMPs is at present limited to preclinical studies. CMPs that induce immune responses cross-reactive with TACA are also referred to as peptide mimotopes. Peptide mimotopes have been described for the GD2 (20-22), GD3 (23), sialylated Lewis a/x (24) and Lewise Y (LeY) antigens (20, 25). Importantly, in preclinical prophylactic and therapeutic vaccination studies, peptide mimotopes were efficacious in eliciting immune responses that reduced tumor burden and inhibited metastatic outgrowth (8, 25, 26). Thus, peptide mimotopes of TACAs represent a new and very promising tool to overcome T-cell independence and to increase the efficiency of the immune response to glycan antigens.

Target carbohydrate antigens expressed on breast cancer cells: Tumors expressing high levels of certain types of TACAs exhibit greater metastasis than those expressing low levels of these antigens, and this negatively impacts prognosis (27-29). In breast cancer, the LeY, STn, KH-1, selected gangliosides, glycosphingolipids and Globo-H carbohydrate antigens are considered prime vaccine candidates because of their tissue distribution (30, 31). In particular, LeY has long been recognized as a potential target for immunotherapy because it is expressed in 70–90% of tumors of epithelial origin (32). The abundant gangliosides include GM3, GM2, GM1, and GD2, GD3 and GT3 (33). Antibodies to TACAs mediate a variety of effector functions and might lend to cross-presentation of tumor antigens to stimulate anti-tumor cellular responses. At present, LeY-conjugate vaccines appear to have only a limited ability to induce anti-LeY immune responses in humans (1). Our *in vitro* studies demonstrate that peptide mimotopes of LeY and gangliosides induce serum antibodies in mice that recognize the appropriate carbohydrate antigens on human or murine breast cancer cell lines (25, 34). Our *in vivo* studies demonstrate that the peptide mimotopes induce sustained immunity to these antigens (8, 25, 26).

Collectively, these data provide the experimental foundation for evaluating peptide mimotopes as potential cancer vaccines in subjects with breast cancer.

Preclinical studies supporting the P10s-PADRE vaccine as a viable candidate for preventing breast cancer recurrence: The desired effect of a cancer vaccine is to modify the clinical outcome of the patient population of interest. Genetic studies have resurrected the concept that the adaptive and innate immune systems play roles in tumor surveillance. Cellular immunity, in which cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells are main effector cells, plays an important role in the antitumor defense mechanism. Tumors over express TACAs, which are reactive with B cells, but the use of TACAs as immunogens is restricted by a lack of B cell cross-talk with T cells. Consequently, this inherent limitation of plain polysaccharide vaccines include limited duration of immunity, the potential for hyoporesponsiveness with repeated vaccinations and ineffectiveness in stimulating a cellular response. To circumvent this drawback we have developed CMPs with overlapping B and T cell epitopes to link TACAs' reactive humoral responses with anti-tumor cellular responses. Among the CMPs we have developed are a series that contain the amino acids Trp-Arg-Tyr as a centralized motif. CMPs with this motif display an ability to induce antibodies cross-reactive with tumor cells, induce cellular responses to tumor cells and induce or activate NK cells with anti-tumor activity. Preclinical studies in mice with vaccines containing P10s or P10 (a longer peptide that contains the P10s sequence) have demonstrated these mimotopes induce a robust immunogenic response that includes cross-reactivity with breast cancer associated TACAs, stimulation of tumor cell reactive cellular responses and/or stimulation of tumor targeting NK cells. Although the mechanism of action appears to vary depending upon the peptide (P10s or P10), coupling agent and adjuvant (keyhole limpet hemocyanin (KLH) vs. PADRE and QS-21) employed, all vaccines tested in mice to date that contain P10s or P10 have consistently inhibited metastatic outgrowth of murine tumor cells expressing TACA structural homologues. Antibodies raised against our P10s-PADRE/QS-21 vaccine are tumor specific that along with NK activation contribute to immune surveillance reminiscent of anti-pathogen vaccines. NK cells recognize many tumor cells but not normal self cells, and they are thought to aid in the elimination of nascent tumors. The major function of NK cells in fighting cancer is likely to be in surveillance and elimination of cells that become malignant before they can cause a tumor. Thus, our P10s-PADRE/QS-21 vaccine represents a new and very promising tool to induce a tumor specific immune responses to breast cancer cells, and potentially can be used to prevent disease relapse in high risk patients.

3. TRIAL OBJECTIVES

a. Primary Objective – Safety: The safety and tolerability of the p10s-PADRE/QS-21 vaccine will be determined by toxicity assessments throughout the duration of the study. Subjects will be evaluated for toxicity using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0. (http://ctep.cancer.gov and **APPENDIX B**). A toxicity of Grade 3 or higher will be considered a dose limiting toxicity (DLT) if it is deemed to be related to the vaccine or any of its components.

b. Secondary Objectives – Immune Response:

1) The ability of the p10s-PADRE/QS-21 vaccine to generate a humoral response

against TACAs will be determined by titering anti-TACA serum IgG or IgM antibodies measured at pre-study and on weeks 2, 3, 4, 7, and 9 from study participant blood samples. IgM and IgG titers to TACAs will be evaluated by enzyme-linked immunosorbent assay (ELISA) and by fluorescence-activated cell sorting (FACS). Titer will be defined as the highest serum dilution yielding an $OD_{405} \geq 0.15$, in accordance with previous studies (1) or a mean fluorescence intensity (MFI) two standard deviations higher than background. A positive TACA-directed immune response will be defined as an anti-TACA serum antibody titer of 1:40 for a baseline pre-vaccination titer of 0 or a ≥ 4-fold increase for a baseline titer > 0 (1).

2) Delayed type hypersensitivity (DTH) responses to the immunizing mimotope and control antigens will be determined by the amount of induration surrounding the injection site at 48 hrs post-injection. An induration diameter of > 5 mm at 48 hrs post injection will be considered a positive response. Control agents to be tested in addition to the p10s-PADRE/QS-21 vaccine include Tetanus-Diphtheria Toxoid Antigen and the Candida antigen.
3) The effect of a late booster immunization of p10s-PADRE/QS-21 on the humoral response against TACAs will be determined by titering anti-TACA serum IgG or IgM antibodies measured on weeks 19 and 21 with the methodology described above.

4. PATIENT POPULATION

Eligibility Criteria: Subjects are eligible for the vaccine study if the following inclusion and exclusion criteria are met:

1) Female subjects of all races with histologically or cytologically confirmed stage IV breast cancer are eligible. The cancer may be newly diagnosed metastatic or relapsed after primary or adjunctive therapy and must not have required a treatment change for 2 months. Disease staging will be done according to the American Joint Commission on Cancer (AJCC), sixth edition. The breast cancer staging information can be found at the following address:
   http://www.cancerstaging.org/education/tnmschema/breast.ppt
2) Age greater than 18 years
3) ECOG Performance Status greater then or equal than 1.
4) Subjects must not have an active infection requiring treatment with parenteral antibiotics.
5) Subjects must not have other significant medical, surgical or psychiatric conditions, or require any medication or treatment which may interfere with compliance of the treatment regimen.
6) Subjects must not have a diagnosis or evidence of organic brain syndrome, significant impairment of basal cognitive function or any psychiatric disorder that might preclude participation in the full protocol.

7) Subjects must have no other current malignancies. Subjects with prior history at any time of any *in situ* cancer, including lobular carcinoma of the breast *in situ*, cervical cancer *in situ*, atypical melanocytic hyperplasia or Clark I melanoma *in situ* or basal or squamous skin cancer are eligible, provided they are disease-free at the time of registration. Subjects with other malignancies are eligible if they have been continuously disease free for $\geq$ 5 years prior to the time of registration.
8) Subjects must not have autoimmune disorders or conditions of immunosuppression. They must not be receiving treatment with systemic corticosteroids, including oral steroids (i.e. prednisone, dexamethasone), continuous use of topical steroid creams or ointments or any steroid containing inhalers. Subjects who have been on systemic steroids will require a 6-week washout period. Subjects who discontinue the use of these classes of medication for at least 6 weeks prior to registration are eligible if, in the judgment of the treating physician, the subject is not likely to require these classes of drugs during the treatment period. Replacement doses of steroids for subjects with adrenal insufficiency are allowed.
9) Women of childbearing potential must not be pregnant (negative serum pregnancy test within 2 weeks of registration and 48 hours of receiving study drug) or breast-feeding, due to the unknown effects of peptide/mimotope vaccines on a fetus or infant.
10) Women of childbearing potential must be counseled to use an accepted and effective method of contraception (including abstinence) while on treatment and for a period of 18 months after completing or discontinuing treatment.
11) Subjects must have obtained a white blood cell (WBC) count $\geq$ 3,000/mm^3 and platelet count $\geq$ 100,000/mm^3 within 2 weeks prior to registration.
12) Subjects must have a serum glutamic-oxaloacetic transaminase (SGOT)/aspartate aminotransferase test (AST) and bilirubin $\leq$ 2 x institutional upper limit (IUL) of normal and serum creatinine $\leq$ 1.8 mg/dl, all obtained within 2 weeks prior to registration.
13) Subjects must be immunocompetent as measured by responsiveness to 2 recall antigens by skin testing.
14) All subjects who wish to participate in the study must sign an informed consent approved by the UAMS Institutional Review Board (IRB).
15) Prestudy laboratory tests must be completed within 2 weeks of registration.

5. INVESTIGATIONAL NEW DRUG – P10S-PADRE/QS-21

a. General Description: P10s-PADRE is a short peptide (P10s) coupled to PADRE, a synthetic, non-natural, peptide that binds with high or intermediate affinity to 15 of 16 of the most common HLA-DR types tested to date. Both components contribute to the efficacy of this molecule in stimulating an immune response. Because of its binding promiscuity, PADRE should overcome the problems posed by the extreme polymorphism of HLA-DR molecules in the human population. Furthermore, the PADRE peptide was specifically engineered as an antigen-presenting molecule for use in humans. Carbohydrate moieties, such as TACAs, typically do not induce T cell responses. Thus, P10s, a TACA peptide mimotope, was developed. By coupling P10s to PADRE, we have increased the likelihood

of generating an immune response, including T cell "help" in our vaccine construct designed for human use.

Stimulon® QS-21 is an immunological adjuvant that has been shown to stimulate both humoral and cell-mediated immunity. QS-21 is a naturally occurring saponin molecule purified from the South American tree *Quillaja saponaria* Molina. It is a triterpene glycoside with the general structure of a quillaic acid 3, 28-O-bis glycoside with the formula C92H148O46, and a molecular weight of 1990 kD.

Complete information on P10s-PADRE and QS-21 may be found in their respective Investigator's Brochures (**APPENDICES C** and **D).**

b. Vaccine Manufacturing: NeoMPS Inc. (San Diego, CA 92126 · USA) will synthesize Mimotope P10s covalently linked with PADRE in powder form according to good manufacturing practices (GMPs) detailed in **APPENDIX E**. Once received, the P10s-PADRE vaccine will be stored frozen at ≤ -20º C for maximum stability. Stimulon® QS-21 will be supplied by Antigenics, Inc. (Lexington, MA 02421 · USA) in powder form and stored frozen at ≤ -20º C for maximum stability as well.

c. Vaccine Formulation: Stock solutions of P10s-PADRE and QS-21 will be made with sterile phosphate buffered saline (PBS) and mixed for injection as defined below:

P10s-PADRE stock solution (filter sterilized) = 10 mg/mL
QS-21 stock solution = 2 mg/mL
Volume needed for 1 injection = 0.750 mL (0.250 mL considered for pipetting error and 0.500 mL for injection)

P10s-PADRE /dose	(500 µg/dose)	(300 µg/dose)	(100 µg/dose)
P10s-PADRE volume	0.075 mL	0.045 mL	0.015 mL
QS-21 volume (100 µg/injection)	0.075 mL	0.075 mL	0.075 mL
Sterile PBS volume	0.600 mL	0.630 mL	0.660 mL

Vaccine Preparation Protocol:

1. Put the appropriate amount of sterile PBS into a tube.
2. Add the calculated volume of the P10s-PADRE stock solution to the sterile PBS.
3. Mix by vortexing for about 15 seconds.
4. Add 0.05 mL QS-21 into the diluted P10s-PADRE solution.
5. Mix by gently vortexing the final vaccine solution for about 15 seconds.
6. Keep on ice until loading a disposable syringe with 0.500 mL vaccine for injection.

d. Label Information: The vaccine drug supply will be labeled with the following message: "Caution: New Drug – Limited by Federal Law to Investigational Use"

e. Agent Ordering: P10s-PADRE and Stimulon® QS-21 will be ordered by the Winthrop P. Rockefeller Cancer Institute Research Pharmacy staff from NeoMPS located at 9395 Cabot Drive, San Diego, CA 92126, and Antigenics, Inc. located at 3 Forbes Road, Lexington, MA 02421, respectively. Both agents will be shipped directly to the Cancer Institute Pharmacy.

f. **Agent Accountability:** P10s-PADRE and Stimulon® QS-21 will be stored in the Cancer Institute pharmacy under the supervision of the research pharmacist who will be responsible for maintaining the supply according to the manufacturer's specifications, dispensing the drug for administration and maintaining all accountability logs. Standard NCI accountability logs will be used, and the UAMS Investigational Agent Accountability Record is provided in **APPENDIX F**.

6. TREATMENT PLAN

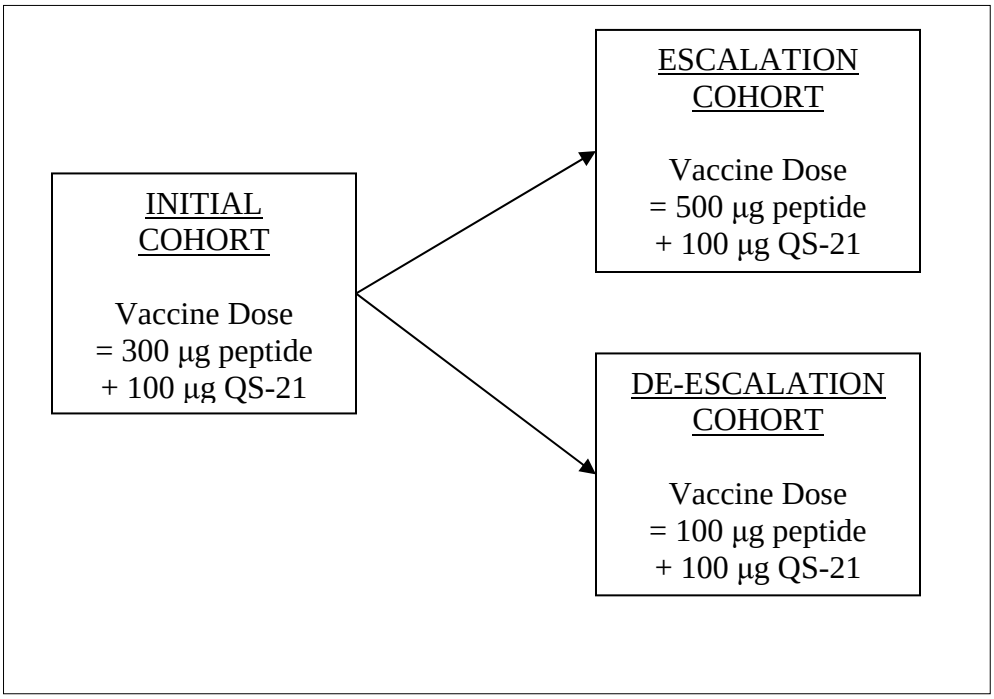
a. **On-study Evaluation:** After signing the IRB-approved informed consent form, research participants will be assigned to a cohort at the time of registration by a clinical research associate (CRA) in the Clinical Research and Data Management (CRDM) office in the Cancer Institute. All research participants will receive the Mimotope P10s-PADRE/QS-21 vaccine via subcutaneous (SC) injection following the schedule on the Study Calendar in Section 8:
   1) P10s-PADRE/QS-21 vaccine will be administered at weeks 1, 2, 3, 7 and 19, for a total of 5 immunizations for each participant. The vaccine will be administered SC at rotating sites that include the arm, thigh or abdomen by nurses in the Infusion Center at the Cancer Institute.
   2) A medical history and physical examination will be done at prestudy and on weeks 1, 2, 3, 7, 9, 19 and 21.
   3) A complete blood count with differential will be done at prestudy and on weeks 1-9 and 19-21. Additionally a chemistry profile and screens for autoimmunity will be done at prestudy and on weeks 3, 7, 9, 19 and 21.
   4) Toxicity evaluations using the NCI CTCAE Version 3.0 will be performed on weeks 1, 2, 3, 7, 19 and 21, prior to each injection or upon presentation of an adverse event (AE). Toxicities to be assessed are the laboratory parameters listed in the study calendar, as well as any sign or symptom found during the history and physical examination not noted at prestudy or on the baseline evaluation. Special attention will be paid to signs and symptoms related to injection reactions, injection site reactions or symptoms or laboratory findings indicating autoimmune toxicities.
   5) Immune responses to the vaccine will be measured by serologic titer in study lab serum samples drawn at prestudy and on weeks 2, 3, 4, 7, 9, 19 and 21. If vaccine is given on these days, the study labs will be drawn before the vaccine is administered.
   6) DTH skin testing will be done at prestudy to determine the immunocompetency of subjects. Tetanus-Diphtheria Toxoid Antigen and the Candida antigen will be

administered intradermally (id) as control antigens at separate locations on the subject's back, and the resulting induration will be read 48 hours later. On weeks 5, 9 and 21 100 μg Mimotope P10s-PADRE will be given id alongside the control antigens to determine subjects' DTH response to vaccine.

b. Prohibited Medications: Systemic steroids are prohibited. If a subject wishing to participate in the study has been on systemic steroids, a 6-week washout will be required prior to participation in the study.

c. Rescue Medications: Subjects who develop symptomatic autoimmune reactions, Grade 3 or greater hypersensitivity reactions or Grade 3 or greater local reactions should be treated as indicated with systemic steroids, topical steroids, epinephrine or Benadryl. These subjects will be removed from the study.

d. Dose Assignment: Subjects will be treated with vaccine admixed with QS-21 on weeks 1, 2, 3, 7 and 19 in cohorts according to the following dosing diagram:



DLT and the immune-response endpoints are defined in Section 3, "Trial Objectives". The decision to escalate or de-escalate the dose, expand the cohort or terminate the study will be based on assessment for DLT, which will require 9 weeks per subject. The time to assess a cohort of 3 for DLTs and immune responses is thus anticipated to be 13 weeks based on an accrual rate of 2 eligible Stage IV subjects per month. Upon evaluation of all subjects in a cohort (3 or 6 per dose level), the decision whether to escalate, de-escalate or stop will proceed according to the cohort-appropriate schedule shown in **Tables 1, 2, and 3.**

Table 1: Toxicity Decision Rules for Initial Cohort (300 µg p10S-PADRE)	
***DLTs/ Cohort*[1]**	***Action***
0/3	Begin accrual to Escalation Cohort
1/3	Expand Initial Cohort to 6 subjects
1/6	Begin accrual to Escalation Cohort
2/6, 3/6, or 4/6	Begin Accrual to De-Escalation Cohort
2/3 or 3/3	Begin Accrual to De-Escalation Cohort
[1] DLT = Dose-Limiting Toxicity	

Table 2: Toxicity Decision Rules for Escalation Cohort (500 µg P10s-PADRE)	
***DLTs/ Cohort*[1]**	***Action***
0/3 or 1/3	Expand cohort to 6 subjects
1/6	Stop: Declare escalation dose to be MTD[2]
2/6, 3/6, or 4/6	Stop: Previous dose level is MTD[2]
2/3 or 3/3	Stop: Previous dose level is MTD[2]
[1] DLT = Dose-Limiting Toxicity [2] MTD = Maximum Tolerated Dose	

Table 3: Toxicity Decision Rules for De-Escalation Cohort (100 µg P10s-PADRE)	
***DLTs/ Cohort*[1]**	***Action***
0/3 or 1/3	Expand cohort to 6 subjects.
1/6	Stop: De-escalation dose level is the MTD[2]
2/6, 3/6, or 4/6	Stop: De-escalation dose level is above MTD[2]
2/3 or 3/3	Stop: De-escalation dose level is above MTD[2]
[1] DLT = Dose-Limiting Toxicity [2] MTD = Maximum Tolerated Dose	

If the initial-cohort dose of 300 µg P10s-PADRE is declared to be the MTD and only 3 subjects were enrolled into the initial cohort, then this cohort will be expanded to 6 to assure that 6 subjects are treated at the MTD. The subjects will be assigned to cohorts by the CRA for the trial, who will notify the research nurse, who will notify the investigator of the cohort assignments. Only one cohort will be open to enrollment at a time. The study will have a hold pending the evaluation of each cohort. If two or more subjects are enrolled on the same day, then their injection schedules will be staggered at least one day apart. The research pharmacist will be notified by orders of the registration and cohort dose. The subjects in a given cohort of 3 can start injections as close as one day apart. Expansion of the cohort to 6, or enrollment to the next cohort, may start once the previous 3 subjects complete their week-9 serology and are evaluated for DLT. Subjects who withdraw from the dose-escalation study without a DLT will be replaced. Subjects who withdraw with a DLT will be considered evaluable for MTD determination.

7. RISKS AND TOXICITIES TO BE MONITORED

a. Potential Toxicities, Risks and Precautions:

Procedure	Risks	Measures to Minimize Risks
Complete history and physical exam, including blood chemistries	Identification of previously unknown condition	Qualified health care provider to evaluate potential subject Research records are kept in a locked area accessible only by study personnel.
Administration of study vaccine Mimotope P10s-PADRE	Experimental agent may be toxic or harmful. First time use in humans Risk of local reactions (i.e. swelling, redness, tenderness, itching, extravasations) Potential for side effects ranging from hematologic toxicities and hypersensitivity reactions to anaphylaxis Unanticipated risks Unknown risks	Careful monitoring by clinic visits and 24 hour, 7 days per week physicians on call for unexpected problems Only non-pregnant, non-lactating females may participate. The use of contraception during the study and the use of contraception for 18 months post completion of the trial are required. Frequent laboratory tests including complete blood count (CBC) with differential, liver function tests, etc. Close and frequent monitoring of subjects by qualified staff DTH assay to monitor for hypersensitivity reactions Emergency equipment including crash carts, advanced cardiac life support (ACLS) certified staff and rescue medications such as Benadryl, epinephrine, high dose steroids, etc. will be on-site during administration. The Medical Monitor will review all toxicities on a regular basis and will be available to aid subjects as needed. The study drug may be discontinued. This research is being conducted at an experienced clinical research center.

Procedure	Risks	Measures to Minimize Risks
		Reporting and monitoring mechanisms are in place for AEs, serious adverse events (SAEs) and unanticipated problems.
Administration of 100 µg QS-21, SC	Dermatology/Skin: local erythema, rash, pruritis	Careful monitoring by clinic visits and 24 hour, 7 days per week physicians on call for unexpected problems
	Gastrointestinal: diarrhea, anorexia, nausea, vomiting, abnormal taste	Only non-pregnant, non-lactating females may participate. The use of contraception during the study and the use of contraception for 18 months post completion of the trial are required.
	Hepatic: elevated hepatic enzymes, hypo-albuminemia with prolonged treatment	Frequent laboratory tests including CBC with differential, liver function tests, etc.
	Neurology: confusion, neuropathies	Close and frequent monitoring of subjects by qualified staff.
	Pulmonary: dyspnea (due to fluid retention and capillary leak syndrome), pleuritis	DTH assay to monitor for hypersensitivity reactions
	Cardiovascular: hypertension, cardiac arrhythmias, atrial fibrillation, pericarditis	Emergency equipment including crash carts, ACLS certified staff and rescue medications such as Benadryl, epinephrine, high dose steroids, etc. will be on-site during administration.
	Pain: headache, arthralgias, bone pain, abdominal pain, chest pain, myalgia	The Medical Monitor will review all toxicities on a regular basis and will be available to aid subjects as needed.
	Coagulation: partial thromboplastin time (PTT), prothrombin time (PT), thrombo-embolic phenomena	The study drug may be discontinued.
	Fever, flu-like syndrome (chills, rigors, myalgias), fatigue, headache,	This research is being conducted at an experienced clinical research center. Reporting and monitoring mechanisms are in

Procedure	Risks	Measures to Minimize Risks
	abnormal labs including blood urea nitrogen (BUN) and albumin	place for AEs, SAEs and unanticipated problems.
Collection of blood samples	Pain, bruising at the injection site and rarely infection Discovery of previously unknown conditions Possible breach of confidentiality	Experienced personnel will perform the phlebotomies using approved techniques. Pressure and dressings will be used to minimize pain, bruising and infection. Research records are kept in a locked area accessible only by study personnel. Subject study numbers will be used for identification of samples so that they may be retained for future research and confidentiality is ensured.
Serum pregnancy testing	Discovery of previously unknown conditions Possible breach of confidentiality	Research records are kept in a locked area accessible only by study personnel. Subjects will only be identified by study numbers on all research documents.
Serum for immunologic evaluation	Discovery of previously unknown conditions Possible breach of confidentiality	Research records are kept in a locked area accessible only by study personnel. Subjects will only be identified by study numbers on all research documents.
Skin test and DTH assay – performed at various locations on subjects' backs	Pain, bruising at the injection site, and rarely infection Potential for allergic reaction including anaphylaxis	Experienced personnel will perform the injections using approved techniques. Pressure and dressings will be used to minimize pain, bruising and infection. Emergency equipment including crash carts, ACLS certified staff and rescue medications such as Benadryl, epinephrine, high dose steroids, etc. will be on-site during administration. This research is being conducted at an experienced clinical research center.

Procedure	Risks	Measures to Minimize Risks
	Discovery of previously unknown conditions	Reporting and monitoring mechanisms are in AEs, SAEs and unanticipated problems. Research records are kept in a locked area accessible only by study personnel. Subjects will only be identified by study numbers on all research documents.
Collection of data	Possible breach of confidentiality	Research records are kept in a locked area accessible only by study personnel. Subjects will only be identified by study numbers on all research documents. Investigators will provide certification of completion of human subjects protection training course. UAMS shall retain the records and reports for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the Food and Drug Administration (FDA) has been so notified. After such time all study records will be destroyed as well as the links between identifiers of the research subjects and their research study numbers according to UAMS' record destruction policy (**APPENDIX G**).

Subjects will receive the 5 planned vaccine doses unless they withdraw from the study or develop Grade 3 toxicity of any type, at which time they will discontinue the injections. There will be no dose modifications for toxicity. Special attention will be given to toxicities mediated by autoimmune mechanisms, such as colitis, thyroiditis or systemic lupus erythematosus (SLE), as well as to injection-site local reactions or allergic reactions. The NCI CTCAE Version 3.0 will be used for toxicity and SAE reporting. A copy of the CTCAE Version 3.0 can be downloaded from the Cancer Therapy Evaluation Program (CTEP) home page (http://ctep.cancer.gov/reporting/ctc.html or viewed in **APPENDIX B**. All appropriate treatment areas have access to a copy of the CTCAE Version 3.0.

Any subject may voluntarily revoke consent and withdraw from the study at any time. A subject may be terminated early for the following conditions: (i) non-compliance, (ii) an unrelated intercurrent illness that may affect assessment or place the subject at risk for AEs

or require systemic steroids, (iii) deterioration in performance status so as to make participation a hardship for the subject, or (iv) for any reason that the investigator feels it is not in the subject's best interest to continue.

b. Benefits: As this is the first time Mimotope P10s-PADRE will be administered to humans, there are no clearly defined benefits to subjects of this study; however, this vaccine may potentiate an immune response which could improve median progression-free survival and overall survival of cancer patients.

8. STUDY CALENDAR

TEST/EVENT	WEEK [6]																
	Prestudy[1]	48 hrs later	1	2	3	4	5	48 hrs later	6	7	8	9	48 hrs later	19	20	21	48 hrs later
Vaccination with Mimotope P10s-PADRE/QS-21			x	x	x					x				x			
Adverse Events			x	x	x					x		x		x		x	
Concomitant medications [7]	x		x	x	x					x		x		x		x	
History/Physical Exam/	x		x	x	x					x		x		x		x	
CBC with Differential	x		x	x	x	x	x		x	x	x	x		x	x	x	
SGOT	x				x					x		x		x		x	
Alkaline Phosphatase	x				x					x		x		x		x	
LDH (lactate dehydrogenase)	x				x					x		x		x		x	
GGT (gamma-glutamyl transferase test)	x				x					x		x		x		x	
Creatinine	x				x					x		x		x		x	
Calcium	x				x					x		x		x		x	
Albumin	x				x					x		x		x		x	
Amylase	x				x					x		x		x		x	
TSH (thyroid stimulating hormone)	x				x					x		x		x		x	
T_4	x				x					x		x		x		x	
Anti-nuclear Antibody (ANA)	x				x					x		x		x		x	
PT/PTT	x				x					x		x		x		x	
Serum Pregnancy Test[2]	x																
Study Lab[3]	x			x	x	x				x		x		x		x	
DTH Skin Test[4,5]	x[4]						x[5]					x[5]				x[5]	
Read DTH Skin Test[4,5]		x[4]						x[5]					x[5]				x[5]

[1]Prestudy is to be completed within 14 days of registration.
[2]For women of child bearing potential, a serum pregnancy test must be done at prestudy and within 48 hours prior to dosing. One test may suffice for both.
[3]10 mL serum samples will be collected in red top tubes for TACA assessment and effector assays. Specimens will be picked up by Dr. Kieber-Emmons or a member of his research staff. Call 526-5930 for pick-up.
[4]The DTH skin test at prestudy will test the subjects' immunocompetency. Control antigens,Tetanus-Diptheria Toxid Antigen and Candida antigen, will be administered id and the resulting induration will be ready at 48 - 72 hours post injection.
[5] The DTH skin tests on weeks 5, 9 and 21 will test the DTH response to Mimotope P10s-PADRE. Tetanus-Diphtheria Toxoid Antigen and Candida antigen will be administered id alongside Mimotope P10s-PADRE as control antigens to assess immunocompetency. DTH skin test indurations will be read at 48 hours post injection.
[6] Visits must occur at set time points mentioned in the study calendar (+/- 3 days) with the exception of reading the skin test with must occur 48-72 post administration
[7] Concomitant medications will be collected from registration to end of treatment.

9. CRITERIA FOR EVALUATION

a. Determination of DLT: See Section 7, "Risks and Toxicities to be Monitored". Any Grade 3 toxicity will be dose limiting.

b. Immunological Evaluation: Serum will be collected at the Study Lab time points indicated in the study calendar in Section 8, flash-frozen in aliquots and stored at -80ºC. IgM and IgG titers to TACAs will be evaluated by ELISA and FACS analysis. A positive TACA-directed immune response will be defined as an anti-TACA serum antibody titer of 1:40 for a baseline pre-vaccination titer of 0 or a $\geq$ 4-fold increase over baseline titer > 0 (1, 11). Subjects will be judged to have had an adequate immune response if they have a positive TACA-directed immune response at any one of the first five designated time points following vaccine administration (Weeks 2, 3, 4, 7 and 9). The value of a booster immunization will be determined by anti-TACA IgM and IgG titers obtained from study labs collected on week 21.

c. Determination of DTH Responses: To evaluate DTH responses, study subjects will be skin-tested according to the time points in the study calendar against the Mimotope P10s-PADRE, Tetanus-Diphtheria Toxoid Antigen and Candida antigen. The latter 2 antigens serve as control antigens. DTH responses to the control antigens will be assessed at pre-study to determine immunocompetency of study subjects. All antigens will be administered id at separate locations on the subject's back. Induration will be measured using calipers and reported in mm across two diameters at 48 hours post injection. An induration of > 5 mm will be considered positive.

10. STATISTICAL CONSIDERATIONS

a. Sample Size, Study Duration and General Considerations: Because each subject will receive multiple vaccine injections at a constant dose over an extended period of time, the second and third subject of *the same* dose cohort will be enrolled, as available, before the first subject has finished all scheduled injections. However, if two or more subjects are enrolled on the same day, then their injection schedules will be staggered at least one day

apart. The decision to escalate or de-escalate the dose, expand the cohort, or terminate the study will be based on assessment for DLTs, which will require 9 weeks per subject. The time to assess a cohort of 3 for DLTs is thus anticipated to be 13 weeks based on an accrual rate of 2 eligible Stage IV subjects per month. Assuming no DLTs occur in the initial and final cohorts, this study will require a minimum of 6 or a maximum of 12 subjects. Subjects who withdraw from the dose-escalation study without a DLT will be replaced. Subjects who withdraw with a DLT will be considered evaluable for MTD determination. Given the recruitment and immunization/evaluation schedule, we expect this study to be completed in a minimum of 6 and a maximum of 12 months.

It should be noted that the primary objective of this study is to ensure the safety of the mimotope vaccine. Because of sample size, these studies only provide a qualitative assessment of vaccine immunogenicity. However, our immunization schedule should favor the generation of antibodies (1, 11, 35). A positive TACA-directed immune response will be defined as an anti-TACA serum antibody titer of 1:40 for a baseline pre-vaccination titer of 0 or a $\geq$ 4-fold increase for a baseline titer > 0 (1, 11). A subject will be judged to have had an adequate immune response if they have a positive TACA-directed immune response at any one of the first five indicated time points following vaccine administration on the study calendar (Section 8, Study Lab Weeks 2, 3, 4, 7 and 9). Upon evaluation of all subjects in a cohort (3-6/dose), dose escalation will proceed according to the schedule shown in **Tables 1, 2, and 3**. Except for the fact that they allow for possible de-escalation from the initial dose of 300 μg, **Tables 1, 2, and 3** constitute the toxicity-based "traditional" design of Storer (36). A secondary endpoint will be the DTH response to Mimotope P10s-PADRE.

b. Data Analysis Plan: Toxicity will be graded according to the NCI CTCAE Version 3.0. All observed toxicities will be enumerated terms of type (organ affected or laboratory determination such as absolute neutrophil count), severity (by NCI Common Toxicity Criteria (CTC) and nadir or maximum values for the laboratory measures), time of onset (i.e. dose number), duration and reversibility or outcome. A toxicity of Grade 3 or higher will be scored as a DLT if it is deemed to be related to the vaccine or any of its components. TACA-directed immune titers will be tabulated for each subject at baseline and at indicated time points on the study calendar. Immune titers will be compared to baseline values and scored as TACA-directed immune responses (positive or negative). The components of the DTH response, namely, the induration in mm across two diameters, will be tabulated by antigen (mimotope vs. control) for each subject at the indicated time points on the study calendar. An induration of > 5 mm will be considered positive.

To meet the secondary objective of determining the humoral response against TACAs, the anti-TACA serum titers measured from subjects' blood samples will be collected at pre-study and on weeks 2, 3, 4, 7, and 9. IgM and IgG titers to TACAs will be evaluated by ELISA and FACS analysis. Titer will be defined as the highest serum dilution yielding an $OD_{405} \geq 0.15$, in accordance with previous studies (1) or an MFI two standard deviations higher than background. A positive TACA-directed immune response will be defined as an anti-TACA serum antibody titer of 1:40 for a baseline pre-vaccination titer of 0 or a $\geq$ 4-fold increase for a baseline titer > 0. The number and proportion of positive TACA-directed immune responses at each time point will be reported. Medians and quartiles of titer will also be

reported at each time point for any dose cohort of size 6.

To meet the secondary objective of analyzing the DTH response, the difference in longer induration diameters, the difference in shorter induration diameters and the difference in diameter products (longer x shorter) will be calculated at each time point and plotted via scatter plot against dose. Medians and quartiles will also be reported for any dose cohort of size 6.

To meet the secondary objective of determining sustainability of the immune response, the subject's TACA-directed immune titer at Week 19 will be compared to her TACA-directed immune titer at Week 9. If the week 19 titer is more than four-fold less than the week 9 titer, or if the week 19 titer is less than 1:10 relative to the week 9 titer, then the subject's immune response will be considered as not sustained; otherwise, it will be considered a sustained immune response. The number and percentage of subjects with a sustained immune response will be reported in the aggregate and by dose cohort if more than one dose cohort is enrolled. In addition, the ratio of week 19 and week 9 titers will be plotted as dot plots and summarized as the mean, median and range.

To meet the secondary objective of determining the immune response to the week 19 booster immunization, the subject's TACA-directed immune titer at Week 21 will be expressed as a ratio relative to her TACA-directed immune titer at Week 19. This ratio will be summarized as the mean, median and range, and plotted as dot plots. Subjects in whom the week 21 titer is more than 2-fold higher than the week 19 titer will be considered as having shown a boosted response to the booster immunization; the number and percentage of subjects showing a boosted response will be reported in the aggregate and by dose cohort if more than one cohort is enrolled.

Any deviations from the above analysis plan will be reported to the FDA as part of the investigational new drug (IND) application, along with the reason for the deviation. Inasmuch as the above analysis plan is central to supporting the IND application, any deviation would consist of an addition to the existing plan, not a modification of it.

c. Missing, Unused and Spurious Data: Missing data will be treated as missing, and will not be imputed. All data collected will necessarily be reported to the FDA as part of the IND application, so there will be no unused data. Spurious data will be corrected at the source document according the following procedure: a single straight line will be drawn through each spurious datum, and then the correct value will be written in next to it and initialed by the investigator who made the correction. Any data documented as spurious that is unable to be corrected at the source will be treated as missing.

11. REGISTRATION GUIDELINES

Screening logs will be maintained by the study nurses. Subject registration will occur after the IRB-approved consent is signed and eligibility has been confirmed. The subjects will be

registered in the CRDM office and assigned a study number by the CRA. The study number will be used for identification of the research subject during the study.

12. DATA SUBMISSION SCHEDULE

Data must be submitted according to protocol requirements for ALL subjects registered, whether or not assigned treatment is administered. This includes subjects deemed to be ineligible to participate in the study or for whom documentation is inadequate to determine eligibility. Data obtained during the study will be collected within 14 days of each subject visit and entered into the protocol database within 14 days of collection. Subjects will be registered in C3PR, a cancer Biomedical Informatics Grid (*caBIG*®, NCI) application. Data will be entered into OpenClinica through electronic web-based case report forms (CRFs) which replicate the paper CRFs attached to this protocol. OpenClinica is a secure open source system for electronic data capture and clinical data management.

UAMS shall retain the records and reports for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. After such time all study records will be destroyed as well as the links between identifiers of the research subjects and their research study numbers according to the UAMS record destruction policy (**APPENDIX G).**

FORM SCHEDULE

Forms	Visits																	
	Prestudy	48 hrs later	W1	W2	W3	W4	W5	48 hrs later	W6	W7	W8	W9	48 hrs later	W19	W20	W21	48 hrs later	Post-treatment
Administration (A)			A	A	A					A				A				
Adverse Event (AE)	AE		AE	AE	AE	AE	AE		AE	AE	AE	AE		AE	AE	AE		
Biological Markers (BM)	BM		BM	BM	BM	BM	BM		BM	BM	BM	BM		BM	BM	BM		
Concomitant Medications (CM)	CM		CM	CM	CM	CM	CM		CM	CM	CM	CM		CM	CM	CM		
Demographics (D)	D																	
Disease History (DH)	DH																	
Eligibility Checklist (EC)	EC																	
End of Treatment (ET)			ET	ET	ET	ET	ET		ET	ET	ET	ET		ET	ET	ET		
Medical History (MH)	MH																	
Physical Exam (PE)	PE		PE	PE	PE					PE		PE		PE		PE		
Prior Therapy (PT)	PT																	
Radiology (XR)	XR																	

Skin test (ST)	ST	ST					ST	ST				ST	ST			ST	ST	
Survival (S)																		S
Vital Signs (VS)	VS		VS	VS	VS	VS	VS		VS	VS	VS	VS		VS	VS	VS		

13. SPECIAL INSTRUCTIONS

Blood samples for research labwork will be collected in the clinic in 10 mL red-top tubes and transported to the tissue bank specimen processing area. These specimens will be picked up by Dr. Kieber-Emmons or a member of his research staff. Call 501-526-5930 for pick-up.

14. ETHICAL AND REGULATORY CONSIDERATIONS

The following must be observed to comply with FDA regulations for the conduct and monitoring of clinical investigations. The following also represents sound research practice:

All study personnel must have completed training in good clinical practice (GCP) and protection of human subjects.

a. Recruitment and Informed Consent: Research subjects will be recruited from the breast cancer clinics (Medical Oncology and Ladies Oncology Clinics) at the Winthrop P. Rockefeller Cancer Institute on the UAMS campus. The research subjects will be identified by preview of the clinics' schedules for Dr. Hutchins and Dr. Makhoul by the research nurse. Prior to any research activities, the research subject will be approached for participation in the study by her physician, who will discuss the protocol along with the risks and potential benefits of participating in it. A clear statement will be made concerning the voluntary nature of her participation and that her decision will have no effect on her remaining care. The research nurse will follow with a detailed review of the informed consent document. The research subject will be encouraged to have family or friends participate in any or all of the process. The research subject will be given time to ask questions, will be questioned to be certain she understands the information, and if she agrees to proceed, will sign consent. In general, registration and prestudy work will begin the next business day, allowing additional time for the research subject to reflect and request additional questions or withdraw. The consent process will be documented in the medical record. A copy of the informed consent document will be given to the research subject, and additional copies will be sent to the medical records department for distribution to the research pharmacy. The original informed consent will be filed with the regulatory documents in CRDM. The consent process will occur in a private exam room or in the private office of the research nurse. There will be no additional recruitment materials. The principles of informed consent are described by Federal Regulatory Guidelines (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). These principles must be followed to comply with FDA regulations for the conduct and monitoring of clinical investigations.

b. Institutional Review: This study will be approved by the UAMS IRB as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). This study will also undergo scientific review by the Cancer Institute's Protocol Review and Monitoring Committee (PRMC). Approval by both the IRB and PRMC is required before the clinical trial can be activated.

c. Investigational Agent Accountability: For each investigational drug, drug disposition (drug receipt, dispensing, transfer or return) will be maintained on the UAMS Investigational Agent Accountability Record (**APPENDIX F)**. Drug supplies will be kept in a secure, limited access storage area under the recommended storage conditions in the research pharmacy in the Winthrop P. Rockefeller Cancer Institute under the direction of the research pharmacist. During the course of the study, the following information will be noted on the Investigational Agent Accountability Record; the study number, the research subject's initials, the research subject's assigned number, the dose of drug, the date(s) and quantity of drug dispensed to the subject, the balance forward, the lot number and the recorder's initials. These Investigational Agent Accountability Records will be readily available for inspection and are open to FDA inspection at any time.

15. ADVERSE EVENTS

See full policy **APPENDIX H**.

a. Adverse Event (AE): Any unfavorable and unintended sign, symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure. Each AE is a unique representation of a specific event used for medical documentation and scientific analysis. *[ICH E6 1.2]*

b. Serious Adverse Event (SAE): Any adverse drug experience occurring at any dose that results in any of the following outcomes: death, a life-threatening adverse drug experience, inpatient hospitalization, prolongation of existing hospitalization, a persistent or significant disability/incapacity or a congenital anomaly/birth defect. Important medical events that may not result in death, be life threatening or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. FDA requires IND sponsors to report SAEs through the expedited reporting system. *[21CFR312.32 (a) ICH E6 1.50 Partially IRB Handbook policy 10.3]*

Any adverse experience that meets reporting guidelines for SAEs must be reported to the CRA for the study in the CRDM office within 24 hours of knowledge of the event. The CRA will follow the AE monitoring plan (**APPENDIX H**). All SAEs will be reported to the Principal Investigator (PI), Co-PI, Department of Defense (DOD), FDA, IRB and Medical Monitor according to this plan.

All AEs and SAEs must be reported to the IRB within 10 days. All AEs and SAEs must also be recorded in the appropriate section of the CRF. The report should include, whenever possible, the investigator's written medical judgment as to the relationship of the AE/SAE to study medications(s) (i.e., "probable", "possible" or "unrelated").

16. MONITORING

a. Medical Monitor: The Medical Monitor is Dr. Joe Beck. His CV and the UAMS Medical Monitor standard operating procedure (SOP) can be found in **APPENDICES I and J**.

b. Data Monitor: UAMS is the IND Sponsor. One (or more) Data Monitor(s) will be appointed by the monitoring division of the UAMS Research Support Center (RSC) to assure that the rights and well-being of human subjects are protected, that the data are accurate, complete and verifiable from source documents and that the trial is conducted in compliance with currently approved protocol/amendments, with GCP, and with the applicable regulatory requirements set forth in *21 CFR 312*.

The Data Monitor(s) will be familiar with the investigational products, the protocol, the informed consent form, any other information provided to the subjects, SOPs, GCP and applicable regulatory requirements.

Data Monitor(s) will have access to research subjects' medical records and other study-related records. The investigator agrees to cooperate with the Data Monitor(s) and Medical Monitor to ensure that any problems detected in the course of these monitoring visits are resolved. Personal contact between the Data Monitor, Medical Monitor and the investigator will be maintained throughout the clinical trial to assure that the investigator is fulfilling his/her obligations and that the facilities used in the clinical trial remain acceptable.

1) **Pre-investigation Site Visit**: A pre-investigation site visit will be performed by the Data Monitor in order to inspect the facility where the study is going to be conducted, and to assure that the investigator and his/her staff understand the protocol and agree to comply with the current regulations for clinical trial conduct in human subjects (*21 CFR 312, 21 CFR 50, 21 CFR 56, 21 CFR 11, 21 CFR 21*). The Data Monitor will document the IRB approval and generate a special report that will allow subject enrollment on the trial to begin.

2) **Periodic Site Visits:** The first visit of the Data Monitor will occur after the first research subject has completed her treatment as specified by the protocol. Subsequent monitoring visits will take place after enrolling each additional cohort or at intervals no longer than every 8 weeks. The Data Monitor will review the CRFs, source data/documents and other trial-related records for accuracy, consistency and completeness. Enrollment of research subjects after meeting eligibility criteria and signing a consent form will be documented. Missing visits, withdrawals and subject recruitment rate will be monitored.

3) **Investigational Products**: The Data Monitor will verify that the storage conditions are appropriate and that the investigational drug is being dispensed to eligible subjects according to the study protocol. The Data Monitor will verify that there are accurate records of the receipt, use and return of the investigational product.

4) **Monitoring Report**: After each monitoring visit (no more than 8 weeks apart) a separate monitoring report will be generated and submitted to the investigator and Medical Monitor. This report will include significant findings related to deficiencies and deviations from the protocol, SOPs, GCP and the applicable regulatory requirements and actions taken to prevent recurrence of the detected deviations. The report will make recommendations for actions to be taken to secure compliance. The study team, which includes the PI, Co-PIs, biostatistician, Medical Monitor and CRA, will meet after each cohort completes the protocol to review AEs and review monitoring reports in order to make adjustments necessary to protect the research subjects and the integrity of the trial.

5) **Research Subjects and Data Safety**: If 3 SAEs occur with attribution to the study drug, the trial will be suspended until further review is completed by the Medical Monitor, PI, sponsor and FDA. This will be accomplished by the study team, the PI, Co-PI, biostatistician, Medical Monitor and CRA, either at the regular meeting or a special meeting called by the Medical Monitor or the PI because of the SAEs.

6) **Audits**: An audit by the UAMS RSC will be scheduled after the completion of the first cohort. The audit will follow RSC's standard auditing procedure.

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APPENDIX

APPENDIX A - ABBREVIATIONS

ACLS – advanced cardiac life support

AE – adverse event

AJCC – American Joint Commission on Cancer

ANA – anti-nuclear antibody

AST – aspartate aminotransferase test

BUN – blood urea nitrogen

CBC – complete blood count

CDC – complement dependent cytotoxicity

CMPs – carbohydrate mimetic peptids

Co-PI – co-principal investigator/co-investigator

CRDM- Clinical Research Data Management

CRA – Clinical Research Associate

CRF – case report form

CTC – common toxicity criteria

CTCAE – Common Terminology Criteria for Adverse Events

CTEP – Cancer Therapy Evaluation Program

CTL – cytotoxic T lymphocyte

dl – deciliter

DLT – dose limiting toxicity

DOD – Department of Defense

DTH – delayed type hypersensitivity

ELISA – enzyme-linked immunosorbent assay

FACS – fluorescence activated cell sorting

FDA – food and drug administration

GCP – good clinical practice

GGT – gamma-glutamyl transferase test

GMP – good manufacturing practice

id – intradermally

IgG – immunoglobulin g

IgM – immunoglobulin m
IND - investigational new drug
IRB - institutional review board
IUL – institutional upper limit
kD – kilodalton
KLH – keyhole limpet hemocyanin
LDH – lactate dehydrogenase
LeY – Lewis Y antigen
MFI – mean fluorescence intensity
mg – milligram
mL – milliliter
mm – millimeter
MTD – maximum tolerated dose
µg – microgram
NCI – National Cancer Institute
NK – natural killer
PBS – phosphate buffered saline
PI – prinicipal investigator
PRMC – protocol review and monitoring committee
PT – prothrombin time
PTT – partial thromboplastin time
RSC – UAMS Research Support Center
SAE – serious adverse event
SC - subcutaneous
SGOT – serum glutamic-oxaloacetic transaminase
SLE – systemic lupus erythematosus
SOP – standard operating procedure
STn antigen – sialosyl Tn antigen
TACAs - tumor associated carbohydrate antigens
TSH – thyroid stimulating hormone
UAMS - University of Arkansas for Medical Sciences
WBC – white blood cell

APPENDIX B - NCI COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS (CTCAE) VERSION 3.0

ALLERGY/IMMUNOLOGY						Page 1 of 1
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Allergic reaction/ hypersensitivity (including drug fever)	Allergic reaction	Transient flushing or rash; drug fever <38°C (<100.4°F)	Rash; flushing; urticaria; dyspnea; drug fever ≥38°C (≥100.4°F)	Symptomatic bronchospasm, with or without urticaria; parenteral medication(s) indicated; allergy-related edema/angioedema; hypotension	Anaphylaxis	Death
REMARK: Urticaria with manifestations of allergic or hypersensitivity reaction is graded as Allergic reaction/hypersensitivity (including drug fever).						
ALSO CONSIDER: Cytokine release syndrome/acute infusion reaction.						
Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)	Rhinitis	Mild, intervention not indicated	Moderate, intervention indicated	—	—	—
REMARK: Rhinitis associated with obstruction or stenosis is graded as Obstruction/stenosis of airway – *Select* in the PULMONARY/UPPER RESPIRATORY CATEGORY.						
Autoimmune reaction	Autoimmune reaction	Asymptomatic and serologic or other evidence of autoimmune reaction, with normal organ function and intervention not indicated	Evidence of autoimmune reaction involving a non-essential organ or function (e.g., hypothyroidism)	Reversible autoimmune reaction involving function of a major organ or other adverse event (e.g., transient colitis or anemia)	Autoimmune reaction with life-threatening consequences	Death
ALSO CONSIDER: Colitis; Hemoglobin; Hemolysis (e.g., immune hemolytic anemia, drug-related hemolysis); Thyroid function, low (hypothyroidism).						
Serum sickness	Serum sickness	—	—	Present	—	Death
NAVIGATION NOTE: Splenic function is graded in the BLOOD/BONE MARROW CATEGORY.						
NAVIGATION NOTE: Urticaria as an isolated symptom is graded as Urticaria (hives, welts, wheals) in the DERMATOLOGY/SKIN CATEGORY.						
Vasculitis	Vasculitis	Mild, intervention not indicated	Symptomatic, non-steroidal medical intervention indicated	Steroids indicated	Ischemic changes; amputation indicated	Death
Allergy/Immunology – Other (Specify, __)	Allergy – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

AUDITORY/EAR

Page 1 of 2

		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
NAVIGATION NOTE: Earache (otalgia) is graded as Pain – *Select* in the PAIN CATEGORY.						
Hearing: patients with/without baseline audiogram and enrolled in a monitoring program[1]	Hearing (monitoring program)	Threshold shift or loss of 15 – 25 dB relative to baseline, averaged at 2 or more contiguous test frequencies in at least one ear; or subjective change in the absence of a Grade 1 threshold shift	Threshold shift or loss of >25 – 90 dB, averaged at 2 contiguous test frequencies in at least one ear	Adult only: Threshold shift of >25 – 90 dB, averaged at 3 contiguous test frequencies in at least one ear Pediatric: Hearing loss sufficient to indicate therapeutic intervention, including hearing aids (e.g., ≥20 dB bilateral HL in the speech frequencies; ≥30 dB unilateral HL; and requiring additional speech-language related services)	Adult only: Profound bilateral hearing loss (>90 dB) Pediatric: Audiologic indication for cochlear implant and requiring additional speech-language related services	—
REMARK: Pediatric recommendations are identical to those for adults, unless specified. For children and adolescents (≤18 years of age) without a baseline test, pre-exposure/pre-treatment hearing should be considered to be <5 dB loss.						
Hearing: patients without baseline audiogram and not enrolled in a monitoring program[1]	Hearing (without monitoring program)	—	Hearing loss not requiring hearing aid or intervention (i.e., not interfering with ADL)	Hearing loss requiring hearing aid or intervention (i.e., interfering with ADL)	Profound bilateral hearing loss (>90 dB)	—
REMARK: Pediatric recommendations are identical to those for adults, unless specified. For children and adolescents (≤18 years of age) without a baseline test, pre-exposure/pre-treatment hearing should be considered to be <5 dB loss.						
Otitis, external ear (non-infectious)	Otitis, external	External otitis with erythema or dry desquamation	External otitis with moist desquamation, edema, enhanced cerumen or discharge; tympanic membrane perforation; tympanostomy	External otitis with mastoiditis; stenosis or osteomyelitis	Necrosis of soft tissue or bone	Death
ALSO CONSIDER: Hearing: patients with/without baseline audiogram and enrolled in a monitoring program[1]; Hearing: patients without baseline audiogram and not enrolled in a monitoring program[1].						
Otitis, middle ear (non-infectious)	Otitis, middle	Serous otitis	Serous otitis, medical intervention indicated	Otitis with discharge; mastoiditis	Necrosis of the canal soft tissue or bone	Death

AUDITORY/EAR

Page 2 of 2

		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Tinnitus	Tinnitus	—	Tinnitus not interfering with ADL	Tinnitus interfering with ADL	Disabling	—
ALSO CONSIDER: Hearing: patients with/without baseline audiogram and enrolled in a monitoring program[1]; Hearing: patients without baseline audiogram and not enrolled in a monitoring program[1].						
Auditory/Ear – Other (Specify, __)	Auditory/Ear – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

[1] Drug-induced ototoxicity should be distinguished from age-related threshold decrements or unrelated cochlear insult. When considering whether an adverse event has occurred, it is first necessary to classify the patient into one of two groups. (1) The patient is under standard treatment/enrolled in a clinical trial <2.5 years, and has a 15 dB or greater threshold shift averaged across two contiguous frequencies; or (2) The patient is under standard treatment/enrolled in a clinical trial >2.5 years, and the difference between the expected age-related and the observed threshold shifts is 15 dB or greater averaged across two contiguous frequencies. Consult standard references for appropriate age- and gender-specific hearing norms, e.g., Morrell, et al. Age- and gender-specific reference ranges for hearing level and longitudinal changes in hearing level. Journal of the Acoustical Society of America 100:1949-1967, 1996; or Shotland, et al. Recommendations for cancer prevention trials using potentially ototoxic test agents. Journal of Clinical Oncology 19:1658-1663, 2001.

In the absence of a baseline prior to initial treatment, subsequent audiograms should be referenced to an appropriate database of normals. ANSI. (1996)

American National Standard: Determination of occupational noise exposure and estimation of noise-induced hearing impairment, ANSI S 3.44-1996. (Standard S 3.44). New York: American National Standards Institute. The recommended ANSI S3.44 database is Annex B.

BLOOD/BONE MARROW						Page 1 of 1
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Bone marrow cellularity	Bone marrow cellularity	Mildly hypocellular or ≤25% reduction from normal cellularity for age	Moderately hypocellular or >25 – ≤50% reduction from normal cellularity for age	Severely hypocellular or >50 – ≤75% reduction cellularity from normal for age	—	Death
CD4 count	CD4 count	<LLN – 500/mm^3 <LLN – 0.5 x 10^9 /L	<500 – 200/mm^3 <0.5 – 0.2 x 10^9 /L	<200 – 50/mm^3 <0.2 x 0.05 – 10^9 /L	<50/mm^3 <0.05 x 10^9 /L	Death
Haptoglobin	Haptoglobin	<LLN	—	Absent	—	Death
Hemoglobin	Hemoglobin	<LLN – 10.0 g/dL <LLN – 6.2 mmol/L <LLN – 100 g/L	<10.0 – 8.0 g/dL <6.2 – 4.9 mmol/L <100 – 80g/L	<8.0 – 6.5 g/dL <4.9 – 4.0 mmol/L <80 – 65 g/L	<6.5 g/dL <4.0 mmol/L <65 g/L	Death
Hemolysis (e.g., immune hemolytic anemia, drug-related hemolysis)	Hemolysis	Laboratory evidence of hemolysis only (e.g., direct antiglobulin test [DAT, Coombs'] schistocytes)	Evidence of red cell destruction and ≥2 gm decrease in hemoglobin, no transfusion	Transfusion or medical intervention (e.g., steroids) indicated	Catastrophic consequences of hemolysis (e.g., renal failure, hypotension, bronchospasm, emergency splenectomy)	Death
ALSO CONSIDER: Haptoglobin; Hemoglobin.						
Iron overload	Iron overload	—	Asymptomatic iron overload, intervention not indicated	Iron overload, intervention indicated	Organ impairment (e.g., endocrinopathy, cardiopathy)	Death
Leukocytes (total WBC)	Leukocytes	<LLN – 3000/mm^3 <LLN – 3.0 x 10^9 /L	<3000 – 2000/mm^3 <3.0 – 2.0 x 10^9 /L	<2000 – 1000/mm^3 <2.0 – 1.0 x 10^9 /L	<1000/mm^3 <1.0 x 10^9 /L	Death
Lymphopenia	Lymphopenia	<LLN – 800/mm^3 <LLN x 0.8 – 10^9 /L	<800 – 500/mm^3 <0.8 – 0.5 x 10^9 /L	<500 – 200 mm^3 <0.5 – 0.2 x 10^9 /L	<200/mm^3 <0.2 x 10^9 /L	Death
Myelodysplasia	Myelodysplasia	—	—	Abnormal marrow cytogenetics (marrow blasts ≤5%)	RAEB or RAEB-T (marrow blasts >5%)	Death
Neutrophils/granulocytes (ANC/AGC)	Neutrophils	<LLN – 1500/mm^3 <LLN – 1.5 x 10^9 /L	<1500 – 1000/mm^3 <1.5 – 1.0 x 10^9 /L	<1000 – 500/mm^3 <1.0 – 0.5 x 10^9 /L	<500/mm^3 <0.5 x 10^9 /L	Death
Platelets	Platelets	<LLN – 75,000/mm^3 <LLN – 75.0 x 10^9 /L	<75,000 – 50,000/mm^3 <75.0 – 50.0 x 10^9 /L	<50,000 – 25,000/mm^3 <50.0 – 25.0 x 10^9 /L	<25,000/mm^3 <25.0 x 10^9 /L	Death
Splenic function	Splenic function	Incidental findings (e.g., Howell-Jolly bodies)	Prophylactic antibiotics indicated	—	Life-threatening consequences	Death
Blood/Bone Marrow – Other (Specify, __)	Blood – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

CARDIAC ARRHYTHMIA

Page 1 of 2

		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Conduction abnormality/ atrioventricular heart block – *Select:* – Asystole – AV Block-First degree – AV Block-Second degree Mobitz Type I (Wenckebach) – AV Block-Second degree Mobitz Type II – AV Block-Third degree (Complete AV block) – Conduction abnormality NOS – Sick Sinus Syndrome – Stokes-Adams Syndrome – Wolff-Parkinson-White Syndrome	Conduction abnormality – *Select*	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Incompletely controlled medically or controlled with device (e.g., pacemaker)	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)	Death
Palpitations	Palpitations	Present	Present with associated symptoms (e.g., lightheadedness, shortness of breath)	—	—	—
REMARK: Grade palpitations only in the absence of a documented arrhythmia.						
Prolonged QTc interval	Prolonged QTc	QTc >0.45 – 0.47 second	QTc >0.47 – 0.50 second; ≥0.06 second above baseline	QTc >0.50 second	QTc >0.50 second; life-threatening signs or symptoms (e.g., arrhythmia, CHF, hypotension, shock syncope); Torsade de pointes	Death
Supraventricular and nodal arrhythmia – *Select:* – Atrial fibrillation – Atrial flutter – Atrial tachycardia/Paroxysmal Atrial Tachycardia – Nodal/Junctional – Sinus arrhythmia – Sinus bradycardia – Sinus tachycardia – Supraventricular arrhythmia NOS – Supraventricular extrasystoles (Premature Atrial Contractions; Premature Nodal/Junctional Contractions) – Supraventricular tachycardia	Supraventricular arrhythmia – *Select*	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Symptomatic and incompletely controlled medically, or controlled with device (e.g., pacemaker)	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)	Death
NAVIGATION NOTE: Syncope is graded as Syncope (fainting) in the NEUROLOGY CATEGORY.						

CARDIAC ARRHYTHMIA

Page 2 of 2

		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Vasovagal episode	Vasovagal episode	—	Present without loss of consciousness	Present with loss of consciousness	Life-threatening consequences	Death
Ventricular arrhythmia – *Select:* – Bigeminy – Idioventricular rhythm – PVCs – Torsade de pointes – Trigeminy – Ventricular arrhythmia NOS – Ventricular fibrillation – Ventricular flutter – Ventricular tachycardia	Ventricular arrhythmia – *Select*	Asymptomatic, no intervention indicated	Non-urgent medical intervention indicated	Symptomatic and incompletely controlled medically or controlled with device (e.g., defibrillator)	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)	Death
Cardiac Arrhythmia – Other (Specify, __)	Cardiac Arrhythmia – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

CARDIAC GENERAL						Page 1 of 3
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
NAVIGATION NOTE: Angina is graded as Cardiac ischemia/infarction in the CARDIAC GENERAL CATEGORY.						
Cardiac ischemia/infarction	Cardiac ischemia/infarction	Asymptomatic arterial narrowing without ischemia	Asymptomatic and testing suggesting ischemia; stable angina	Symptomatic and testing consistent with ischemia; unstable angina; intervention indicated	Acute myocardial infarction	Death
Cardiac troponin I (cTnI)	cTnI	—	—	Levels consistent with unstable angina as defined by the manufacturer	Levels consistent with myocardial infarction as defined by the manufacturer	Death
Cardiac troponin T (cTnT)	cTnT	0.03 – <0.05 ng/mL	0.05 – <0.1 ng/mL	0.1 – <0.2 ng/mL	0.2 ng/mL	Death
Cardiopulmonary arrest, cause unknown (non-fatal)	Cardiopulmonary arrest	—	—	—	Life-threatening	—
REMARK: Grade 4 (non-fatal) is the only appropriate grade. CTCAE provides three alternatives for reporting Death: 1. A CTCAE term associated with Grade 5. 2. A CTCAE 'Other (Specify, __)' within any CATEGORY. 3. Death not associated with CTCAE term – *Select* in the DEATH CATEGORY.						
NAVIGATION NOTE: Chest pain (non-cardiac and non-pleuritic) is graded as Pain – *Select* in the PAIN CATEGORY.						
NAVIGATION NOTE: CNS ischemia is graded as CNS cerebrovascular ischemia in the NEUROLOGY CATEGORY.						
Hypertension	Hypertension	Asymptomatic, transient (<24 hrs) increase by >20 mmHg (diastolic) or to >150/100 if previously WNL; intervention not indicated Pediatric: Asymptomatic, transient (<24 hrs) BP increase >ULN; intervention not indicated	Recurrent or persistent (≥24 hrs) or symptomatic increase by >20 mmHg (diastolic) or to >150/100 if previously WNL; monotherapy may be indicated Pediatric: Recurrent or persistent (≥24 hrs) BP >ULN; monotherapy may be indicated	Requiring more than one drug or more intensive therapy than previously Pediatric: Same as adult	Life-threatening consequences (e.g., hypertensive crisis) Pediatric: Same as adult	Death
REMARK: Use age and gender-appropriate normal values >95th percentile ULN for pediatric patients.						

CARDIAC GENERAL						Page 2 of 3
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Hypotension	Hypotension	Changes, intervention not indicated	Brief (<24 hrs) fluid replacement or other therapy; no physiologic consequences	Sustained (≥24 hrs) therapy, resolves without persisting physiologic consequences	Shock (e.g., acidemia; impairment of vital organ function)	Death
ALSO CONSIDER: Syncope (fainting).						
Left ventricular diastolic dysfunction	Left ventricular diastolic dysfunction	Asymptomatic diagnostic finding; intervention not indicated	Asymptomatic, intervention indicated	Symptomatic CHF responsive to intervention	Refractory CHF, poorly controlled; intervention such as ventricular assist device or heart transplant indicated	Death
Left ventricular systolic dysfunction	Left ventricular systolic dysfunction	Asymptomatic, resting ejection fraction (EF) <60 – 50%; shortening fraction (SF) <30 – 24%	Asymptomatic, resting EF <50 – 40%; SF <24 – 15%	Symptomatic CHF responsive to intervention; EF <40 – 20% SF <15%	Refractory CHF or poorly controlled; EF <20%; intervention such as ventricular assist device, ventricular reduction surgery, or heart transplant indicated	Death
NAVIGATION NOTE: Myocardial infarction is graded as Cardiac ischemia/infarction in the CARDIAC GENERAL CATEGORY.						
Myocarditis	Myocarditis	—	—	CHF responsive to intervention	Severe or refractory CHF	Death
Pericardial effusion (non-malignant)	Pericardial effusion	Asymptomatic effusion	—	Effusion with physiologic consequences	Life-threatening consequences (e.g., tamponade); emergency intervention indicated	Death
Pericarditis	Pericarditis	Asymptomatic, ECG or physical exam (rub) changes consistent with pericarditis	Symptomatic pericarditis (e.g., chest pain)	Pericarditis with physiologic consequences (e.g., pericardial constriction)	Life-threatening consequences; emergency intervention indicated	Death
NAVIGATION NOTE: Pleuritic pain is graded as Pain – *Select* in the PAIN CATEGORY.						
Pulmonary hypertension	Pulmonary hypertension	Asymptomatic without therapy	Asymptomatic, therapy indicated	Symptomatic hypertension, responsive to therapy	Symptomatic hypertension, poorly controlled	Death
Restrictive cardiomyopathy	Restrictive cardiomyopathy	Asymptomatic, therapy not indicated	Asymptomatic, therapy indicated	Symptomatic CHF responsive to intervention	Refractory CHF, poorly controlled; intervention such as ventricular assist device, or heart transplant indicated	Death

CARDIAC GENERAL						Page 3 of 3
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Right ventricular dysfunction (cor pulmonale)	Right ventricular dysfunction	Asymptomatic without therapy	Asymptomatic, therapy indicated	Symptomatic cor pulmonale, responsive to intervention	Symptomatic cor pulmonale poorly controlled; intervention such as ventricular assist device, or heart transplant indicated	Death
Valvular heart disease	Valvular heart disease	Asymptomatic valvular thickening with or without mild valvular regurgitation or stenosis; treatment other than endocarditis prophylaxis not indicated	Asymptomatic; moderate regurgitation or stenosis by imaging	Symptomatic; severe regurgitation or stenosis; symptoms controlled with medical therapy	Life-threatening; disabling; intervention (e.g., valve replacement, valvuloplasty) indicated	Death
Cardiac General – Other (Specify, __)	Cardiac General – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

COAGULATION

Page 1 of 1

Adverse Event	Short Name	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
DIC (disseminated intravascular coagulation)	DIC	—	Laboratory findings with no bleeding	Laboratory findings and bleeding	Laboratory findings, life-threatening or disabling consequences (e.g., CNS hemorrhage, organ damage, or hemodynamically significant blood loss)	Death
REMARK: DIC (disseminated intravascular coagulation) must have increased fibrin split products or D-dimer.						
ALSO CONSIDER: Platelets.						
Fibrinogen	Fibrinogen	<1.0 – 0.75 x LLN or <25% decrease from baseline	<0.75 – 0.5 x LLN or 25 – <50% decrease from baseline	<0.5 – 0.25 x LLN or 50 – <75% decrease from baseline	<0.25 x LLN or 75% decrease from baseline or absolute value <50 mg/dL	Death
REMARK: Use % decrease only when baseline is <LLN (local laboratory value).						
INR (International Normalized Ratio of prothrombin time)	INR	>1 – 1.5 x ULN	>1.5 – 2 x ULN	>2 x ULN	—	—
ALSO CONSIDER: Hemorrhage, CNS; Hemorrhage, GI – *Select*; Hemorrhage, GU – *Select*; Hemorrhage, pulmonary/upper respiratory – *Select*.						
PTT (Partial Thromboplastin Time)	PTT	>1 – 1.5 x ULN	>1.5 – 2 x ULN	>2 x ULN	—	—
ALSO CONSIDER: Hemorrhage, CNS; Hemorrhage, GI – *Select*; Hemorrhage, GU – *Select*; Hemorrhage, pulmonary/upper respiratory – *Select*.						
Thrombotic microangiopathy (e.g., thrombotic thrombocytopenic purpura [TTP] or hemolytic uremic syndrome [HUS])	Thrombotic microangiopathy	Evidence of RBC destruction (schistocytosis) without clinical consequences	—	Laboratory findings present with clinical consequences (e.g., renal insufficiency, petechiae)	Laboratory findings and life-threatening or disabling consequences, (e.g., CNS hemorrhage/ bleeding or thrombosis/ embolism or renal failure)	Death
REMARK: Must have microangiopathic changes on blood smear (e.g., schistocytes, helmet cells, red cell fragments).						
ALSO CONSIDER: Creatinine; Hemoglobin; Platelets.						
Coagulation – Other (Specify, __)	Coagulation – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

CONSTITUTIONAL SYMPTOMS						Page 1 of 2
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Fatigue (asthenia, lethargy, malaise)	Fatigue	Mild fatigue over baseline	Moderate or causing difficulty performing some ADL	Severe fatigue interfering with ADL	Disabling	—
Fever (in the absence of neutropenia, where neutropenia is defined as ANC <1.0 x 10^9/L)	Fever	38.0 – 39.0°C (100.4 – 102.2°F)	>39.0 – 40.0°C (102.3 – 104.0°F)	>40.0°C (>104.0°F) for ≤24 hrs	>40.0°C (>104.0°F) for >24 hrs	Death
REMARK: The temperature measurements listed are oral or tympanic.						
ALSO CONSIDER: Allergic reaction/hypersensitivity (including drug fever).						
NAVIGATION NOTE: Hot flashes are graded as Hot flashes/flushes in the ENDOCRINE CATEGORY.						
Hypothermia	Hypothermia	—	35 – >32°C 95 – >89.6°F	32 – >28°C 89.6 – >82.4° F	≤28 °C 82.4°F or life-threatening consequences (e.g., coma, hypotension, pulmonary edema, acidemia, ventricular fibrillation)	Death
Insomnia	Insomnia	Occasional difficulty sleeping, not interfering with function	Difficulty sleeping, interfering with function but not interfering with ADL	Frequent difficulty sleeping, interfering with ADL	Disabling	—
REMARK: If pain or other symptoms interfere with sleep, do NOT grade as insomnia. Grade primary event(s) causing insomnia.						
Obesity[2]	Obesity	—	BMI 25 – 29.9 kg/m^2	BMI 30 – 39.99 kg/m^2	BMI ≥40 kg/m^2	—
REMARK: BMI = (weight [kg]) / (height [m])^2						
Odor (patient odor)	Patient odor	Mild odor	Pronounced odor	—	—	—
Rigors/chills	Rigors/chills	Mild	Moderate, narcotics indicated	Severe or prolonged, not responsive to narcotics	—	—

[2] NHLBI Obesity Task Force. "Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults," *The Evidence Report,* Obes Res 6:51S-209S, 1998.

CONSTITUTIONAL SYMPTOMS						Page 2 of 2
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Sweating (diaphoresis)	Sweating	Mild and occasional	Frequent or drenching	—	—	—
ALSO CONSIDER: Hot flashes/flushes.						
Weight gain	Weight gain	5 – <10% of baseline	10 – <20% of baseline	≥20% of baseline	—	—
REMARK: Edema, depending on etiology, is graded in the CARDIAC GENERAL or LYMPHATICS CATEGORIES.						
ALSO CONSIDER: Ascites (non-malignant); Pleural effusion (non-malignant).						
Weight loss	Weight loss	5 to <10% from baseline; intervention not indicated	10 – <20% from baseline; nutritional support indicated	≥20% from baseline; tube feeding or TPN indicated	—	—
Constitutional Symptoms – Other (Specify, __)	Constitutional Symptoms – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

DEATH

Page 1 of 1

Adverse Event	Short Name	Grade 1	2	3	4	5
Death not associated with CTCAE term – *Select*: – Death NOS – Disease progression NOS – Multi-organ failure – Sudden death	Death not associated with CTCAE term – *Select*	—	—	—	—	Death

REMARK: Grade 5 is the only appropriate grade. 'Death not associated with CTCAE term – *Select*' is to be used where a death:

1. Cannot be attributed to a CTCAE term associated with Grade 5.
2. Cannot be reported within any CATEGORY using a CTCAE 'Other (Specify, __)'.

DERMATOLOGY/SKIN

Page 1 of 3

Adverse Event	Short Name	Grade 1	2	3	4	5
Atrophy, skin	Atrophy, skin	Detectable	Marked	—	—	—
Atrophy, subcutaneous fat	Atrophy, subcutaneous fat	Detectable	Marked	—	—	—
ALSO CONSIDER: Induration/fibrosis (skin and subcutaneous tissue).						
Bruising (in absence of Grade 3 or 4 thrombocytopenia)	Bruising	Localized or in a dependent area	Generalized	—	—	—
Burn	Burn	Minimal symptoms; intervention not indicated	Medical intervention; minimal debridement indicated	Moderate to major debridement or reconstruction indicated	Life-threatening consequences	Death
REMARK: Burn refers to all burns including radiation, chemical, etc.						
Cheilitis	Cheilitis	Asymptomatic	Symptomatic, not interfering with ADL	Symptomatic, interfering with ADL	—	—
Dry skin	Dry skin	Asymptomatic	Symptomatic, not interfering with ADL	Interfering with ADL	—	—
Flushing	Flushing	Asymptomatic	Symptomatic	—	—	—
Hair loss/alopecia (scalp or body)	Alopecia	Thinning or patchy	Complete	—	—	—
Hyperpigmentation	Hyperpigmentation	Slight or localized	Marked or generalized	—	—	—
Hypopigmentation	Hypopigmentation	Slight or localized	Marked or generalized	—	—	—
Induration/fibrosis (skin and subcutaneous tissue)	Induration	Increased density on palpation	Moderate impairment of function not interfering with ADL; marked increase in density and firmness on palpation with or without minimal retraction	Dysfunction interfering with ADL; very marked density, retraction or fixation	—	—
ALSO CONSIDER: Fibrosis-cosmesis; Fibrosis-deep connective tissue.						
Injection site reaction/ extravasation changes	Injection site reaction	Pain; itching; erythema	Pain or swelling, with inflammation or phlebitis	Ulceration or necrosis that is severe; operative intervention indicated	—	—
ALSO CONSIDER: Allergic reaction/hypersensitivity (including drug fever); Ulceration.						

DERMATOLOGY/SKIN						Page 2 of 3
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Nail changes	Nail changes	Discoloration; ridging (koilonychias); pitting	Partial or complete loss of nail(s); pain in nailbed(s)	Interfering with ADL	—	—
NAVIGATION NOTE: Petechiae is graded as Petechiae/purpura (hemorrhage/bleeding into skin or mucosa) in the HEMORRHAGE/BLEEDING CATEGORY.						
Photosensitivity	Photosensitivity	Painless erythema	Painful erythema	Erythema with desquamation	Life-threatening; disabling	Death
Pruritus/itching	Pruritus	Mild or localized	Intense or widespread	Intense or widespread and interfering with ADL	—	—
ALSO CONSIDER: Rash/desquamation.						
Rash/desquamation	Rash	Macular or papular eruption or erythema without associated symptoms	Macular or papular eruption or erythema with pruritus or other associated symptoms; localized desquamation or other lesions covering <50% of body surface area (BSA)	Severe, generalized erythroderma or macular, papular or vesicular eruption; desquamation covering ≥50% BSA	Generalized exfoliative, ulcerative, or bullous dermatitis	Death
REMARK: Rash/desquamation may be used for GVHD.						
Rash: acne/acneiform	Acne	Intervention not indicated	Intervention indicated	Associated with pain, disfigurement, ulceration, or desquamation	—	Death
Rash: dermatitis associated with radiation – *Select*: – Chemoradiation – Radiation	Dermatitis – *Select*	Faint erythema or dry desquamation	Moderate to brisk erythema; patchy moist desquamation, mostly confined to skin folds and creases; moderate edema	Moist desquamation other than skin folds and creases; bleeding induced by minor trauma or abrasion	Skin necrosis or ulceration of full thickness dermis; spontaneous bleeding from involved site	Death
Rash: erythema multiforme (e.g., Stevens-Johnson syndrome, toxic epidermal necrolysis)	Erythema multiforme	—	Scattered, but not generalized eruption	Severe (e.g., generalized rash or painful stomatitis); IV fluids, tube feedings, or TPN indicated	Life-threatening; disabling	Death
Rash: hand-foot skin reaction	Hand-foot	Minimal skin changes or dermatitis (e.g., erythema) without pain	Skin changes (e.g., peeling, blisters, bleeding, edema) or pain, not interfering with function	Ulcerative dermatitis or skin changes with pain interfering with function	—	—

DERMATOLOGY/SKIN						Page 3 of 3
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Skin breakdown/ decubitus ulcer	Decubitus	—	Local wound care; medical intervention indicated	Operative debridement or other invasive intervention indicated (e.g., hyperbaric oxygen)	Life-threatening consequences; major invasive intervention indicated (e.g., tissue reconstruction, flap, or grafting)	Death
REMARK: Skin breakdown/decubitus ulcer is to be used for loss of skin integrity or decubitus ulcer from pressure or as the result of operative or medical intervention.						
Striae	Striae	Mild	Cosmetically significant	—	—	—
Telangiectasia	Telangiectasia	Few	Moderate number	Many and confluent	—	—
Ulceration	Ulceration	—	Superficial ulceration <2 cm size; local wound care; medical intervention indicated	Ulceration ≥2 cm size; operative debridement, primary closure or other invasive intervention indicated (e.g., hyperbaric oxygen)	Life-threatening consequences; major invasive intervention indicated (e.g., complete resection, tissue reconstruction, flap, or grafting)	Death
Urticaria (hives, welts, wheals)	Urticaria	Intervention not indicated	Intervention indicated for <24 hrs	Intervention indicated for ≥24 hrs	—	—
ALSO CONSIDER: Allergic reaction/hypersensitivity (including drug fever).						
Wound complication, non-infectious	Wound complication, non-infectious	Incisional separation of ≤25% of wound, no deeper than superficial fascia	Incisional separation >25% of wound with local care; asymptomatic hernia	Symptomatic hernia without evidence of strangulation; fascial disruption/dehiscence without evisceration; primary wound closure or revision by operative intervention indicated; hospitalization or hyperbaric oxygen indicated	Symptomatic hernia with evidence of strangulation; fascial disruption with evisceration; major reconstruction flap, grafting, resection, or amputation indicated	Death
REMARK: Wound complication, non-infectious is to be used for separation of incision, hernia, dehiscence, evisceration, or second surgery for wound revision.						
Dermatology/Skin – Other (Specify, __)	Dermatology – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

ENDOCRINE						Page 1 of 2
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Adrenal insufficiency	Adrenal insufficiency	Asymptomatic, intervention not indicated	Symptomatic, intervention indicated	Hospitalization	Life-threatening; disabling	Death
REMARK: Adrenal insufficiency includes any of the following signs and symptoms: abdominal pain, anorexia, constipation, diarrhea, hypotension, pigmentation of mucous membranes, pigmentation of skin, salt craving, syncope (fainting), vitiligo, vomiting, weakness, weight loss. Adrenal insufficiency must be confirmed by laboratory studies (low cortisol frequently accompanied by low aldosterone).						
ALSO CONSIDER: Potassium, serum-high (hyperkalemia); Thyroid function, low (hypothyroidism).						
Cushingoid appearance (e.g., moon face, buffalo hump, centripetal obesity, cutaneous striae)	Cushingoid	—	Present	—	—	—
ALSO CONSIDER: Glucose, serum-high (hyperglycemia); Potassium, serum-low (hypokalemia).						
Feminization of male	Feminization of male	—	—	Present	—	—
NAVIGATION NOTE: Gynecomastia is graded in the SEXUAL/REPRODUCTIVE FUNCTION CATEGORY.						
Hot flashes/flushes[3]	Hot flashes	Mild	Moderate	Interfering with ADL	—	—
Masculinization of female	Masculinization of female	—	—	Present	—	—
Neuroendocrine: ACTH deficiency	ACTH	Asymptomatic	Symptomatic, not interfering with ADL; intervention indicated	Symptoms interfering with ADL; hospitalization indicated	Life-threatening consequences (e.g., severe hypotension)	Death
Neuroendocrine: ADH secretion abnormality (e.g., SIADH or low ADH)	ADH	Asymptomatic	Symptomatic, not interfering with ADL; intervention indicated	Symptoms interfering with ADL	Life-threatening consequences	Death
Neuroendocrine: gonadotropin secretion abnormality	Gonadotropin	Asymptomatic	Symptomatic, not interfering with ADL; intervention indicated	Symptoms interfering with ADL; osteopenia; fracture; infertility	—	—
Neuroendocrine: growth hormone secretion abnormality	Growth hormone	Asymptomatic	Symptomatic, not interfering with ADL; intervention indicated	—	—	—
Neuroendocrine: prolactin hormone secretion abnormality	Prolactin	Asymptomatic	Symptomatic, not interfering with ADL; intervention indicated	Symptoms interfering with ADL; amenorrhea; galactorrhea	—	Death

[3] Sloan JA, Loprinzi CL, Novotny PJ, Barton DL, Lavasseur BI, Windschitl HJ, "Methodologic Lessons Learned from Hot Flash Studies," *J Clin Oncol* 2001 Dec 1;19(23):4280-90

ENDOCRINE						Page 2 of 2
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Pancreatic endocrine: glucose intolerance	Diabetes	Asymptomatic, intervention not indicated	Symptomatic; dietary modification or oral agent indicated	Symptoms interfering with ADL; insulin indicated	Life-threatening consequences (e.g., ketoacidosis, hyperosmolar non-ketotic coma)	Death
Parathyroid function, low (hypoparathyroidism)	Hypoparathyroidism	Asymptomatic, intervention not indicated	Symptomatic; intervention indicated	—	—	—
Thyroid function, high (hyperthyroidism, thyrotoxicosis)	Hyperthyroidism	Asymptomatic, intervention not indicated	Symptomatic, not interfering with ADL; thyroid suppression therapy indicated	Symptoms interfering with ADL; hospitalization indicated	Life-threatening consequences (e.g., thyroid storm)	Death
Thyroid function, low (hypothyroidism)	Hypothyroidism	Asymptomatic, intervention not indicated	Symptomatic, not interfering with ADL; thyroid replacement indicated	Symptoms interfering with ADL; hospitalization indicated	Life-threatening myxedema coma	Death
Endocrine – Other (Specify, __)	Endocrine – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

GASTROINTESTINAL						Page 1 of 10
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
NAVIGATION NOTE: Abdominal pain or cramping is graded as Pain – *Select* in the PAIN CATEGORY.						
Anorexia	Anorexia	Loss of appetite without alteration in eating habits	Oral intake altered without significant weight loss or malnutrition; oral nutritional supplements indicated	Associated with significant weight loss or malnutrition (e.g., inadequate oral caloric and/or fluid intake); IV fluids, tube feedings or TPN indicated	Life-threatening consequences	Death
ALSO CONSIDER: Weight loss.						
Ascites (non-malignant)	Ascites	Asymptomatic	Symptomatic, medical intervention indicated	Symptomatic, invasive procedure indicated	Life-threatening consequences	Death
REMARK: Ascites (non-malignant) refers to documented non-malignant ascites or unknown etiology, but unlikely malignant, and includes chylous ascites.						
Colitis	Colitis	Asymptomatic, pathologic or radiographic findings only	Abdominal pain; mucus or blood in stool	Abdominal pain, fever, change in bowel habits with ileus; peritoneal signs	Life-threatening consequences (e.g., perforation, bleeding, ischemia, necrosis, toxic megacolon)	Death
ALSO CONSIDER: Hemorrhage, GI – *Select*.						
Constipation	Constipation	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema	Persistent symptoms with regular use of laxatives or enemas indicated	Symptoms interfering with ADL; obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction, toxic megacolon)	Death
ALSO CONSIDER: Ileus, GI (functional obstruction of bowel, i.e., neuroconstipation); Obstruction, GI – *Select*.						
Dehydration	Dehydration	Increased oral fluids indicated; dry mucous membranes; diminished skin turgor	IV fluids indicated <24 hrs	IV fluids indicated ≥24 hrs	Life-threatening consequences (e.g., hemodynamic collapse)	Death
ALSO CONSIDER: Diarrhea; Hypotension; Vomiting.						
Dental: dentures or prosthesis	Dentures	Minimal discomfort, no restriction in activities	Discomfort preventing use in some activities (e.g., eating), but not others (e.g., speaking)	Unable to use dentures or prosthesis at any time	—	—

GASTROINTESTINAL						Page 2 of 10
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Dental: periodontal disease	Periodontal	Gingival recession or gingivitis; limited bleeding on probing; mild local bone loss	Moderate gingival recession or gingivitis; multiple sites of bleeding on probing; moderate bone loss	Spontaneous bleeding; severe bone loss with or without tooth loss; osteonecrosis of maxilla or mandible	—	—
REMARK: Severe periodontal disease leading to osteonecrosis is graded as Osteonecrosis (avasoular necrosis) in the MUSCULOSKELETAL CATEGORY.						
Dental: teeth	Teeth	Surface stains; dental caries; restorable, without extractions	Less than full mouth extractions; tooth fracture or crown amputation or repair indicated	Full mouth extractions indicated	—	—
Dental: teeth development	Teeth development	Hypoplasia of tooth or enamel not interfering with function	Functional impairment correctable with oral surgery	Maldevelopment with functional impairment not surgically correctable	—	—
Diarrhea	Diarrhea	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 – 6 stools per day over baseline; IV fluids indicated <24hrs; moderate increase in ostomy output compared to baseline; not interfering with ADL	Increase of ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; hospitalization; severe increase in ostomy output compared to baseline; interfering with ADL	Life-threatening consequences (e.g., hemodynamic collapse)	Death
REMARK: Diarrhea includes diarrhea of small bowel or colonic origin, and/or ostomy diarrhea.						
ALSO CONSIDER: Dehydration; Hypotension.						
Distension/bloating, abdominal	Distension	Asymptomatic	Symptomatic, but not interfering with GI function	Symptomatic, interfering with GI function	—	—
ALSO CONSIDER: Ascites (non-malignant); Ileus, GI (functional obstruction of bowel, i.e., neuroconstipation); Obstruction, GI – *Select*.						

GASTROINTESTINAL						Page 3 of 10
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Dry mouth/salivary gland (xerostomia)	Dry mouth	Symptomatic (dry or thick saliva) without significant dietary alteration; unstimulated saliva flow >0.2 ml/min	Symptomatic and significant oral intake alteration (e.g., copious water, other lubricants, diet limited to purees and/or soft, moist foods); unstimulated saliva 0.1 to 0.2 ml/min	Symptoms leading to inability to adequately aliment orally; IV fluids, tube feedings, or TPN indicated; unstimulated saliva <0.1 ml/min	—	—
REMARK: Dry mouth/salivary gland (xerostomia) includes descriptions of grade using both subjective and objective assessment parameters. Record this event consistently throughout a patient's participation on study. If salivary flow measurements are used for initial assessment, subsequent assessments must use salivary flow.						
ALSO CONSIDER: Salivary gland changes/saliva.						
Dysphagia (difficulty swallowing)	Dysphagia	Symptomatic, able to eat regular diet	Symptomatic and altered eating/swallowing (e.g., altered dietary habits, oral supplements); IV fluids indicated <24 hrs	Symptomatic and severely altered eating/swallowing (e.g., inadequate oral caloric or fluid intake); IV fluids, tube feedings, or TPN indicated ≥24 hrs	Life-threatening consequences (e.g., obstruction, perforation)	Death
REMARK: Dysphagia (difficulty swallowing) is to be used for swallowing difficulty from oral, pharyngeal, esophageal, or neurologic origin. Dysphagia requiring dilation is graded as Stricture/stenosis (including anastomotic), GI – *Select*.						
ALSO CONSIDER: Dehydration; Esophagitis.						
Enteritis (inflammation of the small bowel)	Enteritis	Asymptomatic, pathologic or radiographic findings only	Abdominal pain; mucus or blood in stool	Abdominal pain, fever, change in bowel habits with ileus; peritoneal signs	Life-threatening consequences (e.g., perforation, bleeding, ischemia, necrosis)	Death
ALSO CONSIDER: Hemorrhage, GI – *Select*; Typhlitis (cecal inflammation).						
Esophagitis	Esophagitis	Asymptomatic pathologic, radiographic, or endoscopic findings only	Symptomatic; altered eating/swallowing (e.g., altered dietary habits, oral supplements); IV fluids indicated <24 hrs	Symptomatic and severely altered eating/swallowing (e.g., inadequate oral caloric or fluid intake); IV fluids, tube feedings, or TPN indicated ≥24 hrs	Life-threatening consequences	Death
REMARK: Esophagitis includes reflux esophagitis.						
ALSO CONSIDER: Dysphagia (difficulty swallowing).						

GASTROINTESTINAL						Page 4 of 10
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Fistula, GI – *Select*: – Abdomen NOS – Anus – Biliary tree – Colon/cecum/appendix – Duodenum – Esophagus – Gallbladder – Ileum – Jejunum – Oral cavity – Pancreas – Pharynx – Rectum – Salivary gland – Small bowel NOS – Stomach	Fistula, GI – *Select*	Asymptomatic, radiographic findings only	Symptomatic; altered GI function (e.g., altered dietary habits, diarrhea, or GI fluid loss); IV fluids indicated <24 hrs	Symptomatic and severely altered GI function (e.g., altered dietary habits, diarrhea, or GI fluid loss); IV fluids, tube feedings, or TPN indicated ≥24 hrs	Life-threatening consequences	Death
REMARK: A fistula is defined as an abnormal communication between two body cavities, potential spaces, and/or the skin. The site indicated for a fistula should be the site from which the abnormal process is believed to have originated. For example, a tracheo-esophageal fistula arising in the context of a resected or irradiated esophageal cancer is graded as Fistula, GI – esophagus.						
Flatulence	Flatulence	Mild	Moderate	—	—	—
Gastritis (including bile reflux gastritis)	Gastritis	Asymptomatic radiographic or endoscopic findings only	Symptomatic; altered gastric function (e.g., inadequate oral caloric or fluid intake); IV fluids indicated <24 hrs	Symptomatic and severely altered gastric function (e.g., inadequate oral caloric or fluid intake); IV fluids, tube feedings, or TPN indicated ≥24 hrs	Life-threatening consequences; operative intervention requiring complete organ resection (e.g., gastrectomy)	Death
ALSO CONSIDER: Hemorrhage, GI – *Select*; Ulcer, GI – *Select*.						
NAVIGATION NOTE: Head and neck soft tissue necrosis is graded as Soft tissue necrosis – *Select* in the MUSCULOSKELETAL/SOFT TISSUE CATEGORY.						
Heartburn/dyspepsia	Heartburn	Mild	Moderate	Severe	—	—
Hemorrhoids	Hemorrhoids	Asymptomatic	Symptomatic; banding or medical intervention indicated	Interfering with ADL; interventional radiology, endoscopic, or operative intervention indicated	Life-threatening consequences	Death

GASTROINTESTINAL						Page 5 of 10
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Ileus, GI (functional obstruction of bowel, i.e., neuroconstipation)	Ileus	Asymptomatic, radiographic findings only	Symptomatic; altered GI function (e.g., altered dietary habits); IV fluids indicated <24 hrs	Symptomatic and severely altered GI function; IV fluids, tube feeding, or TPN indicated ≥24 hrs	Life-threatening consequences	Death
REMARK: Ileus, GI is to be used for altered upper or lower GI function (e.g., delayed gastric or colonic emptying). ALSO CONSIDER: Constipation; Nausea; Obstruction, GI – *Select*; Vomiting.						
Incontinence, anal	Incontinence, anal	Occasional use of pads required	Daily use of pads required	Interfering with ADL; operative intervention indicated	Permanent bowel diversion indicated	Death
REMARK: Incontinence, anal is to be used for loss of sphincter control as sequelae of operative or therapeutic intervention.						
Leak (including anastomotic), GI – *Select*: – Biliary tree – Esophagus – Large bowel – Leak NOS – Pancreas – Pharynx – Rectum – Small bowel – Stoma – Stomach	Leak, GI – *Select*	Asymptomatic radiographic findings only	Symptomatic; medical intervention indicated	Symptomatic and interfering with GI function; invasive or endoscopic intervention indicated	Life-threatening consequences	Death
REMARK: Leak (including anasomotic), GI – *Select* is to be used for clinical signs/symptoms or radiographic confirmation of anastomotic or conduit leak (e.g., biliary, esophageal, intestinal, pancreatic, pharyngeal, rectal), but without development of fistula.						
Malabsorption	Malabsorption	—	Altered diet; oral therapies indicated (e.g., enzymes, medications, dietary supplements)	Inability to aliment adequately via GI tract (i.e., TPN indicated)	Life-threatening consequences	Death

GASTROINTESTINAL						Page 6 of 10
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Mucositis/stomatitis (clinical exam) – *Select*: – Anus – Esophagus – Large bowel – Larynx – Oral cavity – Pharynx – Rectum – Small bowel – Stomach – Trachea	Mucositis (clinical exam) – *Select*	Erythema of the mucosa	Patchy ulcerations or pseudomembranes	Confluent ulcerations or pseudomembranes; bleeding with minor trauma	Tissue necrosis; significant spontaneous bleeding; life-threatening consequences	Death
REMARK: Mucositis/stomatitis (functional/symptomatic) may be used for mucositis of the upper aero-digestive tract caused by radiation, agents, or GVHD.						
Mucositis/stomatitis (functional/symptomatic) – *Select*: – Anus – Esophagus – Large bowel – Larynx – Oral cavity – Pharynx – Rectum – Small bowel – Stomach – Trachea	Mucositis (functional/ symptomatic) – *Select*	Upper aerodigestive tract sites: Minimal symptoms, normal diet; minimal respiratory symptoms but not interfering with function Lower GI sites: Minimal discomfort, intervention not indicated	Upper aerodigestive tract sites: Symptomatic but can eat and swallow modified diet; respiratory symptoms interfering with function but not interfering with ADL Lower GI sites: Symptomatic, medical intervention indicated but not interfering with ADL	Upper aerodigestive tract sites: Symptomatic and unable to adequately aliment or hydrate orally; respiratory symptoms interfering with ADL Lower GI sites: Stool incontinence or other symptoms interfering with ADL	Symptoms associated with life-threatening consequences	Death
Nausea	Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition; IV fluids indicated <24 hrs	Inadequate oral caloric or fluid intake; IV fluids, tube feedings, or TPN indicated ≥24 hrs	Life-threatening consequences	Death
ALSO CONSIDER: Anorexia; Vomiting.						

GASTROINTESTINAL						Page 7 of 10
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Necrosis, GI – *Select*: – Anus – Colon/cecum/appendix – Duodenum – Esophagus – Gallbladder – Hepatic – Ileum – Jejunum – Oral – Pancreas – Peritoneal cavity – Pharynx – Rectum – Small bowel NOS – Stoma – Stomach	Necrosis, GI – *Select*	—	—	Inability to aliment adequately by GI tract (e.g., requiring enteral or parenteral nutrition); interventional radiology, endoscopic, or operative intervention indicated	Life-threatening consequences; operative intervention requiring complete organ resection (e.g., total colectomy)	Death
ALSO CONSIDER: Visceral arterial ischemia (non-myocardial).						
Obstruction, GI – *Select*: – Cecum – Colon – Duodenum – Esophagus – Gallbladder – Ileum – Jejunum – Rectum – Small bowel NOS – Stoma – Stomach	Obstruction, GI – *Select*	Asymptomatic radiographic findings only	Symptomatic; altered GI function (e.g., altered dietary habits, vomiting, diarrhea, or GI fluid loss); IV fluids indicated <24 hrs	Symptomatic and severely altered GI function (e.g., altered dietary habits, vomiting, diarrhea, or GI fluid loss); IV fluids, tube feedings, or TPN indicated ≥24 hrs; operative intervention indicated	Life-threatening consequences; operative intervention requiring complete organ resection (e.g., total colectomy)	Death
NAVIGATION NOTE: Operative injury is graded as Intra-operative injury – *Select Organ or Structure* in the SURGERY/INTRA-OPERATIVE INJURY CATEGORY.						
NAVIGATION NOTE: Pelvic pain is graded as Pain – *Select* in the PAIN CATEGORY.						

GASTROINTESTINAL						Page 8 of 10
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Perforation, GI – *Select*: – Appendix – Biliary tree – Cecum – Colon – Duodenum – Esophagus – Gallbladder – Ileum – Jejunum – Rectum – Small bowel NOS – Stomach	Perforation, GI – *Select*	Asymptomatic radiographic findings only	Medical intervention indicated; IV fluids indicated <24 hrs	IV fluids, tube feedings, or TPN indicated ≥24 hrs; operative intervention indicated	Life-threatening consequences	Death
Proctitis	Proctitis	Rectal discomfort, intervention not indicated	Symptoms not interfering with ADL; medical intervention indicated	Stool incontinence or other symptoms interfering with ADL; operative intervention indicated	Life-threatening consequences (e.g., perforation)	Death
Prolapse of stoma, GI	Prolapse of stoma, GI	Asymptomatic	Extraordinary local care or maintenance; minor revision indicated	Dysfunctional stoma; major revision indicated	Life-threatening consequences	Death
REMARK: Other stoma complications may be graded as Fistula, GI – *Select*; Leak (including anastomotic), GI – *Select*; Obstruction, GI – *Select*; Perforation, GI – *Select*; Stricture/stenosis (including anastomotic), GI – *Select*.						
NAVIGATION NOTE: Rectal or perirectal pain (proctalgia) is graded as Pain – *Select* in the PAIN CATEGORY.						
Salivary gland changes/saliva	Salivary gland changes	Slightly thickened saliva; slightly altered taste (e.g., metallic)	Thick, ropy, sticky saliva; markedly altered taste; alteration in diet indicated; secretion-induced symptoms not interfering with ADL	Acute salivary gland necrosis; severe secretion-induced symptoms interfering with ADL	Disabling	—
ALSO CONSIDER: Dry mouth/salivary gland (xerostomia); Mucositis/stomatitis (clinical exam) – *Select*; Mucositis/stomatitis (functional/symptomatic) – *Select*; Taste alteration (dysgeusia).						
NAVIGATION NOTE: Splenic function is graded in the BLOOD/BONE MARROW CATEGORY.						

GASTROINTESTINAL						Page 9 of 10
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Stricture/stenosis (including anastomotic), GI – *Select*: – Anus – Biliary tree – Cecum – Colon – Duodenum – Esophagus – Ileum – Jejunum – Pancreas/pancreatic duct – Pharynx – Rectum – Small bowel NOS – Stoma – Stomach	Stricture, GI – *Select*	Asymptomatic radiographic findings only	Symptomatic; altered GI function (e.g., altered dietary habits, vomiting, bleeding, diarrhea); IV fluids indicated <24 hrs	Symptomatic and severely altered GI function (e.g., altered dietary habits, diarrhea, or GI fluid loss); IV fluids, tube feedings, or TPN indicated ≥24 hrs; operative intervention indicated	Life-threatening consequences; operative intervention requiring complete organ resection (e.g., total colectomy)	Death
Taste alteration (dysgeusia)	Taste alteration	Altered taste but no change in diet	Altered taste with change in diet (e.g., oral supplements); noxious or unpleasant taste; loss of taste	—	—	—
Typhlitis (cecal inflammation)	Typhlitis	Asymptomatic, pathologic or radiographic findings only	Abdominal pain; mucus or blood in stool	Abdominal pain, fever, change in bowel habits with ileus; peritoneal signs	Life-threatening consequences (e.g., perforation, bleeding, ischemia, necrosis); operative intervention indicated	Death
ALSO CONSIDER: Colitis; Hemorrhage, GI – *Select* ; Ileus, GI (functional obstruction of bowel, i.e., neuroconstipation).						

GASTROINTESTINAL						Page 10 of 10
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Ulcer, GI – *Select*: – Anus – Cecum – Colon – Duodenum – Esophagus – Ileum – Jejunum – Rectum – Small bowel NOS – Stoma – Stomach	Ulcer, GI – *Select*	Asymptomatic, radiographic or endoscopic findings only	Symptomatic; altered GI function (e.g., altered dietary habits, oral supplements); IV fluids indicated <24 hrs	Symptomatic and severely altered GI function (e.g., inadequate oral caloric or fluid intake); IV fluids, tube feedings, or TPN indicated ≥24 hrs	Life-threatening consequences	Death
ALSO CONSIDER: Hemorrhage, GI – *Select*.						
Vomiting	Vomiting	1 episode in 24 hrs	2 – 5 episodes in 24 hrs; IV fluids indicated <24 hrs	≥6 episodes in 24 hrs; IV fluids, or TPN indicated ≥24 hrs	Life-threatening consequences	Death
ALSO CONSIDER: Dehydration.						
Gastrointestinal – Other (Specify, __)	GI – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

GROWTH AND DEVELOPMENT						Page 1 of 1
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Bone age (alteration in bone age)	Bone age	—	±2 SD (standard deviation) from normal	—	—	—
Bone growth: femoral head; slipped capital femoral epiphysis	Femoral head growth	Mild valgus/varus deformity	Moderate valgus/varus deformity, symptomatic, interfering with function but not interfering with ADL	Mild slipped capital femoral epiphysis; operative intervention (e.g., fixation) indicated; interfering with ADL	Disabling; severe slipped capital femoral epiphysis >60%; avascular necrosis	—
Bone growth: limb length discrepancy	Limb length	Mild length discrepancy <2 cm	Moderate length discrepancy 2 – 5 cm; shoe lift indicated	Severe length discrepancy >5 cm; operative intervention indicated; interfering with ADL	Disabling; epiphysiodesis	—
Bone growth: spine kyphosis/lordosis	Kyphosis/lordosis	Mild radiographic changes	Moderate accentuation; interfering with function but not interfering with ADL	Severe accentuation; operative intervention indicated; interfering with ADL	Disabling (e.g., cannot lift head)	—
Growth velocity (reduction in growth velocity)	Reduction in growth velocity	10 – 29% reduction in growth from the baseline growth curve	30 – 49% reduction in growth from the baseline growth curve	≥50% reduction in growth from the baseline growth curve	—	—
Puberty (delayed)	Delayed puberty	—	No breast development by age 13 yrs for females; no Tanner Stage 2 development by age 14.5 yrs for males	No sexual development by age 14 yrs for girls, age 16 yrs for boys; hormone replacement indicated	—	—
REMARK: Do not use testicular size for Tanner Stage in male cancer survivors.						
Puberty (precocious)	Precocious puberty	—	Physical signs of puberty <7 years for females, <9 years for males	—	—	—
Short stature	Short stature	Beyond two standard deviations of age and gender mean height	Altered ADL	—	—	—
REMARK: Short stature is secondary to growth hormone deficiency. ALSO CONSIDER: Neuroendocrine: growth hormone secretion abnormality.						
Growth and Development – Other (Specify, __)	Growth and Development – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

HEMORRHAGE/BLEEDING						Page 1 of 4
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Hematoma	Hematoma	Minimal symptoms, invasive intervention not indicated	Minimally invasive evacuation or aspiration indicated	Transfusion, interventional radiology, or operative intervention indicated	Life-threatening consequences; major urgent intervention indicated	Death
REMARK: Hematoma refers to extravasation at wound or operative site or secondary to other intervention. Transfusion implies pRBC.						
ALSO CONSIDER: Fibrinogen; INR (International Normalized Ratio of prothrombin time); Platelets; PTT (Partial Thromboplastin Time).						
Hemorrhage/bleeding associated with surgery, intra-operative or postoperative	Hemorrhage with surgery	—	—	Requiring transfusion of 2 units non-autologous (10 cc/kg for pediatrics) pRBCs beyond protocol specification; postoperative interventional radiology, endoscopic, or operative intervention indicated	Life-threatening consequences	Death
REMARK: Postoperative period is defined as ≤72 hours after surgery. Verify protocol-specific acceptable guidelines regarding pRBC transfusion.						
ALSO CONSIDER: Fibrinogen; INR (International Normalized Ratio of prothrombin time); Platelets; PTT (Partial Thromboplastin Time).						
Hemorrhage, CNS	CNS hemorrhage	Asymptomatic, radiographic findings only	Medical intervention indicated	Ventriculostomy, ICP monitoring, intraventricular thrombolysis, or operative intervention indicated	Life-threatening consequences; neurologic deficit or disability	Death
ALSO CONSIDER: Fibrinogen; INR (International Normalized Ratio of prothrombin time); Platelets; PTT (Partial Thromboplastin Time).						

HEMORRHAGE/BLEEDING						Page 2 of 4
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Hemorrhage, GI – *Select*: – Abdomen NOS – Anus – Biliary tree – Cecum/appendix – Colon – Duodenum – Esophagus – Ileum – Jejunum – Liver – Lower GI NOS – Oral cavity – Pancreas – Peritoneal cavity – Rectum – Stoma – Stomach – Upper GI NOS – Varices (esophageal) – Varices (rectal)	Hemorrhage, GI – *Select*	Mild, intervention (other than iron supplements) not indicated	Symptomatic and medical intervention or minor cauterization indicated	Transfusion, interventional radiology, endoscopic, or operative intervention indicated; radiation therapy (i.e., hemostasis of bleeding site)	Life-threatening consequences; major urgent intervention indicated	Death
REMARK: Transfusion implies pRBC.						
ALSO CONSIDER: Fibrinogen; INR (International Normalized Ratio of prothrombin time); Platelets; PTT (Partial Thromboplastin Time).						

HEMORRHAGE/BLEEDING						Page 3 of 4
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Hemorrhage, GU – *Select*: – Bladder – Fallopian tube – Kidney – Ovary – Prostate – Retroperitoneum – Spermatic cord – Stoma – Testes – Ureter – Urethra – Urinary NOS – Uterus – Vagina – Vas deferens	Hemorrhage, GU – *Select*	Minimal or microscopic bleeding; intervention not indicated	Gross bleeding, medical intervention, or urinary tract irrigation indicated	Transfusion, interventional radiology, endoscopic, or operative intervention indicated; radiation therapy (i.e., hemostasis of bleeding site)	Life-threatening consequences; major urgent intervention indicated	Death
REMARK: Transfusion implies pRBC. ALSO CONSIDER: Fibrinogen; INR (International Normalized Ratio of prothrombin time); Platelets; PTT (Partial Thromboplastin Time).						
Hemorrhage, pulmonary/ upper respiratory – *Select*: – Bronchopulmonary NOS – Bronchus – Larynx – Lung – Mediastinum – Nose – Pharynx – Pleura – Respiratory tract NOS – Stoma – Trachea	Hemorrhage pulmonary – *Select*	Mild, intervention not indicated	Symptomatic and medical intervention indicated	Transfusion, interventional radiology, endoscopic, or operative intervention indicated; radiation therapy (i.e., hemostasis of bleeding site)	Life-threatening consequences; major urgent intervention indicated	Death
REMARK: Transfusion implies pRBC. ALSO CONSIDER: Fibrinogen; INR (International Normalized Ratio of prothrombin time); Platelets; PTT (Partial Thromboplastin Time).						
Petechiae/purpura (hemorrhage/bleeding into skin or mucosa)	Petechiae	Few petechiae	Moderate petechiae; purpura	Generalized petechiae or purpura	—	—
ALSO CONSIDER: Fibrinogen; INR (International Normalized Ratio of prothrombin time); Platelets; PTT (Partial Thromboplastin Time).						

HEMORRHAGE/BLEEDING						Page 4 of 4
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
NAVIGATION NOTE: Vitreous hemorrhage is graded in the OCULAR/VISUAL CATEGORY.						
Hemorrhage/Bleeding – Other (Specify, __)	Hemorrhage – Other (Specify)	Mild without transfusion	—	Transfusion indicated	Catastrophic bleeding, requiring major non-elective intervention	Death

HEPATOBILIARY/PANCREAS

Page 1 of 1

Adverse Event	Short Name	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
NAVIGATION NOTE: Biliary tree damage is graded as Fistula, GI – *Select*; Leak (including anastomotic), GI – *Select*; Necrosis, GI – *Select*; Obstruction, GI – *Select*; Perforation, GI – *Select*; Stricture/stenosis (including anastomotic), GI – *Select* in the GASTROINTESTINAL CATEGORY.						
Cholecystitis	Cholecystitis	Asymptomatic, radiographic findings only	Symptomatic, medical intervention indicated	Interventional radiology, endoscopic, or operative intervention indicated	Life-threatening consequences (e.g., sepsis or perforation)	Death
ALSO CONSIDER: Infection (documented clinically or microbiologically) with Grade 3 or 4 neutrophils – *Select*; Infection with normal ANC or Grade 1 or 2 neutrophils – *Select*; Infection with unknown ANC – *Select*.						
Liver dysfunction/failure (clinical)	Liver dysfunction	—	Jaundice	Asterixis	Encephalopathy or coma	Death
REMARK: Jaundice is not an AE, but occurs when the liver is not working properly or when a bile duct is blocked. It is graded as a result of liver dysfunction/failure or elevated bilirubin.						
ALSO CONSIDER: Bilirubin (hyperbilirubinemia).						
Pancreas, exocrine enzyme deficiency	Pancreas, exocrine enzyme deficiency	—	Increase in stool frequency, bulk, or odor; steatorrhea	Sequelae of absorption deficiency (e.g., weight loss)	Life-threatening consequences	Death
ALSO CONSIDER: Diarrhea.						
Pancreatitis	Pancreatitis	Asymptomatic, enzyme elevation and/or radiographic findings	Symptomatic, medical intervention indicated	Interventional radiology or operative intervention indicated	Life-threatening consequences (e.g., circulatory failure, hemorrhage, sepsis)	Death
ALSO CONSIDER: Amylase.						
NAVIGATION NOTE: Stricture (biliary tree, hepatic or pancreatic) is graded as Stricture/stenosis (including anastomotic), GI – *Select* in the GASTROINTESTINAL CATEGORY.						
Hepatobiliary/Pancreas – Other (Specify. __)	Hepatobiliary – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

INFECTION

Page 1 of 3

Adverse Event	Short Name	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Colitis, infectious (e.g., Clostridium difficile)	Colitis, infectious	Asymptomatic, pathologic or radiographic findings only	Abdominal pain with mucus and/or blood in stool	IV antibiotics or TPN indicated	Life-threatening consequences (e.g., perforation, bleeding, ischemia, necrosis or toxic megacolon); operative resection or diversion indicated	Death
ALSO CONSIDER: Hemorrhage, GI – *Select*; Typhlitis (cecal inflammation).						
Febrile neutropenia (fever of unknown origin without clinically or microbiologically documented infection) (ANC <1.0 x 10^9/L, fever ≥38.5°C)	Febrile neutropenia	—	—	Present	Life-threatening consequences (e.g., septic shock, hypotension, acidosis, necrosis)	Death
ALSO CONSIDER: Neutrophils/granulocytes (ANC/AGC).						
Infection (documented clinically or microbiologically) with Grade 3 or 4 neutrophils (ANC <1.0 x 10^9/L) – *Select* '*Select*' AEs appear at the end of the CATEGORY.	Infection (documented clinically) with Grade 3 or 4 ANC – *Select*	—	Localized, local intervention indicated	IV antibiotic, antifungal, or antiviral intervention indicated; interventional radiology or operative intervention indicated	Life-threatening consequences (e.g., septic shock, hypotension, acidosis, necrosis)	Death
REMARK: Fever with Grade 3 or 4 neutrophils in the absence of documented infection is graded as Febrile neutropenia (fever of unknown origin without clinically or microbiologically documented infection).						
ALSO CONSIDER: Neutrophils/granulocytes (ANC/AGC).						
Infection with normal ANC or Grade 1 or 2 neutrophils – *Select* '*Select*' AEs appear at the end of the CATEGORY.	Infection with normal ANC – *Select*	—	Localized, local intervention indicated	IV antibiotic, antifungal, or antiviral intervention indicated; interventional radiology or operative intervention indicated	Life-threatening consequences (e.g., septic shock, hypotension, acidosis, necrosis)	Death

INFECTION

Page 2 of 3

Adverse Event	Short Name	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Infection with unknown ANC – *Select* '*Select*' AEs appear at the end of the CATEGORY.	Infection with unknown ANC – *Select*	—	Localized, local intervention indicated	IV antibiotic, antifungal, or antiviral intervention indicated; interventional radiology or operative intervention indicated	Life-threatening consequences (e.g., septic shock, hypotension, acidosis, necrosis)	Death
REMARK: Infection with unknown ANC – *Select* is to be used in the rare case when ANC is unknown.						
Opportunistic infection associated with ≥Grade 2 Lymphopenia	Opportunistic infection	—	Localized, local intervention indicated	IV antibiotic, antifungal, or antiviral intervention indicated; interventional radiology or operative intervention indicated	Life-threatening consequences (e.g., septic shock, hypotension, acidosis, necrosis)	Death
ALSO CONSIDER: Lymphopenia.						
Viral hepatitis	Viral hepatitis	Present; transaminases and liver function normal	Transaminases abnormal, liver function normal	Symptomatic liver dysfunction; fibrosis by biopsy; compensated cirrhosis	Decompensated liver function (e.g., ascites, coagulopathy, encephalopathy, coma)	Death
REMARK: Non-viral hepatitis is graded as Infection – *Select*.						
ALSO CONSIDER: Albumin, serum-low (hypoalbuminemia); ALT, SGPT (serum glutamic pyruvic transaminase); AST, SGOT (serum glutamic oxaloacetic transaminase); Bilirubin (hyperbilirubinemia); Encephalopathy.						
Infection – Other (Specify, __)	Infection – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

INFECTION – *SELECT*

Page 3 of 3

AUDITORY/EAR
- External ear (otitis externa)
- Middle ear (otitis media)

CARDIOVASCULAR
- Artery
- Heart (endocarditis)
- Spleen
- Vein

DERMATOLOGY/SKIN
- Lip/perioral
- Peristomal
- Skin (cellulitis)
- Ungual (nails)

GASTROINTESTINAL
- Abdomen NOS
- Anal/perianal
- Appendix
- Cecum
- Colon
- Dental-tooth
- Duodenum
- Esophagus
- Ileum
- Jejunum
- Oral cavity-gums (gingivitis)
- Peritoneal cavity
- Rectum
- Salivary gland
- Small bowel NOS
- Stomach

GENERAL
- Blood
- Catheter-related
- Foreign body (e.g., graft, implant, prosthesis, stent)
- Wound

HEPATOBILIARY/PANCREAS
- Biliary tree
- Gallbladder (cholecystitis)
- Liver
- Pancreas

LYMPHATIC
- Lymphatic

MUSCULOSKELETAL
- Bone (osteomyelitis)
- Joint
- Muscle (infection myositis)
- Soft tissue NOS

NEUROLOGY
- Brain (encephalitis, infectious)
- Brain + Spinal cord (encephalomyelitis)
- Meninges (meningitis)
- Nerve-cranial
- Nerve-peripheral
- Spinal cord (myelitis)

OCULAR
- Conjunctiva
- Cornea
- Eye NOS
- Lens

PULMONARY/UPPER RESPIRATORY
- Bronchus
- Larynx
- Lung (pneumonia)
- Mediastinum NOS
- Mucosa
- Neck NOS
- Nose
- Paranasal
- Pharynx
- Pleura (empyema)
- Sinus
- Trachea
- Upper aerodigestive NOS
- Upper airway NOS

RENAL/GENITOURINARY
- Bladder (urinary)
- Kidney
- Prostate
- Ureter
- Urethra
- Urinary tract NOS

SEXUAL/REPRODUCTIVE FUNCTION
- Cervix
- Fallopian tube
- Pelvis NOS
- Penis
- Scrotum
- Uterus
- Vagina
- Vulva

LYMPHATICS

Page 1 of 2

Adverse Event	Short Name	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Chyle or lymph leakage	Chyle or lymph leakage	Asymptomatic, clinical or radiographic findings	Symptomatic, medical intervention indicated	Interventional radiology or operative intervention indicated	Life-threatening complications	Death
ALSO CONSIDER: Chylothorax.						
Dermal change lymphedema, phlebolymphedema	Dermal change	Trace thickening or faint discoloration	Marked discoloration; leathery skin texture; papillary formation	—	—	—
REMARK: Dermal change lymphedema, phlebolymphedema refers to changes due to venous stasis.						
ALSO CONSIDER: Ulceration.						
Edema: head and neck	Edema: head and neck	Localized to dependent areas, no disability or functional impairment	Localized facial or neck edema with functional impairment	Generalized facial or neck edema with functional impairment (e.g., difficulty in turning neck or opening mouth compared to baseline)	Severe with ulceration or cerebral edema; tracheotomy or feeding tube indicated	Death
Edema: limb	Edema: limb	5 – 10% inter-limb discrepancy in volume or circumference at point of greatest visible difference; swelling or obscuration of anatomic architecture on close inspection; pitting edema	>10 – 30% inter-limb discrepancy in volume or circumference at point of greatest visible difference; readily apparent obscuration of anatomic architecture; obliteration of skin folds; readily apparent deviation from normal anatomic contour	>30% inter-limb discrepancy in volume; lymphorrhea; gross deviation from normal anatomic contour; interfering with ADL	Progression to malignancy (i.e., lymphangiosarcoma); amputation indicated; disabling	Death
Edema: trunk/genital	Edema: trunk/genital	Swelling or obscuration of anatomic architecture on close inspection; pitting edema	Readily apparent obscuration of anatomic architecture; obliteration of skin folds; readily apparent deviation from normal anatomic contour	Lymphorrhea; interfering with ADL; gross deviation from normal anatomic contour	Progression to malignancy (i.e., lymphangiosarcoma); disabling	Death
Edema: viscera	Edema: viscera	Asymptomatic; clinical or radiographic findings only	Symptomatic; medical intervention indicated	Symptomatic and unable to aliment adequately orally; interventional radiology or operative intervention indicated	Life-threatening consequences	Death

LYMPHATICS

Page 2 of 2

Adverse Event	Short Name	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Lymphedema-related fibrosis	Lymphedema-related fibrosis	Minimal to moderate redundant soft tissue, unresponsive to elevation or compression, with moderately firm texture or spongy feel	Marked increase in density and firmness, with or without tethering	Very marked density and firmness with tethering affecting ≥40% of the edematous area	—	—
Lymphocele	Lymphocele	Asymptomatic, clinical or radiographic findings only	Symptomatic; medical intervention indicated	Symptomatic and interventional radiology or operative intervention indicated	—	—
Phlebolymphatic cording	Phlebolymphatic cording	Asymptomatic, clinical findings only	Symptomatic; medical intervention indicated	Symptomatic and leading to contracture or reduced range of motion	—	—
Lymphatics – Other (Specify, __)	Lymphatics – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

METABOLIC/LABORATORY						Page 1 of 3
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Acidosis (metabolic or respiratory)	Acidosis	pH <normal, but ≥7.3	—	pH <7.3	pH <7.3 with life-threatening consequences	Death
Albumin, serum-low (hypoalbuminemia)	Hypoalbuminemia	<LLN – 3 g/dL <LLN – 30 g/L	<3 – 2 g/dL <30 – 20 g/L	<2 g/dL <20 g/L	—	Death
Alkaline phosphatase	Alkaline phosphatase	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.0 x ULN	—
Alkalosis (metabolic or respiratory)	Alkalosis	pH >normal, but ≤7.5	—	pH >7.5	pH >7.5 with life-threatening consequences	Death
ALT, SGPT (serum glutamic pyruvic transaminase)	ALT	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.0 x ULN	—
Amylase	Amylase	>ULN – 1.5 x ULN	>1.5 – 2.0 x ULN	>2.0 – 5.0 x ULN	>5.0 x ULN	—
AST, SGOT (serum glutamic oxaloacetic transaminase)	AST	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.0 x ULN	—
Bicarbonate, serum-low	Bicarbonate, serum-low	<LLN – 16 mmol/L	<16 – 11 mmol/L	<11 – 8 mmol/L	<8 mmol/L	Death
Bilirubin (hyperbilirubinemia)	Bilirubin	>ULN – 1.5 x ULN	>1.5 – 3.0 x ULN	>3.0 – 10.0 x ULN	>10.0 x ULN	—
REMARK: Jaundice is not an AE, but may be a manifestation of liver dysfunction/failure or elevated bilirubin. If jaundice is associated with elevated bilirubin, grade bilirubin.						
Calcium, serum-low (hypocalcemia)	Hypocalcemia	<LLN – 8.0 mg/dL <LLN – 2.0 mmol/L Ionized calcium: <LLN – 1.0 mmol/L	<8.0 – 7.0 mg/dL <2.0 – 1.75 mmol/L Ionized calcium: <1.0 – 0.9 mmol/L	<7.0 – 6.0 mg/dL <1.75 – 1.5 mmol/L Ionized calcium: <0.9 – 0.8 mmol/L	<6.0 mg/dL <1.5 mmol/L Ionized calcium: <0.8 mmol/L	Death
REMARK: Calcium can be falsely low if hypoalbuminemia is present. Serum albumin is <4.0 g/dL, hypocalcemia is reported after the following corrective calculation has been performed: Corrected Calcium (mg/dL) = Total Calcium (mg/dL) – 0.8 [Albumin (g/dL) – 4] [4]. Alternatively, direct measurement of ionized calcium is the definitive method to diagnose metabolically relevant alterations in serum calcium.						

[4] Crit Rev Clin Lab Sci 1984;21(1):51-97

METABOLIC/LABORATORY						Page 2 of 3
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Calcium, serum-high (hypercalcemia)	Hypercalcemia	>ULN – 11.5 mg/dL >ULN – 2.9 mmol/L Ionized calcium: >ULN – 1.5 mmol/L	>11.5 – 12.5 mg/dL >2.9 – 3.1 mmol/L Ionized calcium: >1.5 – 1.6 mmol/L	>12.5 – 13.5 mg/dL >3.1 – 3.4 mmol/L Ionized calcium: >1.6 – 1.8 mmol/L	>13.5 mg/dL >3.4 mmol/L Ionized calcium: >1.8 mmol/L	Death
Cholesterol, serum-high (hypercholesteremia)	Cholesterol	>ULN – 300 mg/dL >ULN – 7.75 mmol/L	>300 – 400 mg/dL >7.75 – 10.34 mmol/L	>400 – 500 mg/dL >10.34 – 12.92 mmol/L	>500 mg/dL >12.92 mmol/L	Death
CPK (creatine phosphokinase)	CPK	>ULN – 2.5 x ULN	>2.5 x ULN – 5 x ULN	>5 x ULN – 10 x ULN	>10 x ULN	Death
Creatinine	Creatinine	>ULN – 1.5 x ULN	>1.5 – 3.0 x ULN	>3.0 – 6.0 x ULN	>6.0 x ULN	Death
REMARK: Adjust to age-appropriate levels for pediatric patients. ALSO CONSIDER: Glomerular filtration rate.						
GGT (γ-Glutamyl transpeptidase)	GGT	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 20.0 x ULN	>20.0 x ULN	—
Glomerular filtration rate	GFR	<75 – 50% LLN	<50 – 25% LLN	<25% LLN, chronic dialysis not indicated	Chronic dialysis or renal transplant indicated	Death
ALSO CONSIDER: Creatinine.						
Glucose, serum-high (hyperglycemia)	Hyperglycemia	>ULN – 160 mg/dL >ULN – 8.9 mmol/L	>160 – 250 mg/dL >8.9 – 13.9 mmol/L	>250 – 500 mg/dL >13.9 – 27.8 mmol/L	>500 mg/dL >27.8 mmol/L or acidosis	Death
REMARK: Hyperglycemia, in general, is defined as fasting unless otherwise specified in protocol.						
Glucose, serum-low (hypoglycemia)	Hypoglycemia	<LLN – 55 mg/dL <LLN – 3.0 mmol/L	<55 – 40 mg/dL <3.0 – 2.2 mmol/L	<40 – 30 mg/dL <2.2 – 1.7 mmol/L	<30 mg/dL <1.7 mmol/L	Death
Hemoglobinuria	Hemoglobinuria	Present	—	—	—	Death
Lipase	Lipase	>ULN – 1.5 x ULN	>1.5 – 2.0 x ULN	>2.0 – 5.0 x ULN	>5.0 x ULN	—
Magnesium, serum-high (hypermagnesemia)	Hypermagnesemia	>ULN – 3.0 mg/dL >ULN – 1.23 mmol/L	—	>3.0 – 8.0 mg/dL >1.23 – 3.30 mmol/L	>8.0 mg/dL >3.30 mmol/L	Death
Magnesium, serum-low (hypomagnesemia)	Hypomagnesemia	<LLN – 1.2 mg/dL <LLN – 0.5 mmol/L	<1.2 – 0.9 mg/dL <0.5 – 0.4 mmol/L	<0.9 – 0.7 mg/dL <0.4 – 0.3 mmol/L	<0.7 mg/dL <0.3 mmol/L	Death
Phosphate, serum-low (hypophosphatemia)	Hypophosphatemia	<LLN – 2.5 mg/dL <LLN – 0.8 mmol/L	<2.5 – 2.0 mg/dL <0.8 – 0.6 mmol/L	<2.0 – 1.0 mg/dL <0.6 – 0.3 mmol/L	<1.0 mg/dL <0.3 mmol/L	Death
Potassium, serum-high (hyperkalemia)	Hyperkalemia	>ULN – 5.5 mmol/L	>5.5 – 6.0 mmol/L	>6.0 – 7.0 mmol/L	>7.0 mmol/L	Death

METABOLIC/LABORATORY						Page 3 of 3
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Potassium, serum-low (hypokalemia)	Hypokalemia	<LLN – 3.0 mmol/L	—	<3.0 – 2.5 mmol/L	<2.5 mmol/L	Death
Proteinuria	Proteinuria	1+ or 0.15 – 1.0 g/24 hrs	2+ to 3+ or >1.0 – 3.5 g/24 hrs	4+ or >3.5 g/24 hrs	Nephrotic syndrome	Death
Sodium, serum-high (hypernatremia)	Hypernatremia	>ULN – 150 mmol/L	>150 – 155 mmol/L	>155 – 160 mmol/L	>160 mmol/L	Death
Sodium, serum-low (hyponatremia)	Hyponatremia	<LLN – 130 mmol/L	—	<130 – 120 mmol/L	<120 mmol/L	Death
Triglyceride, serum-high (hypertriglyceridemia)	Hypertriglyceridemia	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 10 x ULN	>10 x ULN	Death
Uric acid, serum-high (hyperuricemia)	Hyperuricemia	>ULN – 10 mg/dL ≤0.59 mmol/L without physiologic consequences	—	>ULN – 10 mg/dL ≤0.59 mmol/L with physiologic consequences	>10 mg/dL >0.59 mmol/L	Death
ALSO CONSIDER: Creatinine; Potassium, serum-high (hyperkalemia); Renal failure; Tumor lysis syndrome.						
Metabolic/Laboratory – Other (Specify, __)	Metabolic/Lab – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

MUSCULOSKELETAL/SOFT TISSUE						Page 1 of 4
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Arthritis (non-septic)	Arthritis	Mild pain with inflammation, erythema, or joint swelling, but not interfering with function	Moderate pain with inflammation, erythema, or joint swelling interfering with function, but not interfering with ADL	Severe pain with inflammation, erythema, or joint swelling and interfering with ADL	Disabling	Death
REMARK: Report only when the diagnosis of arthritis (e.g., inflammation of a joint or a state characterized by inflammation of joints) is made. Arthralgia (sign or symptom of pain in a joint, especially non-inflammatory in character) is graded as Pain – *Select* in the PAIN CATEGORY.						
Bone: spine-scoliosis	Scoliosis	≤20 degrees; clinically undetectable	>20 – 45 degrees; visible by forward flexion; interfering with function but not interfering with ADL	>45 degrees; scapular prominence in forward flexion; operative intervention indicated; interfering with ADL	Disabling (e.g., interfering with cardiopulmonary function)	Death
Cervical spine-range of motion	Cervical spine ROM	Mild restriction of rotation or flexion between 60 – 70 degrees	Rotation <60 degrees to right or left; <60 degrees of flexion	Ankylosed/fused over multiple segments with no C-spine rotation	—	—
REMARK: 60 – 65 degrees of rotation is required for reversing a car; 60 – 65 degrees of flexion is required to tie shoes.						
Exostosis	Exostosis	Asymptomatic	Involving multiple sites; pain or interfering with function	Excision indicated	Progression to malignancy (i.e., chondrosarcoma)	Death
Extremity-lower (gait/walking)	Gait/walking	Limp evident only to trained observer and able to walk ≥1 kilometer; cane indicated for walking	Noticeable limp, or limitation of limb function, but able to walk ≥0.1 kilometer (1 city block); quad cane indicated for walking	Severe limp with stride modified to maintain balance (widened base of support, marked reduction in step length); ambulation limited to walker; crutches indicated	Unable to walk	—
ALSO CONSIDER: Ataxia (incoordination); Muscle weakness, generalized or specific area (not due to neuropathy) – *Select*.						
Extremity-upper (function)	Extremity-upper (function)	Able to perform most household or work activities with affected limb	Able to perform most household or work activities with compensation from unaffected limb	Interfering with ADL	Disabling; no function of affected limb	—
Fibrosis-cosmesis	Fibrosis-cosmesis	Visible only on close examination	Readily apparent but not disfiguring	Significant disfigurement; operative intervention indicated if patient chooses	—	—

MUSCULOSKELETAL/SOFT TISSUE						Page 2 of 4
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Fibrosis-deep connective tissue	Fibrosis-deep connective tissue	Increased density, "spongy" feel	Increased density with firmness or tethering	Increased density with fixation of tissue; operative intervention indicated; interfering with ADL	Life-threatening; disabling; loss of limb; interfering with vital organ function	Death
ALSO CONSIDER: Induration/fibrosis (skin and subcutaneous tissue); Muscle weakness, generalized or specific area (not due to neuropathy) – *Select*; Neuropathy: motor; Neuropathy: sensory.						
Fracture	Fracture	Asymptomatic, radiographic findings only (e.g., asymptomatic rib fracture on plain x-ray, pelvic insufficiency fracture on MRI, etc.)	Symptomatic but non-displaced; immobilization indicated	Symptomatic and displaced or open wound with bone exposure; operative intervention indicated	Disabling; amputation indicated	Death
Joint-effusion	Joint-effusion	Asymptomatic, clinical or radiographic findings only	Symptomatic; interfering with function but not interfering with ADL	Symptomatic and interfering with ADL	Disabling	Death
ALSO CONSIDER: Arthritis (non-septic).						
Joint-function[5]	Joint-function	Stiffness interfering with athletic activity; ≤25% loss of range of motion (ROM)	Stiffness interfering with function but not interfering with ADL; >25 – 50% decrease in ROM	Stiffness interfering with ADL; >50 – 75% decrease in ROM	Fixed or non-functional joint (arthrodesis); >75% decrease in ROM	—
ALSO CONSIDER: Arthritis (non-septic).						
Local complication – device/prosthesis-related	Device/prosthesis	Asymptomatic	Symptomatic, but not interfering with ADL; local wound care; medical intervention indicated	Symptomatic, interfering with ADL; operative intervention indicated (e.g., hardware/device replacement or removal, reconstruction)	Life-threatening; disabling; loss of limb or organ	Death
Lumbar spine-range of motion	Lumbar spine ROM	Stiffness and difficulty bending to the floor to pick up a very light object but able to do activity	Some lumbar spine flexion but requires a reaching aid to pick up a very light object from the floor	Ankylosed/fused over multiple segments with no L-spine flexion (i.e., unable to reach to floor to pick up a very light	—	—

[5] Adapted from the *International SFTR Method of Measuring and Recording Joint Motion, International Standard Orthopedic Measurements (ISOM),* Jon J. Gerhardt and Otto A. Russee, Bern, Switzerland, Han Huber 9 Publisher), 1975.

MUSCULOSKELETAL/SOFT TISSUE						Page 3 of 4
		Grade				
Adverse Event	Short Name	1	2	3	4	5
				object)		
Muscle weakness, generalized or specific area (not due to neuropathy) – *Select:* – Extraocular – Extremity-lower – Extremity-upper – Facial – Left-sided – Ocular – Pelvic – Right-sided – Trunk – Whole body/generalized	Muscle weakness – *Select*	Asymptomatic, weakness on physical exam	Symptomatic and interfering with function, but not interfering with ADL	Symptomatic and interfering with ADL	Life-threatening; disabling	Death
ALSO CONSIDER: Fatigue (asthenia, lethargy, malaise).						
Muscular/skeletal hypoplasia	Muscular/skeletal hypoplasia	Cosmetically and functionally insignificant hypoplasia	Deformity, hypoplasia, or asymmetry able to be remediated by prosthesis (e.g., shoe insert) or covered by clothing	Functionally significant deformity, hypoplasia, or asymmetry, unable to be remediated by prosthesis or covered by clothing	Disabling	—
Myositis (inflammation/damage of muscle)	Myositis	Mild pain, not interfering with function	Pain interfering with function, but not interfering with ADL	Pain interfering with ADL	Disabling	Death
REMARK: Myositis implies muscle damage (i.e., elevated CPK).						
ALSO CONSIDER: CPK (creatine phosphokinase); Pain – *Select*.						
Osteonecrosis (avascular necrosis)	Osteonecrosis	Asymptomatic, radiographic findings only	Symptomatic and interfering with function, but not interfering with ADL; minimal bone removal indicated (i.e., minor sequestrectomy)	Symptomatic and interfering with ADL; operative intervention or hyperbaric oxygen indicated	Disabling	Death

MUSCULOSKELETAL/SOFT TISSUE						Page 4 of 4
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Osteoporosis[6]	Osteoporosis	Radiographic evidence of osteoporosis or Bone Mineral Density (BMD) t-score –1 to –2.5 (osteopenia) and no loss of height or therapy indicated	BMD t-score < –2.5; loss of height <2 cm; anti-osteoporotic therapy indicated	Fractures; loss of height ≥2 cm	Disabling	Death
Seroma	Seroma	Asymptomatic	Symptomatic; medical intervention or simple aspiration indicated	Symptomatic, interventional radiology or operative intervention indicated	—	—
Soft tissue necrosis – *Select:* – Abdomen – Extremity-lower – Extremity-upper – Head – Neck – Pelvic – Thorax	Soft tissue necrosis – *Select*	—	Local wound care; medical intervention indicated	Operative debridement or other invasive intervention indicated (e.g., hyperbaric oxygen)	Life-threatening consequences; major invasive intervention indicated (e.g., tissue reconstruction, flap, or grafting)	Death
Trismus (difficulty, restriction or pain when opening mouth)	Trismus	Decreased range of motion without impaired eating	Decreased range of motion requiring small bites, soft foods or purees	Decreased range of motion with inability to adequately aliment or hydrate orally	—	—
NAVIGATION NOTE: Wound-infectious is graded as Infection – *Select* in the INFECTION CATEGORY.						
NAVIGATION NOTE: Wound non-infectious is graded as Wound complication, non-infectious in the DERMATOLOGY/SKIN CATEGORY.						
Musculoskeletal/Soft Tissue – Other (Specify. ___)	Musculoskeletal – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

[6] "Assessment of Fracture Risk and its Application to Screening for Postmenopausal Osteoporosis," Report of a *WHO Study Group Technical Report Series*, No. 843, 1994, v + 129 pages [C*, E, F, R, S], ISBN 92 4 120843 0, Sw.fr. 22.-/US $19.80; in developing countries: Sw.fr. 15.40, Order no. 1100843

NEUROLOGY						Page 1 of 5
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
NAVIGATION NOTE: ADD (Attention Deficit Disorder) is graded as Cognitive disturbance.						
NAVIGATION NOTE: Aphasia, receptive and/or expressive, is graded as Speech impairment (e.g., dysphasia or aphasia).						
Apnea	Apnea	—	—	Present	Intubation indicated	Death
Arachnoiditis/ meningismus/radiculitis	Arachnoiditis	Symptomatic, not interfering with function; medical intervention indicated	Symptomatic (e.g., photophobia, nausea) interfering with function but not interfering with ADL	Symptomatic, interfering with ADL	Life-threatening; disabling (e.g., paraplegia)	Death
ALSO CONSIDER: Fever (in the absence of neutropenia, where neutropenia is defined as ANC <1.0 x 10^9/L); Infection (documented clinically or microbiologically) with Grade 3 or 4 neutrophils (ANC <1.0 x 109/L) – *Select*; Infection with normal ANC or Grade 1 or 2 neutrophils – *Select*; Infection with unknown ANC – *Select*; Pain – *Select*; Vomiting.						
Ataxia (incoordination)	Ataxia	Asymptomatic	Symptomatic, not interfering with ADL	Symptomatic, interfering with ADL; mechanical assistance indicated	Disabling	Death
REMARK: Ataxia (incoordination) refers to the consequence of medical or operative intervention.						
Brachial plexopathy	Brachial plexopathy	Asymptomatic	Symptomatic, not interfering with ADL	Symptomatic, interfering with ADL	Disabling	Death
CNS cerebrovascular ischemia	CNS ischemia	—	Asymptomatic, radiographic findings only	Transient ischemic event or attack (TIA) ≤24 hrs duration	Cerebral vascular accident (CVA, stroke), neurologic deficit >24 hrs	Death
NAVIGATION NOTE: CNS hemorrhage/bleeding is graded as Hemorrhage, CNS in the HEMORRHAGE/BLEEDING CATEGORY.						
CNS necrosis/cystic progression	CNS necrosis	Asymptomatic, radiographic findings only	Symptomatic, not interfering with ADL; medical intervention indicated	Symptomatic and interfering with ADL; hyperbaric oxygen indicated	Life-threatening; disabling; operative intervention indicated to prevent or treat CNS necrosis/cystic progression	Death
Cognitive disturbance	Cognitive disturbance	Mild cognitive disability; not interfering with work/school/life performance; specialized educational services/devices not indicated	Moderate cognitive disability; interfering with work/school/life performance but capable of independent living; specialized resources on part-time basis indicated	Severe cognitive disability; significant impairment of work/school/life performance	Unable to perform ADL; full-time specialized resources or institutionalization indicated	Death
REMARK: Cognitive disturbance may be used for Attention Deficit Disorder (ADD).						

NEUROLOGY						Page 2 of 5
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Confusion	Confusion	Transient confusion, disorientation, or attention deficit	Confusion, disorientation, or attention deficit interfering with function, but not interfering with ADL	Confusion or delirium interfering with ADL	Harmful to others or self; hospitalization indicated	Death
REMARK: Attention Deficit Disorder (ADD) is graded as Cognitive disturbance.						
NAVIGATION NOTE: Cranial neuropathy is graded as Neuropathy-cranial – *Select*.						
Dizziness	Dizziness	With head movements or nystagmus only; not interfering with function	Interfering with function, but not interfering with ADL	Interfering with ADL	Disabling	—
REMARK: Dizziness includes disequilibrium, lightheadedness, and vertigo. ALSO CONSIDER: Neuropathy: cranial – *Select*; Syncope (fainting).						
NAVIGATION NOTE: Dysphasia, receptive and/or expressive, is graded as Speech impairment (e.g., dysphasia or aphasia).						
Encephalopathy	Encephalopathy	—	Mild signs or symptoms; not interfering with ADL	Signs or symptoms interfering with ADL; hospitalization indicated	Life-threatening; disabling	Death
ALSO CONSIDER: Cognitive disturbance; Confusion; Dizziness; Memory impairment; Mental status; Mood alteration – *Select*; Psychosis (hallucinations/delusions); Somnolence/depressed level of consciousness.						
Extrapyramidal/ involuntary movement/ restlessness	Involuntary movement	Mild involuntary movements not interfering with function	Moderate involuntary movements interfering with function, but not interfering with ADL	Severe involuntary movements or torticollis interfering with ADL	Disabling	Death
NAVIGATION NOTE: Headache/neuropathic pain (e.g., jaw pain, neurologic pain, phantom limb pain, post-infectious neuralgia, or painful neuropathies) is graded as Pain – *Select* in the PAIN CATEGORY.						
Hydrocephalus	Hydrocephalus	Asymptomatic, radiographic findings only	Mild to moderate symptoms not interfering with ADL	Severe symptoms or neurological deficit interfering with ADL	Disabling	Death
Irritability (children <3 years of age)	Irritability	Mild; easily consolable	Moderate; requiring increased attention	Severe; inconsolable	—	—
Laryngeal nerve dysfunction	Laryngeal nerve	Asymptomatic, weakness on clinical examination/testing only	Symptomatic, but not interfering with ADL; intervention not indicated	Symptomatic, interfering with ADL; intervention indicated (e.g., thyroplasty, vocal cord injection)	Life-threatening; tracheostomy indicated	Death

NEUROLOGY						Page 3 of 5
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Leak, cerebrospinal fluid (CSF)	CSF leak	Transient headache; postural care indicated	Symptomatic, not interfering with ADL; blood patch indicated	Symptomatic, interfering with ADL; operative intervention indicated	Life-threatening; disabling	Death
REMARK: Leak, cerebrospinal fluid (CSF) may be used for CSF leak associated with operation and persisting >72 hours.						
Leukoencephalopathy (radiographic findings)	Leukoencephalopathy	Mild increase in subarachnoid space (SAS); mild ventriculomegaly; small (+/- multiple) focal T2 hyperintensities, involving periventricular white matter or <1/3 of susceptible areas of cerebrum	Moderate increase in SAS; moderate ventriculomegaly; focal T2 hyperintensities extending into centrum ovale or involving 1/3 to 2/3 of susceptible areas of cerebrum	Severe increase in SAS; severe ventriculomegaly; near total white matter T2 hyperintensities or diffuse low attenuation (CT)	—	—
REMARK: Leukoencephalopathy is a diffuse white matter process, specifically NOT associated with necrosis. Leukoencephalopathy (radiographic findings) does not include lacunas, which are areas that become void of neural tissue.						
Memory impairment	Memory impairment	Memory impairment not interfering with function	Memory impairment interfering with function, but not interfering with ADL	Memory impairment interfering with ADL	Amnesia	—
Mental status[7]	Mental status	—	1 – 3 point below age and educational norm in Folstein Mini-Mental Status Exam (MMSE)	>3 point below age and educational norm in Folstein MMSE	—	—
Mood alteration – *Select*: – Agitation – Anxiety – Depression – Euphoria	Mood alteration – *Select*	Mild mood alteration not interfering with function	Moderate mood alteration interfering with function, but not interfering with ADL; medication indicated	Severe mood alteration interfering with ADL	Suicidal ideation; danger to self or others	Death
Myelitis	Myelitis	Asymptomatic, mild signs (e.g., Babinski's or Lhermitte's sign)	Weakness or sensory loss not interfering with ADL	Weakness or sensory loss interfering with ADL	Disabling	Death

[7] Folstein MF, Folstein, SE and McHugh PR (1975) "Mini-Mental State: A Practical Method for Grading the State of Patients for the Clinician," *Journal of Psychiatric Research*, 12: 189-198

NEUROLOGY						Page 4 of 5
		Grade				
Adverse Event	Short Name	1	2	3	4	5
NAVIGATION NOTE: Neuropathic pain is graded as Pain – *Select* in the PAIN CATEGORY.						
Neuropathy: cranial – *Select*:	Neuropathy: cranial – *Select*	Asymptomatic, detected on exam/testing only	Symptomatic, not interfering with ADL	Symptomatic, interfering with ADL	Life-threatening; disabling	Death
– CN I Smell – CN II Vision – CN III Pupil, upper eyelid, extra ocular movements – CN IV Downward, inward movement of eye – CN V Motor-jaw muscles; Sensory-facial – CN VI Lateral deviation of eye – CN VII Motor-face; Sensory-taste – CN VIII Hearing and balance – CN IX Motor-pharynx; Sensory-ear, pharynx, tongue – CN X Motor-palate; pharynx, larynx – CN XI Motor-sternomastoid and trapezius – CN XII Motor-tongue						
Neuropathy: motor	Neuropathy-motor	Asymptomatic, weakness on exam/testing only	Symptomatic weakness interfering with function, but not interfering with ADL	Weakness interfering with ADL; bracing or assistance to walk (e.g., cane or walker) indicated	Life-threatening; disabling (e.g., paralysis)	Death
REMARK: Cranial nerve motor neuropathy is graded as Neuropathy: cranial – *Select*. ALSO CONSIDER: Laryngeal nerve dysfunction; Phrenic nerve dysfunction.						
Neuropathy: sensory	Neuropathy-sensory	Asymptomatic; loss of deep tendon reflexes or paresthesia (including tingling) but not interfering with function	Sensory alteration or paresthesia (including tingling), interfering with function, but not interfering with ADL	Sensory alteration or paresthesia interfering with ADL	Disabling	Death
REMARK: Cranial nerve sensory neuropathy is graded as Neuropathy: cranial – *Select*.						
Personality/behavioral	Personality	Change, but not adversely affecting patient or family	Change, adversely affecting patient or family	Mental health intervention indicated	Change harmful to others or self; hospitalization indicated	Death
Phrenic nerve dysfunction	Phrenic nerve	Asymptomatic weakness on exam/testing only	Symptomatic but not interfering with ADL; intervention not indicated	Significant dysfunction; intervention indicated (e.g., diaphragmatic plication)	Life-threatening respiratory compromise; mechanical ventilation indicated	Death
Psychosis (hallucinations/ delusions)	Psychosis	—	Transient episode	Interfering with ADL; medication, supervision	Harmful to others or self; life-threatening	Death

NEUROLOGY						Page 5 of 5
		Grade				
Adverse Event	Short Name	1	2	3	4	5
				or restraints indicated	consequences	
Pyramidal tract dysfunction (e.g., ↑ tone, hyperreflexia, positive Babinski, ↓ fine motor coordination)	Pyramidal tract dysfunction	Asymptomatic, abnormality on exam or testing only	Symptomatic; interfering with function but not interfering with ADL	Interfering with ADL	Disabling; paralysis	Death
Seizure	Seizure	—	One brief generalized seizure; seizure(s) well controlled by anticonvulsants or infrequent focal motor seizures not interfering with ADL	Seizures in which consciousness is altered; poorly controlled seizure disorder, with breakthrough generalized seizures despite medical intervention	Seizures of any kind which are prolonged, repetitive, or difficult to control (e.g., status epilepticus, intractable epilepsy)	Death
Somnolence/depressed level of consciousness	Somnolence	—	Somnolence or sedation interfering with function, but not interfering with ADL	Obtundation or stupor; difficult to arouse; interfering with ADL	Coma	Death
Speech impairment (e.g., dysphasia or aphasia)	Speech impairment	—	Awareness of receptive or expressive dysphasia, not impairing ability to communicate	Receptive or expressive dysphasia, impairing ability to communicate	Inability to communicate	—
REMARK: Speech impairment refers to a primary CNS process, not neuropathy or end organ dysfunction. ALSO CONSIDER: Laryngeal nerve dysfunction; Voice changes/dysarthria (e.g., hoarseness, loss, or alteration in voice, laryngitis).						
Syncope (fainting)	Syncope (fainting)	—	—	Present	Life-threatening consequences	Death
ALSO CONSIDER: CNS cerebrovascular ischemia; Conduction abnormality/atrioventricular heart block – *Select*; Dizziness; Supraventricular and nodal arrhythmia – *Select*; Vasovagal episode; Ventricular arrhythmia – *Select*.						
NAVIGATION NOTE: Taste alteration (CN VII, IX) is graded as Taste alteration (dysgeusia) in the GASTROINTESTINAL CATEGORY.						
Tremor	Tremor	Mild and brief or intermittent but not interfering with function	Moderate tremor interfering with function, but not interfering with ADL	Severe tremor interfering with ADL	Disabling	—
Neurology – Other (Specify, __)	Neurology – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

OCULAR/VISUAL						Page 1 of 3
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Cataract	Cataract	Asymptomatic, detected on exam only	Symptomatic, with moderate decrease in visual acuity (20/40 or better); decreased visual function correctable with glasses	Symptomatic with marked decrease in visual acuity (worse than 20/40); operative intervention indicated (e.g., cataract surgery)	—	—
Dry eye syndrome	Dry eye	Mild, intervention not indicated	Symptomatic, interfering with function but not interfering with ADL; medical intervention indicated	Symptomatic or decrease in visual acuity interfering with ADL; operative intervention indicated	—	—
Eyelid dysfunction	Eyelid dysfunction	Asymptomatic	Symptomatic, interfering with function but not ADL; requiring topical agents or epilation	Symptomatic; interfering with ADL; surgical intervention indicated	—	—
REMARK: Eyelid dysfunction includes canalicular stenosis, ectropion, entropion, erythema, madarosis, symblepharon, telangiectasis, thickening, and trichiasis. ALSO CONSIDER: Neuropathy: cranial – *Select*.						
Glaucoma	Glaucoma	Elevated intraocular pressure (EIOP) with single topical agent for intervention; no visual field deficit	EIOP causing early visual field deficit (i.e., nasal step or arcuate deficit); multiple topical or oral agents indicated	EIOP causing marked visual field deficits (i.e., involving both superior and inferior visual fields); operative intervention indicated	EIOP resulting in blindness (20/200 or worse); enucleation indicated	—
Keratitis (corneal inflammation/corneal ulceration)	Keratitis	Abnormal ophthalmologic changes only; intervention not indicated	Symptomatic and interfering with function, but not interfering with ADL	Symptomatic and interfering with ADL; operative intervention indicated	Perforation or blindness (20/200 or worse)	—
NAVIGATION NOTE: Ocular muscle weakness is graded as Muscle weakness, generalized or specific area (not due to neuropathy) – *Select* in the MUSCULOSKELETAL/SOFT TISSUE CATEGORY.						
Night blindness (nyctalopia)	Nyctalopia	Symptomatic, not interfering with function	Symptomatic and interfering with function but not interfering with ADL	Symptomatic and interfering with ADL	Disabling	—

OCULAR/VISUAL						Page 2 of 3
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Nystagmus	Nystagmus	Asymptomatic	Symptomatic and interfering with function but not interfering with ADL	Symptomatic and interfering with ADL	Disabling	—
ALSO CONSIDER: Neuropathy: cranial – *Select*; Ophthalmoplegia/diplopia (double vision).						
Ocular surface disease	Ocular surface disease	Asymptomatic or minimally symptomatic but not interfering with function	Symptomatic, interfering with function but not interfering with ADL; topical antibiotics or other topical intervention indicated	Symptomatic, interfering with ADL; operative intervention indicated	—	—
REMARK: Ocular surface disease includes conjunctivitis, keratoconjunctivitis sicca, chemosis, keratinization, and palpebral conjunctival epithelial metaplasia.						
Ophthalmoplegia/ diplopia (double vision)	Diplopia	Intermittently symptomatic, intervention not indicated	Symptomatic and interfering with function but not interfering with ADL	Symptomatic and interfering with ADL; surgical intervention indicated	Disabling	—
ALSO CONSIDER: Neuropathy: cranial – *Select*.						
Optic disc edema	Optic disc edema	Asymptomatic	Decreased visual acuity (20/40 or better); visual field defect present	Decreased visual acuity (worse than 20/40); marked visual field defect but sparing the central 20 degrees	Blindness (20/200 or worse)	—
ALSO CONSIDER: Neuropathy: cranial – *Select*.						
Proptosis/enophthalmos	Proptosis/enophthalmos	Asymptomatic, intervention not indicated	Symptomatic and interfering with function, but not interfering with ADL	Symptomatic and interfering with ADL	—	—
Retinal detachment	Retinal detachment	Exudative; no central vision loss; intervention not indicated	Exudative and visual acuity 20/40 or better but intervention not indicated	Rhegmatogenous or exudative detachment; operative intervention indicated	Blindness (20/200 or worse)	—
Retinopathy	Retinopathy	Asymptomatic	Symptomatic with moderate decrease in visual acuity (20/40 or better)	Symptomatic with marked decrease in visual acuity (worse than 20/40)	Blindness (20/200 or worse)	—

OCULAR/VISUAL						Page 3 of 3
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Scleral necrosis/melt	Scleral necrosis	Asymptomatic or symptomatic but not interfering with function	Symptomatic, interfering with function but not interfering with ADL; moderate decrease in visual acuity (20/40 or better); medical intervention indicated	Symptomatic, interfering with ADL; marked decrease in visual acuity (worse than 20/40); operative intervention indicated	Blindness (20/200 or worse); painful eye with enucleation indicated	—
Uveitis	Uveitis	Asymptomatic	Anterior uveitis; medical intervention indicated	Posterior or pan-uveitis; operative intervention indicated	Blindness (20/200 or worse)	—
Vision-blurred vision	Blurred vision	Symptomatic not interfering with function	Symptomatic and interfering with function, but not interfering with ADL	Symptomatic and interfering with ADL	Disabling	—
Vision-flashing lights/floaters	Flashing lights	Symptomatic not interfering with function	Symptomatic and interfering with function, but not interfering with ADL	Symptomatic and interfering with ADL	Disabling	—
Vision-photophobia	Photophobia	Symptomatic not interfering with function	Symptomatic and interfering with function, but not interfering with ADL	Symptomatic and interfering with ADL	Disabling	—
Vitreous hemorrhage	Vitreous hemorrhage	Asymptomatic, clinical findings only	Symptomatic, interfering with function, but not interfering with ADL; intervention not indicated	Symptomatic, interfering with ADL; vitrectomy indicated	—	—
Watery eye (epiphora, tearing)	Watery eye	Symptomatic, intervention not indicated	Symptomatic, interfering with function but not interfering with ADL	Symptomatic, interfering with ADL	—	—
Ocular/Visual – Other (Specify, __)	Ocular – Other (Specify)	Symptomatic not interfering with function	Symptomatic and interfering with function, but not interfering with ADL	Symptomatic and interfering with ADL	Blindness (20/200 or worse)	Death

PAIN

Page 1 of 1

Adverse Event	Short Name	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Pain – *Select*: '*Select*' AEs appear at the end of the CATEGORY.	Pain – *Select*	Mild pain not interfering with function	Moderate pain; pain or analgesics interfering with function, but not interfering with ADL	Severe pain; pain or analgesics severely interfering with ADL	Disabling	—
Pain – Other (Specify, __)	Pain – Other (Specify)	Mild pain not interfering with function	Moderate pain; pain or analgesics interfering with function, but not interfering with ADL	Severe pain; pain or analgesics severely interfering with ADL	Disabling	—

PAIN – *SELECT*

AUDITORY/EAR
- External ear
- Middle ear

CARDIOVASCULAR
- Cardiac/heart
- Pericardium

DERMATOLOGY/SKIN
- Face
- Lip
- Oral-gums
- Scalp
- Skin

GASTROINTESTINAL
- Abdomen NOS
- Anus
- Dental/teeth/peridontal
- Esophagus
- Oral cavity
- Peritoneum
- Rectum
- Stomach

GENERAL
- Pain NOS
- Tumor pain

HEPATOBILIARY/PANCREAS
- Gallbladder
- Liver

LYMPHATIC
- Lymph node

MUSCULOSKELETAL
- Back
- Bone
- Buttock
- Extremity-limb
- Intestine
- Joint
- Muscle
- Neck
- Phantom (pain associated with missing limb)

NEUROLOGY
- Head/headache
- Neuralgia/peripheral nerve

OCULAR
- Eye

PULMONARY/UPPER RESPIRATORY
- Chest wall
- Chest/thorax NOS

PULMONARY/UPPER RESPIRATORY (*continued*)
- Larynx
- Pleura
- Sinus
- Throat/pharynx/larynx

RENAL/GENITOURINARY
- Bladder
- Kidney

SEXUAL/REPRODUCTIVE FUNCTION
- Breast
- Ovulatory
- Pelvis
- Penis
- Perineum
- Prostate
- Scrotum
- Testicle
- Urethra
- Uterus
- Vagina

PULMONARY/UPPER RESPIRATORY						Page 1 of 4
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Adult Respiratory Distress Syndrome (ARDS)	ARDS	—	—	Present, intubation not indicated	Present, intubation indicated	Death
ALSO CONSIDER: Dyspnea (shortness of breath); Hypoxia; Pneumonitis/pulmonary infiltrates.						
Aspiration	Aspiration	Asymptomatic ("silent aspiration"); endoscopy or radiographic (e.g., barium swallow) findings	Symptomatic (e.g., altered eating habits, coughing or choking episodes consistent with aspiration); medical intervention indicated (e.g., antibiotics, suction or oxygen)	Clinical or radiographic signs of pneumonia or pneumonitis; unable to aliment orally	Life-threatening (e.g., aspiration pneumonia or pneumonitis)	Death
ALSO CONSIDER: Infection (documented clinically or microbiologically) with Grade 3 or 4 neutrophils (ANC <1.0 x 10^9/L) – *Select;* Infection with normal ANC or Grade 1 or 2 neutrophils – *Select;* Infection with unknown ANC – *Select;* Laryngeal nerve dysfunction; Neuropathy: cranial – *Select;* Pneumonitis/pulmonary infiltrates.						
Atelectasis	Atelectasis	Asymptomatic	Symptomatic (e.g., dyspnea, cough), medical intervention indicated (e.g., bronchoscopic suctioning, chest physiotherapy, suctioning)	Operative (e.g., stent, laser) intervention indicated	Life-threatening respiratory compromise	Death
ALSO CONSIDER: Adult Respiratory Distress Syndrome (ARDS); Cough; Dyspnea (shortness of breath); Hypoxia; Infection (documented clinically or microbiologically) with Grade 3 or 4 neutrophils (ANC <1.0 x 109/L) – *Select;* Infection with normal ANC or Grade 1 or 2 neutrophils – *Select;* Infection with unknown ANC – *Select;* Obstruction/stenosis of airway – *Select;* Pneumonitis/pulmonary infiltrates; Pulmonary fibrosis (radiographic changes).						
Bronchospasm, wheezing	Bronchospasm	Asymptomatic	Symptomatic not interfering with function	Symptomatic interfering with function	Life-threatening	Death
ALSO CONSIDER: Allergic reaction/hypersensitivity (including drug fever); Dyspnea (shortness of breath).						
Carbon monoxide diffusion capacity (DL_{CO})	DL_{CO}	90 – 75% of predicted value	<75 – 50% of predicted value	<50 – 25% of predicted value	<25% of predicted value	Death
ALSO CONSIDER: Hypoxia; Pneumonitis/pulmonary infiltrates; Pulmonary fibrosis (radiographic changes).						
Chylothorax	Chylothorax	Asymptomatic	Symptomatic; thoracentesis or tube drainage indicated	Operative intervention indicated	Life-threatening (e.g., hemodynamic instability or ventilatory support indicated)	Death
Cough	Cough	Symptomatic, non-narcotic medication only indicated	Symptomatic and narcotic medication indicated	Symptomatic and significantly interfering with sleep or ADL	—	—

PULMONARY/UPPER RESPIRATORY						Page 2 of 4
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Dyspnea (shortness of breath)	Dyspnea	Dyspnea on exertion, but can walk 1 flight of stairs without stopping	Dyspnea on exertion but unable to walk 1 flight of stairs or 1 city block (0.1km) without stopping	Dyspnea with ADL	Dyspnea at rest; intubation/ventilator indicated	Death
ALSO CONSIDER: Hypoxia; Neuropathy: motor; Pneumonitis/pulmonary infiltrates; Pulmonary fibrosis (radiographic changes).						
Edema, larynx	Edema, larynx	Asymptomatic edema by exam only	Symptomatic edema, no respiratory distress	Stridor; respiratory distress; interfering with ADL	Life-threatening airway compromise; tracheotomy, intubation, or laryngectomy indicated	Death
ALSO CONSIDER: Allergic reaction/hypersensitivity (including drug fever).						
FEV_1	FEV_1	90 – 75% of predicted value	<75 – 50% of predicted value	<50 – 25% of predicted value	<25% of predicted	Death
Fistula, pulmonary/upper respiratory – *Select* – Bronchus – Larynx – Lung – Oral cavity – Pharynx – Pleura – Trachea	Fistula, pulmonary – *Select*	Asymptomatic, radiographic findings only	Symptomatic, tube thoracostomy or medical management indicated; associated with altered respiratory function but not interfering with ADL	Symptomatic and associated with altered respiratory function interfering with ADL; or endoscopic (e.g., stent) or primary closure by operative intervention indicated	Life-threatening consequences; operative intervention with thoracoplasty, chronic open drainage or multiple thoracotomies indicated	Death
REMARK: A fistula is defined as an abnormal communication between two body cavities, potential spaces, and/or the skin. The site indicated for a fistula should be the site from which the abnormal process is believed to have arisen. For example, a tracheo-esophageal fistula arising in the context of a resected or irradiated esophageal cancer should be graded as Fistula, GI – esophagus in the GASTROINTESTINAL CATEGORY.						
NAVIGATION NOTE: Hemoptysis is graded as Hemorrhage, pulmonary/upper respiratory – *Select* in the HEMORRHAGE/BLEEDING CATEGORY.						
Hiccoughs (hiccups, singultus)	Hiccoughs	Symptomatic, intervention not indicated	Symptomatic, intervention indicated	Symptomatic, significantly interfering with sleep or ADL	—	—
Hypoxia	Hypoxia	—	Decreased O_2 saturation with exercise (e.g., pulse oximeter <88%); intermittent supplemental oxygen	Decreased O_2 saturation at rest; continuous oxygen indicated	Life-threatening; intubation or ventilation indicated	Death

PULMONARY/UPPER RESPIRATORY						Page 3 of 4
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Nasal cavity/paranasal sinus reactions	Nasal/paranasal reactions	Asymptomatic mucosal crusting, blood-tinged secretions	Symptomatic stenosis or edema/narrowing interfering with airflow	Stenosis with significant nasal obstruction; interfering with ADL	Necrosis of soft tissue or bone	Death
ALSO CONSIDER: Infection (documented clinically or microbiologically) with Grade 3 or 4 neutrophils (ANC <1.0 x 10^9/L) – *Select;* Infection with normal ANC or Grade 1 or 2 neutrophils – *Select;* Infection with unknown ANC – *Select.*						
Obstruction/stenosis of airway – *Select:* – Bronchus – Larynx – Pharynx – Trachea	Airway obstruction – *Select*	Asymptomatic obstruction or stenosis on exam, endoscopy, or radiograph	Symptomatic (e.g., noisy airway breathing), but causing no respiratory distress; medical management indicated (e.g., steroids)	Interfering with ADL; stridor or endoscopic intervention indicated (e.g., stent, laser)	Life-threatening airway compromise; tracheotomy or intubation indicated	Death
Pleural effusion (non-malignant)	Pleural effusion	Asymptomatic	Symptomatic, intervention such as diuretics or up to 2 therapeutic thoracenteses indicated	Symptomatic and supplemental oxygen, >2 therapeutic thoracenteses, tube drainage, or pleurodesis indicated	Life-threatening (e.g., causing hemodynamic instability or ventilatory support indicated)	Death
ALSO CONSIDER: Atelectasis; Cough; Dyspnea (shortness of breath); Hypoxia; Pneumonitis/pulmonary infiltrates; Pulmonary fibrosis (radiographic changes).						
NAVIGATION NOTE: Pleuritic pain is graded as Pain – *Select* in the PAIN CATEGORY.						
Pneumonitis/pulmonary infiltrates	Pneumonitis	Asymptomatic, radiographic findings only	Symptomatic, not interfering with ADL	Symptomatic, interfering with ADL; O_2 indicated	Life-threatening; ventilatory support indicated	Death
ALSO CONSIDER: Adult Respiratory Distress Syndrome (ARDS); Cough; Dyspnea (shortness of breath); Hypoxia; Infection (documented clinically or microbiologically) with Grade 3 or 4 neutrophils (ANC <1.0 x 10^9/L) – *Select;* Infection with normal ANC or Grade 1 or 2 neutrophils – *Select;* Infection with unknown ANC – *Select;* Pneumonitis/pulmonary infiltrates; Pulmonary fibrosis (radiographic changes).						
Pneumothorax	Pneumothorax	Asymptomatic, radiographic findings only	Symptomatic; intervention indicated (e.g., hospitalization for observation, tube placement without sclerosis)	Sclerosis and/or operative intervention indicated	Life-threatening, causing hemodynamic instability (e.g., tension pneumothorax); ventilatory support indicated	Death
Prolonged chest tube drainage or air leak after pulmonary resection	Chest tube drainage or leak	—	Sclerosis or additional tube thoracostomy indicated	Operative intervention indicated (e.g., thoracotomy with stapling or sealant application)	Life-threatening; debilitating; organ resection indicated	Death

PULMONARY/UPPER RESPIRATORY						Page 4 of 4
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Prolonged intubation after pulmonary resection (>24 hrs after surgery)	Prolonged intubation	—	Extubated within 24 – 72 hrs postoperatively	Extubated >72 hrs postoperatively, but before tracheostomy indicated	Tracheostomy indicated	Death
NAVIGATION NOTE: Pulmonary embolism is graded as Grade 4 either as Thrombosis/embolism (vascular access-related) or Thrombosis/thrombus/embolism in the VASCULAR CATEGORY.						
Pulmonary fibrosis (radiographic changes)	Pulmonary fibrosis	Minimal radiographic findings (or patchy or bi-basilar changes) with estimated radiographic proportion of total lung volume that is fibrotic of <25%	Patchy or bi-basilar changes with estimated radiographic proportion of total lung volume that is fibrotic of 25 – <50%	Dense or widespread infiltrates/consolidation with estimated radiographic proportion of total lung volume that is fibrotic of 50 – <75%	Estimated radiographic proportion of total lung volume that is fibrotic is ≥75%; honeycombing	Death
REMARK: Fibrosis is usually a "late effect" seen >3 months after radiation or combined modality therapy (including surgery). It is thought to represent scar/fibrotic lung tissue. It may be difficult to distinguish from pneumonitis that is generally seen within 3 months of radiation or combined modality therapy.						
ALSO CONSIDER: Adult Respiratory Distress Syndrome (ARDS); Cough; Dyspnea (shortness of breath); Hypoxia; Infection (documented clinically or microbiologically) with Grade 3 or 4 neutrophils (ANC <1.0 x 10^9/L) – *Select;* Infection with normal ANC or Grade 1 or 2 neutrophils – *Select;* Infection with unknown ANC – *Select*.						
NAVIGATION NOTE: Recurrent laryngeal nerve dysfunction is graded as Laryngeal nerve dysfunction in the NEUROLOGY CATEGORY.						
Vital capacity	Vital capacity	90 – 75% of predicted value	<75 – 50% of predicted value	<50 – 25% of predicted value	<25% of predicted value	Death
Voice changes/dysarthria (e.g., hoarseness, loss or alteration in voice, laryngitis)	Voice changes	Mild or intermittent hoarseness or voice change, but fully understandable	Moderate or persistent voice changes, may require occasional repetition but understandable on telephone	Severe voice changes including predominantly whispered speech; may require frequent repetition or face-to-face contact for understandability; requires voice aid (e.g., electrolarynx) for ≤50% of communication	Disabling; non-understandable voice or aphonic; requires voice aid (e.g., electrolarynx) for >50% of communication or requires >50% written communication	Death
ALSO CONSIDER: Laryngeal nerve dysfunction; Speech impairment (e.g., dysphasia or aphasia).						
Pulmonary/Upper Respiratory – Other (Specify. __)	Pulmonary – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

RENAL/GENITOURINARY						Page 1 of 3
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Bladder spasms	Bladder spasms	Symptomatic, intervention not indicated	Symptomatic, antispasmodics indicated	Narcotics indicated	Major surgical intervention indicated (e.g., cystectomy)	—
Cystitis	Cystitis	Asymptomatic	Frequency with dysuria; macroscopic hematuria	Transfusion; IV pain medications; bladder irrigation indicated	Catastrophic bleeding; major non-elective intervention indicated	Death
ALSO CONSIDER: Infection (documented clinically or microbiologically) with Grade 3 or 4 neutrophils (ANC <1.0 x 109/L) – *Select*; Infection with normal ANC or Grade 1 or 2 neutrophils – *Select*; Infection with unknown ANC – *Select*; Pain – *Select*.						
Fistula, GU – *Select*: – Bladder – Genital tract-female – Kidney – Ureter – Urethra – Uterus – Vagina	Fistula, GU – *Select*	Asymptomatic, radiographic findings only	Symptomatic; noninvasive intervention indicated	Symptomatic interfering with ADL; invasive intervention indicated	Life-threatening consequences; operative intervention requiring partial or full organ resection; permanent urinary diversion	Death
REMARK: A fistula is defined as an abnormal communication between two body cavities, potential spaces, and/or the skin. The site indicated for a fistula should be the site from which the abnormal process is believed to have originated.						
Incontinence, urinary	Incontinence, urinary	Occasional (e.g., with coughing, sneezing, etc.), pads not indicated	Spontaneous, pads indicated	Interfering with ADL; intervention indicated (e.g., clamp, collagen injections)	Operative intervention indicated (e.g., cystectomy or permanent urinary diversion)	—
Leak (including anastomotic), GU – *Select*: – Bladder – Fallopian tube – Kidney – Spermatic cord – Stoma – Ureter – Urethra – Uterus – Vagina – Vas deferens	Leak, GU – *Select*	Asymptomatic, radiographic findings only	Symptomatic; medical intervention indicated	Symptomatic, interfering with GU function; invasive or endoscopic intervention indicated	Life-threatening	Death
REMARK: Leak (including anastomotic), GU – *Select* refers to clinical signs and symptoms or radiographic confirmation of anastomotic leak but without development of fistula.						

RENAL/GENITOURINARY						Page 2 of 3
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Obstruction, GU – *Select*: – Bladder – Fallopian tube – Prostate – Spermatic cord – Stoma – Testes – Ureter – Urethra – Uterus – Vagina – Vas deferens	Obstruction, GU – *Select*	Asymptomatic, radiographic or endoscopic findings only	Symptomatic but no hydronephrosis, sepsis or renal dysfunction; dilation or endosoopic repair or stent placement indicated	Symptomatic and altered organ function (e.g., sepsis or hydronephrosis, or renal dysfunction); operative intervention indicated	Life-threatening consequences; organ failure or operative intervention requiring complete organ resection indicated	Death
NAVIGATION NOTE: Operative injury is graded as Intra-operative injury – *Select Organ or Structure* in the SURGERY/INTRA-OPERATIVE INJURY CATEGORY.						
Perforation, GU – *Select*: – Bladder – Fallopian tube – Kidney – Ovary – Prostate – Spermatic cord – Stoma – Testes – Ureter – Urethra – Uterus – Vagina – Vas deferens	Perforation, GU – *Select*	Asymptomatic radiographic findings only	Symptomatic, associated with altered renal/GU function	Symptomatic, operative intervention indicated	Life-threatening consequences or organ failure; operative intervention requiring organ resection indicated	Death
Prolapse of stoma, GU	Prolapse stoma, GU	Asymptomatic; special intervention, extraordinary care not indicated	Extraordinary local care or maintenance; minor revision under local anesthesia indicated	Dysfunctional stoma; operative intervention or major stomal revision indicated	Life-threatening consequences	Death
REMARK: Other stoma complications may be graded as Fistula, GU – *Select*; Leak (including anastomotic), GU – *Select*; Obstruction, GU – *Select*; Perforation, GU – *Select*; Stricture/stenosis (including anastomotic), GU – *Select*.						
Renal failure	Renal failure	—	—	Chronic dialysis not indicated	Chronic dialysis or renal transplant indicated	Death
ALSO CONSIDER: Glomerular filtration rate.						

RENALGENITOURINARY						Page 3 of 3
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Stricture/stenosis (including anastomotic), GU – *Select*: – Bladder – Fallopian tube – Prostate – Spermatic cord – Stoma – Testes – Ureter – Urethra – Uterus – Vagina – Vas deferens	Stricture, anastomotic, GU – *Select*	Asymptomatic, radiographic or endoscopic findings only	Symptomatic but no hydronephrosis, sepsis or renal dysfunction; dilation or endosoopic repair or stent placement indicated	Symptomatic and altered organ function (e.g., sepsis or hydronephrosis, or renal dysfunction); operative intervention indicated	Life-threatening consequences; organ failure or operative intervention requiring organ resection indicated	Death
ALSO CONSIDER: Obstruction, GU – *Select*.						
Urinary electrolyte wasting (e.g., Fanconi's syndrome, renal tubular acidosis)	Urinary electrolyte wasting	Asymptomatic, intervention not indicated	Mild, reversible and manageable with replacement	Irreversible, requiring continued replacement	—	—
ALSO CONSIDER: Acidosis (metabolic or respiratory); Bicarbonate, serum-low; Calcium, serum-low (hypocalcemia); Phosphate, serum-low (hypophosphatemia).						
Urinary frequency/urgency	Urinary frequency	Increase in frequency or nocturia up to 2 x normal; enuresis	Increase >2 x normal but <hourly	≥1 x/hr; urgency; catheter indicated	—	—
Urinary retention (including neurogenic bladder)	Urinary retention	Hesitancy or dribbling, no significant residual urine; retention occurring during the immediate postoperative period	Hesitancy requiring medication; or operative bladder atony requiring indwelling catheter beyond immediate postoperative period but for <6 weeks	More than daily catheterization indicated; urological intervention indicated (e.g., TURP, suprapubic tube, urethrotomy)	Life-threatening consequences; organ failure (e.g., bladder rupture); operative intervention requiring organ resection indicated	Death
REMARK: The etiology of retention (if known) is graded as Obstruction, GU – *Select*; Stricture/stenosis (including anastomotic), GU – *Select*. ALSO CONSIDER: Obstruction, GU – *Select*; Stricture/stenosis (including anastomotic), GU – *Select*.						
Urine color change	Urine color change	Present	—	—	—	—
REMARK: Urine color refers to change that is not related to other dietary or physiologic cause (e.g., bilirubin, concentrated urine, and hematuria).						
Renal/Genitourinary – Other (Specify, __)	Renal – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

SECONDARY MALIGNANCY						Page 1 of 1
		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Secondary Malignancy – possibly related to cancer treatment (Specify, __)	Secondary Malignancy (possibly related to cancer treatment)	—	—	Non-life-threatening basal or squamous cell carcinoma of the skin	Solid tumor, leukemia or lymphoma	Death
REMARK: Secondary malignancy excludes metastasis from initial primary. Any malignancy possibly related to cancer treatment (including AML/MDS) should be reported via the routine reporting mechanisms outlined in each protocol. Important: Secondary Malignancy is an exception to NCI Expedited Adverse Event Reporting Guidelines. Secondary Malignancy is "Grade 4, present" but NCI does not require AdEERS Expedited Reporting for any (related or unrelated to treatment) Secondary Malignancy. A diagnosis of AML/MDS following treatment with an NCI-sponsored investigational agent is to be reported using the form available from the CTEP Web site at http://ctep.cancer.gov. Cancers not suspected of being treatment-related are not to be reported here.						

SEXUAL/REPRODUCTIVE FUNCTION						Page 1 of 2
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Breast function/lactation	Breast function	Mammary abnormality, not functionally significant	Mammary abnormality, functionally significant	—	—	—
Breast nipple/areolar deformity	Nipple/areolar	Limited areolar asymmetry with no change in nipple/areolar projection	Asymmetry of nipple areolar complex with slight deviation in nipple projection	Marked deviation of nipple projection	—	—
Breast volume/hypoplasia	Breast	Minimal asymmetry; minimal hypoplasia	Asymmetry exists, ≤1/3 of the breast volume; moderate hypoplasia	Asymmetry exists, >1/3 of the breast volume; severe hypoplasia	—	—
REMARK: Breast volume is referenced with both arms straight overhead.						
NAVIGATION NOTE: Dysmenorrhea is graded as Pain – *Select* in the PAIN CATEGORY.						
NAVIGATION NOTE: Dyspareunia is graded as Pain – *Select* in the PAIN CATEGORY.						
NAVIGATION NOTE: Dysuria (painful urination) is graded as Pain – *Select* in the PAIN CATEGORY.						
Erectile dysfunction	Erectile dysfunction	Decrease in erectile function (frequency/rigidity of erections) but erectile aids not indicated	Decrease in erectile function (frequency/rigidity of erections), erectile aids indicated	Decrease in erectile function (frequency/rigidity of erections) but erectile aids not helpful; penile prosthesis indicated	—	—
Ejaculatory dysfunction	Ejaculatory dysfunction	Diminished ejaculation	Anejaculation or retrograde ejaculation	—	—	—
NAVIGATION NOTE: Feminization of male is graded in the ENDOCRINE CATEGORY.						
Gynecomastia	Gynecomastia	—	Asymptomatic breast enlargement	Symptomatic breast enlargement; intervention indicated	—	—
ALSO CONSIDER: Pain – *Select*.						
Infertility/sterility	Infertility/sterility	—	Male: oligospermia/low sperm count Female: diminished fertility/ovulation	Male: sterile/azoospermia Female: infertile/ anovulatory	—	—
Irregular menses (change from baseline)	Irregular menses	1 – 3 months without menses	>3 – 6 months without menses but continuing menstrual cycles	Persistent amenorrhea for >6 months	—	—

SEXUAL/REPRODUCTIVE FUNCTION						Page 2 of 2
		Grade				
Adverse Event	**Short Name**	1	2	3	4	5
Libido	Libido	Decrease in interest but not affecting relationship; intervention not indicated	Decrease in interest and adversely affecting relationship; intervention indicated	—	—	—
NAVIGATION NOTE: Masculinization of female is graded in the ENDOCRINE CATEGORY.						
Orgasmic dysfunction	Orgasmic function	Transient decrease	Decrease in orgasmic response requiring intervention	Complete inability of orgasmic response; not responding to intervention	—	—
NAVIGATION NOTE: Pelvic pain is graded as Pain – *Select* in the PAIN CATEGORY.						
NAVIGATION NOTE: Ulcers of the labia or perineum are graded as Ulceration in DERMATOLOGY/SKIN CATEGORY.						
Vaginal discharge (non-infectious)	Vaginal discharge	Mild	Moderate to heavy; pad use indicated	—	—	—
Vaginal dryness	Vaginal dryness	Mild	Interfering with sexual function; dyspareunia; intervention indicated	—	—	—
ALSO CONSIDER: Pain – *Select*.						
Vaginal mucositis	Vaginal mucositis	Erythema of the mucosa; minimal symptoms	Patchy ulcerations; moderate symptoms or dyspareunia	Confluent ulcerations; bleeding with trauma; unable to tolerate vaginal exam, sexual intercourse or tampon placement	Tissue necrosis; significant spontaneous bleeding; life-threatening consequences	—
Vaginal stenosis/length	Vaginal stenosis	Vaginal narrowing and/or shortening not interfering with function	Vaginal narrowing and/or shortening interfering with function	Complete obliteration; not surgically correctable	—	—
Vaginitis (not due to infection)	Vaginitis	Mild, intervention not indicated	Moderate, intervention indicated	Severe, not relieved with treatment; ulceration, but operative intervention not indicated	Ulceration and operative intervention indicated	—
Sexual/Reproductive Function – Other (Specify, __)	Sexual – Other (Specify)	Mild	Moderate	Severe	Disabling	Death

SURGERY/INTRA-OPERATIVE INJURY						Page 1 of 2
		Grade				
Adverse Event	**Short Name**	1	2	3	4	5
NAVIGATION NOTE: Intra-operative hemorrhage is graded as Hemorrhage/bleeding associated with surgery, intra-operative or postoperative in the HEMORRHAGE/BLEEDING CATEGORY.						
Intra-operative injury – *Select Organ or Structure* *'Select'* AEs appear at the end of the CATEGORY.	Intraop injury – *Select*	Primary repair of injured organ/structure indicated	Partial resection of injured organ/structure indicated	Complete resection or reconstruction of injured organ/structure indicated	Life threatening consequences; disabling	—
REMARK: The *'Select'* AEs are defined as significant, unanticipated injuries that are recognized at the time of surgery. These AEs do not refer to additional surgical procedures that must be performed because of a change in the operative plan based on intra-operative findings. Any sequelae resulting from the intra-operative injury that result in an adverse outcome for the patient must also be recorded and graded under the relevant CTCAE Term.						
Intra-operative Injury – Other (Specify, __)	Intraop Injury – Other (Specify)	Primary repair of injured organ/structure indicated	Partial resection of injured organ/structure indicated	Complete resection or reconstruction of injured organ/structure indicated	Life threatening consequences; disabling	—
REMARK: Intra-operative Injury – Other (Specify, __) is to be used only to report an organ/structure not included in the *'Select'* AEs found at the end of the CATEGORY. Any sequelae resulting from the intra-operative injury that result in an adverse outcome for the patient must also be recorded and graded under the relevant CTCAE Term.						

SURGERY/INTRA-OPERATIVE INJURY – *SELECT*

AUDITORY/EAR
- Inner ear
- Middle ear
- Outer ear NOS
- Outer ear-Pinna

CARDIOVASCULAR
- Artery-aorta
- Artery-carotid
- Artery-cerebral
- Artery-extremity (lower)
- Artery-extremity (upper)
- Artery-hepatic
- Artery-major visceral artery
- Artery-pulmonary
- Artery NOS
- Heart
- Spleen
- Vein-extremity (lower)
- Vein-extremity (upper)
- Vein-hepatic
- Vein-inferior vena cava
- Vein-jugular
- Vein-major visceral vein
- Vein-portal vein
- Vein-pulmonary
- Vein-superior vena cava
- Vein NOS

DERMATOLOGY/SKIN
- Breast
- Nails
- Skin

ENDOCRINE
- Adrenal gland
- Parathyroid
- Pituitary

ENDOCRINE (*continued*)
- Thyroid

HEAD AND NECK
- Gingiva
- Larynx
- Lip/perioral area
- Face NOS
- Nasal cavity
- Nasopharynx
- Neck NOS
- Nose
- Oral cavity NOS
- Parotid gland
- Pharynx
- Salivary duct
- Salivary gland
- Sinus
- Teeth
- Tongue
- Upper aerodigestive NOS

GASTROINTESTINAL
- Abdomen NOS
- Anal sphincter
- Anus
- Appendix
- Cecum
- Colon
- Duodenum
- Esophagus
- Ileum
- Jejunum
- Oral
- Peritoneal cavity
- Rectum
- Small bowel NOS

GASTROINTESTINAL (*continued*)
- Stoma (GI)
- Stomach

HEPATOBILIARY/ PANCREAS
- Biliary tree-common bile duct
- Biliary tree-common hepatic duct
- Biliary tree-left hepatic duct
- Biliary tree-right hepatic duct
- Biliary tree NOS
- Gallbladder
- Liver
- Pancreas
- Pancreatic duct

MUSCULOSKELETAL
- Bone
- Cartilage
- Extremity-lower
- Extremity-upper
- Joint
- Ligament
- Muscle
- Soft tissue NOS
- Tendon

NEUROLOGY
- Brain
- Meninges
- Spinal cord

NERVES:
- Brachial plexus
- CN I (olfactory)
- CN II (optic)
- CN III (oculomotor)
- CN IV (trochlear)

NEUROLOGY (*continued*)

NERVES:
- CN V (trigeminal) motor
- CN V (trigeminal) sensory
- CN VI (abducens)
- CN VII (facial) motor-face
- CN VII (facial) sensory-taste
- CN VIII (vestibulocochlear)
- CN IX (glossopharyngeal) motor pharynx
- CN IX (glossopharyngeal) sensory ear-pharynx-tongue
- CN X (vagus)
- CN XI (spinal accessory)
- CN XII (hypoglossal)
- Cranial nerve or branch NOS
- Lingual
- Lung thoracic
- Peripheral motor NOS
- Peripheral sensory NOS
- Recurrent laryngeal
- Sacral plexus
- Sciatic
- Thoracodorsal

OCULAR
- Conjunctiva
- Cornea
- Eye NOS
- Lens
- Retina

PULMONARY/UPPER RESPIRATORY
- Bronchus
- Lung
- Mediastinum
- Pleura
- Thoracic duct
- Trachea
- Upper airway NOS

RENAL/GENITOURINARY
- Bladder
- Cervix
- Fallopian tube
- Kidney
- Ovary
- Pelvis NOS
- Penis
- Prostate
- Scrotum
- Testis
- Ureter
- Urethra
- Urinary conduit
- Urinary tract NOS
- Uterus
- Vagina
- Vulva

SYNDROMES

Page 1 of 2

Adverse Event	Short Name	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
NAVIGATION NOTE: Acute vascular leak syndrome is graded in the VASCULAR CATEGORY.						
NAVIGATION NOTE: Adrenal insufficiency is graded in the ENDOCRINE CATEGORY.						
NAVIGATION NOTE: Adult Respiratory Distress Syndrome (ARDS) is graded in the PULMONARY/UPPER RESPIRATORY CATEGORY.						
Alcohol intolerance syndrome (antabuse-like syndrome)	Alcohol intolerance syndrome	—	—	Present	—	Death
REMARK: An antabuse-like syndrome occurs with some new anti-androgens (e.g., nilutamide) when patient also consumes alcohol.						
NAVIGATION NOTE: Autoimmune reaction is graded as Autoimmune reaction/hypersensitivity (including drug fever) in the ALLERGY/IMMUNOLOGY CATEGORY.						
Cytokine release syndrome/acute infusion reaction	Cytokine release syndrome	Mild reaction; infusion interruption not indicated; intervention not indicated	Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Life-threatening; pressor or ventilatory support indicated	Death
REMARK: Cytokine release syndromes/acute infusion reactions are different from Allergic/hypersensitive reactions, although some of the manifestations are common to both AEs. An acute infusion reaction may occur with an agent that causes cytokine release (e.g., monoclonal antibodies or other biological agents). Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hrs of completion of infusion. Signs/symptoms may include: Allergic reaction/hypersensitivity (including drug fever); Arthralgia (joint pain); Bronchospasm; Cough; Dizziness; Dyspnea (shortness of breath); Fatigue (asthenia, lethargy, malaise); Headache; Hypertension; Hypotension; Myalgia (muscle pain); Nausea; Pruritis/itching; Rash/desquamation; Rigors/chills; Sweating (diaphoresis); Tachycardia; Tumor pain (onset or exacerbation of tumor pain due to treatment); Urticaria (hives, welts, wheals); Vomiting. ALSO CONSIDER: Allergic reaction/hypersensitivity (including drug fever); Bronchospasm, wheezing; Dyspnea (shortness of breath); Hypertension; Hypotension; Hypoxia; Prolonged QTc interval; Supraventricular and nodal arrhythmia – *Select*; Ventricular arrhythmia – *Select*.						
NAVIGATION NOTE: Disseminated intravascular coagulation (DIC) is graded in the COAGULATION CATEGORY.						
NAVIGATION NOTE: Fanconi's syndrome is graded as Urinary electrolyte wasting (e.g., Fanconi's syndrome, renal tubular acidosis) in the RENAL/GENITOURINARY CATEGORY.						
Flu-like syndrome	Flu-like syndrome	Symptoms present but not interfering with function	Moderate or causing difficulty performing some ADL	Severe symptoms interfering with ADL	Disabling	Death
REMARK: Flu-like syndrome represents a constellation of symptoms which may include cough with catarrhal symptoms, fever, headache, malaise, myalgia, prostration, and is to be used when the symptoms occur in a cluster consistent with one single pathophysiological process.						
NAVIGATION NOTE: Renal tubular acidosis is graded as Urinary electrolyte wasting (e.g., Fanconi's syndrome, renal tubular acidosis) in the RENAL/GENITOURINARY CATEGORY.						

SYNDROMES

Page 2 of 2

Adverse Event	Short Name	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
"Retinoic acid syndrome"	"Retinoic acid syndrome"	Fluid retention; less than 3 kg of weight gain; intervention with fluid restriction and/or diuretics indicated	Mild to moderate signs/ symptoms; steroids indicated	Severe signs/symptoms; hospitalization indicated	Life-threatening; ventilatory support indicated	Death
REMARK: Patients with acute promyelocytic leukemia may experience a syndrome similar to "retinoic acid syndrome" in association with other agents such as arsenic trioxide. The syndrome is usually manifested by otherwise unexplained fever, weight gain, respiratory distress, pulmonary infiltrates and/or pleural effusion, with or without leukocytosis. ALSO CONSIDER: Acute vascular leak syndrome; Pleural effusion (non-malignant); Pneumonitis/pulmonary infiltrates.						
NAVIGATION NOTE: SIADH is graded as Neuroendocrine: ADH secretion abnormality (e.g., SIADH or low ADH) in the ENDOCRINE CATEGORY.						
NAVIGATION NOTE: Stevens-Johnson syndrome is graded as Rash: erythema multiforme (e.g., Stevens-Johnson syndrome, toxic epidermal necrolysis) in the DERMATOLOGY/SKIN CATEGORY.						
NAVIGATION NOTE: Thrombotic microangiopathy is graded as Thrombotic microangiopathy (e.g., thrombotic thrombocytopenic purpura [TTP] or hemolytic uremic syndrome [HUS]) in the COAGULATION CATEGORY.						
Tumor flare	Tumor flare	Mild pain not interfering with function	Moderate pain; pain or analgesics interfering with function, but not interfering with ADL	Severe pain; pain or analgesics interfering with function and interfering with ADL	Disabling	Death
REMARK: Tumor flare is characterized by a constellation of signs and symptoms in direct relation to initiation of therapy (e.g., anti-estrogens/androgens or additional hormones). The symptoms/signs include tumor pain, inflammation of visible tumor, hypercalcemia, diffuse bone pain, and other electrolyte disturbances. ALSO CONSIDER: Calcium, serum-high (hypercalcemia).						
Tumor lysis syndrome	Tumor lysis syndrome	—	—	Present	—	Death
ALSO CONSIDER: Creatinine; Potassium, serum-high (hyperkalemia).						
Syndromes – Other (Specify, __)	Syndromes – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

VASCULAR

Page 1 of 2

		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Acute vascular leak syndrome	Acute vascular leak syndrome	—	Symptomatic, fluid support not indicated	Respiratory compromise or fluids indicated	Life-threatening; pressor support or ventilatory support indicated	Death
Peripheral arterial ischemia	Peripheral arterial ischemia	—	Brief (<24 hrs) episode of ischemia managed non-surgically and without permanent deficit	Recurring or prolonged (≥24 hrs) and/or invasive intervention indicated	Life-threatening, disabling and/or associated with end organ damage (e.g., limb loss)	Death
Phlebitis (including superficial thrombosis)	Phlebitis	—	Present	—	—	—
ALSO CONSIDER: Injection site reaction/extravasation changes.						
Portal vein flow	Portal flow	—	Decreased portal vein flow	Reversal/retrograde portal vein flow	—	—
Thrombosis/embolism (vascular access-related)	Thrombosis/embolism (vascular access)	—	Deep vein thrombosis or cardiac thrombosis; intervention (e.g., anticoagulation, lysis, filter, invasive procedure) not indicated	Deep vein thrombosis or cardiac thrombosis; intervention (e.g., anticoagulation, lysis, filter, invasive procedure) indicated	Embolic event including pulmonary embolism or life-threatening thrombus	Death
Thrombosis/thrombus/ embolism	Thrombosis/thrombus/ embolism	—	Deep vein thrombosis or cardiac thrombosis; intervention (e.g., anticoagulation, lysis, filter, invasive procedure) not indicated	Deep vein thrombosis or cardiac thrombosis; intervention (e.g., anticoagulation, lysis, filter, invasive procedure) indicated	Embolic event including pulmonary embolism or life-threatening thrombus	Death
Vessel injury-artery – *Select*: – Aorta – Carotid – Extremity-lower – Extremity-upper – Other NOS – Visceral	Artery injury – *Select*	Asymptomatic diagnostic finding; intervention not indicated	Symptomatic (e.g., claudication); not interfering with ADL; repair or revision not indicated	Symptomatic interfering with ADL; repair or revision indicated	Life-threatening; disabling; evidence of end organ damage (e.g., stroke, MI, organ or limb loss)	Death
NAVIGATION NOTE: Vessel injury to an artery intra-operatively is graded as Intra-operative injury – *Select Organ or Structure* in the SURGERY/INTRA-OPERATIVE INJURY CATEGORY.						

VASCULAR

Page 2 of 2

		Grade				
Adverse Event	**Short Name**	**1**	**2**	**3**	**4**	**5**
Vessel injury-vein – *Select*: – Extremity-lower – Extremity-upper – IVC – Jugular – Other NOS – SVC – Viscera	Vein injury – *Select*	Asymptomatic diagnostic finding; intervention not indicated	Symptomatic (e.g., claudication); not interfering with ADL; repair or revision not indicated	Symptomatic interfering with ADL; repair or revision indicated	Life-threatening; disabling; evidence of end organ damage	Death
NAVIGATION NOTE: Vessel injury to a vein intra-operatively is graded as Intra-operative injury – *Select Organ or Structure* in the SURGERY/INTRA-OPERATIVE INJURY CATEGORY.						
Visceral arterial ischemia (non-myocardial)	Visceral arterial ischemia	—	Brief (<24 hrs) episode of ischemia managed medically and without permanent deficit	Prolonged (≥24 hrs) or recurring symptoms and/or invasive intervention indicated	Life-threatening; disabling; evidence of end organ damage	Death
ALSO CONSIDER: CNS cerebrovascular ischemia.						
Vascular – Other (Specify, __)	Vascular – Other (Specify)	Mild	Moderate	Severe	Life-threatening; disabling	Death

APPENDIX C – P10S-PADRE INVESTIGATOR'S BROCHURE

APPENDIX D – QS-21 INVESTIGATOR'S BROCHURE

CONFIDENTIAL DOSSIER

INVESTIGATOR'S BROCHURE

For

Stimulon®, QS-21

Mixture of structural isomers : 3-O-β-D-galactopyranosyl-(1→2) -[β-D-xylopyranosyl-(1→3)]- β -D-glucuronopyranosyl-quillaic acid 28-O- β -D-apiofuranosyl-(1→3)- β -D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-3-[5-O- α -L-arabinofuranosyl 3,5-dihydroxy-6-methyl-octanoyl]-3,5-di-hydroxy-6-methyl-octanoyl]- β -D-fucopyranoside. [CAS 141256-04-4];
3-O- β -D-galactopyranosyl-(1→2) -[β -D-xylopyranosyl-(1→3)]- β -D-glucuronopyranosyl-quillaic acid 28-O- β -D-xylopyranosyl-(1→3)- β -D-xylopyranosyl-(1→4)- α -L-rhamnopyranosyl-(1→2)-3-[5-O- α -L-arabinofuranosyl 3,5-dihydroxy-6-methyl-octanoyl]-3,5-di-hydroxy-6-methyl-octanoyl]- β -D-fucopyranoside. [CAS 145633-52-9]

Edition 6 – (06/30/2005)

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TABLE OF CONTENTS

1. SUMMARY

Purified, subunit antigens are generally poor immunogens, and vaccines based on them often require potent adjuvants for induction of optimal immune responses. One such strong adjuvant is Antigenics' Stimulon®, QS-21.

QS-21 is a naturally occurring saponin molecule purified from the South American tree *Quillaja saponaria* Molina. It is a triterpene glycoside with the general structure of a quillaic acid 3, 28-O-bis glycoside with the formula $C_{92}H_{148}O_{46}$, and a molecular weight of 1990 Kd. QS-21 powder goes readily into solution in buffered saline at a pH ranging between 5 and 7. Solubility increases with pH, reaching 17 mg/mL at pH 7. QS-21 solutions are stable to storage at -20°C. The shelf life is dependent upon QS-21 concentration, formulation pH, and storage temperature.

Rabbit toxicity studies with single or multiple injections of various doses of QS-21 alone or combined with vaccine antigens have documented a pattern of mild to moderate inflammation (hemorrhage, necrosis, edema) at the injection site, and no significant organ toxicity. Slight alterations in white blood cell counts and creatinine phosphokinase are common. Similar results on hematology and local injection site inflammation were observed in toxicity studies in rats administered QS-21 in the presence of antigen. Pharmacokinetic data collected after a single IM injection of tritium-labeled QS-21 in rabbits show QS-21 to be highly concentrated in the draining lymph nodes. Excretion occurs primarily through kidneys, and both QS-21 and its metabolites are found in the urine. Studies in mice, rabbits and monkeys with QS-21-adjuvanted vaccines show improvement in humoral and cellular immune responses, especially increase in antibody titers, induction of antigen-specific cytotoxic T lymphocytes (mice, monkeys), immunoglobulin class switching, affinity maturation and broadening of antigen-primed B cell repertoire. Studies in mice with QS-21 adjuvanted vaccines show improvement in serum and mucosal antibody titers after administration via the oral or intranasal route.

QS-21 has been evaluated in over 120 different clinical trials of various experimental vaccines in cancer, infectious disease, and neurodegenerative disorders. Mild to moderate pain, erythema and edema at the injection site are common side effects of QS-21 containing vaccines. Low-grade fever and severe pain at the injection site may occur, but these effects are uncommon and short-lived. No significant hematological and biological alterations have been documented.

2. INTRODUCTION

Stimulon®, QS-21 belongs to the pharmacologic class known as vaccine adjuvants. Purified subunit vaccines, derived from bacterial, parasitic, viral, or cancer antigens, have advantages over live attenuated, inactivated whole organisms, or whole cancer cell vaccines. These advantages include: absence of risk of reversion to virulence, no risk of incomplete inactivation, elimination of cellular DNA, and no inclusion of irrelevant antigens or contaminating proteins. However, these purified subunit vaccines usually are not as efficient at inducing antibody responses, especially in unprimed individuals. They generate lower antibody levels, produce restricted antibody isotype profiles, and fail to induce cellular cytotoxic immune responses. One method to overcome these defects in the immune response to purified subunits is to include an appropriate adjuvant. An ideal adjuvant would enhance the induction and level of antibody responses, develop a broad antibody isotype profile, stimulate a cellular cytotoxic immune response, induce immunologic memory, and lack significant local or systemic reactogenicity and pyrogenicity. Adjuvants typically fall into one of two classes: "vehicles", which prolong release of antigen, and immunomodulators, which stimulate cells of the immune system. QS-21 belongs to the latter class although it is compatible with vehicle type adjuvants.

QS-21 is a purified, naturally occurring saponin molecule from the South American tree *Quillaja saponaria* Molina. The bark of this tree is rich in triterpene glycoside saponins, representing up to 10% of the weight of the bark. The extracts of *Quillaja saponaria* have also been shown to contain components useful in vaccine applications. One of these components, designated as Stimulon®, QS-21, is purified from a water extract of the bark by a chromatographic process yielding predominately a single peak when analyzed by reversed-phase high pressure liquid chromatography (HPLC). The QS-21 (generic name) molecule consists of a mixture of two structural isomers that cannot be separated easily by reversed-phase HPLC:

QS-21-V1: 3-O-β-D-galactopyranosyl-(1→2) -[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-quillaic acid 28-O- β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-3-[5-O-α-L-arabinofuranosyl 3,5-dihydroxy-6-methyl-octanoyl]-3,5-di-hydroxy-6-methyl-octanoyl]- β-D-fucopyranoside. [CAS 141256-04-4]

QS-21-V2: 3-O-β-D-galactopyranosyl-(1→2) -[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-quillaic acid 28-O-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-3-[5-O-α-L-arabinofuranosyl 3,5-dihydroxy-6-methyl-octanoyl]-3,5-di-hydroxy-6-methyl-octanoyl]-β-D-fucopyranoside. [CAS 145633-52-9].

Both isomers have biological activity over a similar dose range for adjuvant function[1], and are believed to be the active ingredient in the product.

The rationale for the use of QS-21 is to improve vaccine performance. Evaluation of this product includes measurements of its effect on vaccine antigen-specific antibody responses, measurements of antigen-specific cell mediated immune responses, and other vaccine-specific responses, as well as antigen-nonspecific innate immune responses. The exact measurements that are utilized depend upon the specific vaccine, antigen, and indication.

3. PHYSICAL, CHEMICAL, AND PHARMACEUTICAL PROPERTIES AND FORMULATION

3.1. Common Name

QS-21

3.2. Chemical Formula

$C_{92}H_{148}O_{46}$

3.3. Molecular Weight

1990

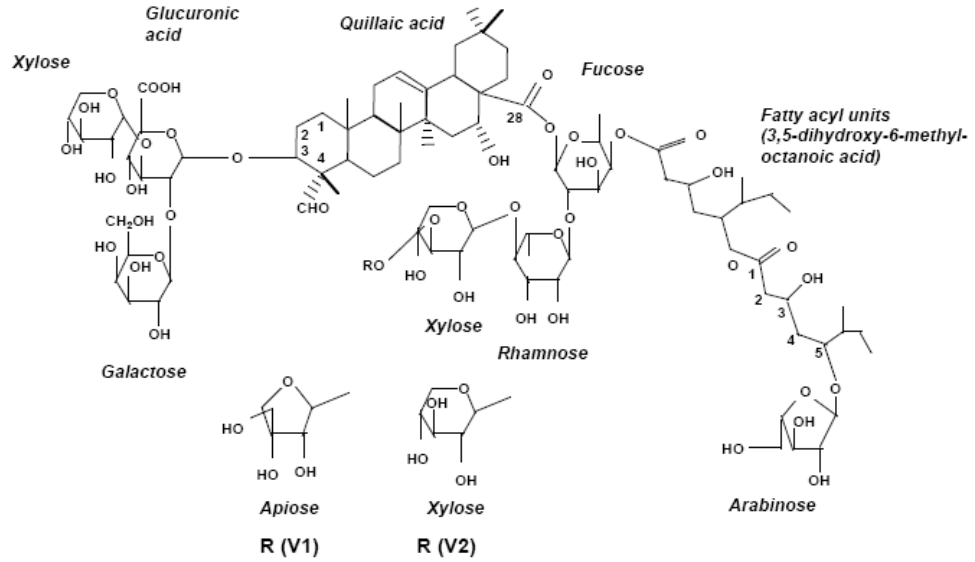
3.4. Isomeric Forms

QS-21 consists of two structural isomers designated QS-21-V1 and QS-21-V2. These have identical chemical formulas and molecular weights and differ in one terminal pentose. V1 and V2 are present in the product at a typical ratio of V1:V2 of approximately 2:1.[1] These isomers are not separable by reversed-phase HPLC, but they can be separated by hydrophilic interaction chromatography or by capillary electrophoresis. Both isomers have adjuvant activity.[1]

3.5. Structural Formula

The QS-21 product consists of a triterpene glycoside with the general structure of an acylated quillaic acid 3,28-O-bis glycoside.[2] The structure of QS-21 and the structural difference in isomers V1 and V2 is shown in **Figure 1.**

Figure 1: Structure of QS-21 Isomers V1 and V2



3.6. Physical Description

Appearance: odorless white powder.

3.7. Pharmaceutical Properties and Formulation

QS-21 is soluble in aqueous solutions with a solubility limit of 17 mg/mL in buffered saline at pH 7.0. It is also soluble in methanol and mixed methanol/water solutions. It is practically insoluble in chloroform.

QS-21 is supplied for investigational use only in two forms: (1) solid powder to be resolubilized for further manufacture of bulk vaccines, and (2) vialed sterile aqueous form in Phosphate Buffered Saline (PBS) to be mixed in the clinic with separate vials of antigen, diluents, and/or excipients.

3.7.1. Solid Powder

QS-21 solid is supplied in an amber glass vial containing at least 100 mg or 1.0 gram of QS-21. It is identified by Antigenics part number 90123.

3.7.1.1. Storage of Solid Powder

QS-21 should be stored at ≤ -20°C in a dry environment. The shelf life at this storage condition is four (4) years. The expiration date for solid powder QS-21 is listed on the vial label and on the certificate of analysis.

3.7.1.2. Formulation from Solid Powder

Vials of solid powder should be allowed to warm to room temperature prior to opening. Weighing should be carried out in a sterile area, using sterile, pyrogen-free labware. If the vial is partially used, it should be resealed with a sterile teflon-coated septa and aluminum crimp and stored again at -20°C. It is recommended that the contents of a partially-used vial be re-tested for endotoxin and bioburden prior to the next use.

QS-21 can be solubilized as a stock solution (1 to 2 mg QS-21 per mL) in buffered saline in a pH range between 5 to 7. It is also soluble in unbuffered saline if the pH is adjusted within the range of 5.0 to 7.0 with dilute sodium hydroxide. The pH of a QS-21 solution should not exceed pH 7 during titration to avoid an alkaline-catalyzed ester hydrolysis. The formulation should preferably be carried out in a glass container.

A QS-21 stock solution can be filter sterilized through a 0.2 micron filter (Gelman Acrodisc® or Millipore Millipak™) with approximately 98% recovery. It is not acceptable to autoclave this product. This stock solution can be stored at 2-8°C after filtration. A stock solution of 0.5 mg/mL QS-21 in PBS at pH 7.0 will degrade 10% after storage at 4°C for 25 months; the kinetics of degradation is first-order. Alternatively, the stock solution may be stored at ≤ -20°C with no significant degradation over the same period.

Stability of the QS-21 in the final antigen/adjuvant formulation will be dependent upon the particular antigen, buffer, final QS-21 concentration, pH, and excipients included. Hence, this should be determined for each specific vaccine formulation. As a general guideline, QS-21 is less stable with increasing pH and decreasing concentration. **Table 1** summarizes the shelf life of QS-21 formulations in PBS at 4°C at 50 µg/mL and 500 µg/mL at pH 6.0 and 7.0, in the absence of added antigen or excipients.

Table 1: Shelf Life of QS-21 in PBS at 4°C

	Shelf Life (time to 10% degradation) at 4°C	
pH	**50 µg/mL QS-21**	**500 µg/mL QS-21**
6.0	679 days (22.6 months)	3670 days (122 months)
7.0	94 days (3.1 months)	750 days (24.8 months)

3.7.2. Sterile Aqueous QS-21

QS-21 is also supplied as single dose vials of sterile, unpreserved aqueous solution of PBS (10 mM sodium phosphate, 150 mM sodium chloride, pH 6.8) at a concentration of 0.5 mg/mL (500 µg/mL). This formulation may have a slightly turbid appearance.

Aqueous QS-21 is supplied in clear glass vials containing ≥ 0.6 mL (total of 300 µg in vial). This allows syringe withdrawal of various doses of adjuvant, permitting dose-ranging studies. Aqueous QS-21 is to be used as an adjuvant and is not provided for direct administration. It is identified by Antigenics part number 90124.

3.7.2.1. Storage of Sterile Aqueous QS-21

Aqueous QS-21 should be stored at ≤ -20ºC; the shelf life at this storage condition is three (3) years. The expiration date is listed on the vials and the certificate of analysis.

3.7.2.2. Aqueous QS-21 Formulation

The QS-21 vial should be resuspended by gentle inversion to assure mixing prior to withdrawal. **Table 2** lists volumes that correspond to various amounts of QS-21. The QS-21 formulation should be withdrawn with a sterile 1 mL tuberculin syringe and needle and mixed with other sterile components. Although the QS-21 in the vial may have a turbid appearance, it is expected to clarify after dilution with antigen or diluents. The QS-21 vials do not contain a preservative, are single-use only, and should not be re-entered.

Stability of the QS-21 in the final antigen/adjuvant formulation will be dependent upon the particular antigen, buffer, final QS-21 concentration, pH, and excipients included. Hence, this should be determined for each specific vaccine formulation. In absence of extended stability testing, a mixed antigen/adjuvant formulation should be used within eight hours of mixing.

Table 2: Volumes of Aqueous QS-21 (0.5 mg/mL) and Given Quantity of QS-21

Volume of Aqueous QS-21 (mL)	Corresponding Quantity of QS-21 (µg)
0.1	50
0.2	100
0.3	150
0.4	200
0.5	250
0.6	300

3.8. Structural Similarities

QS-21 is structurally similar to the active compounds in the commercial veterinary adjuvant Quil-A (Superfos s/a). Both QS-21 and Quil-A are purified from the same source (*Quillaja saponaria*). Quil-A[3] is a partially purified fraction from *Quillaja saponaria* that consists of a highly heterogeneous array of triterpene glycosides, yielding at least 23 peaks on HPLC.[4] QS-21 is highly purified and is one of the HPLC peaks in Quil-A.[4] QS-21 typically respresents less than 10% of Quil-A.

QS-21 is also structurally similar to sterols, including cholesterol. Saponins from *Quillaja saponaria* have been reported to form complexes with cholesterol-containing membranes or liposomes[5].

4. NONCLINICAL STUDIES

4.1. Nonclinical Pharmacology

QS-21 is an immunological adjuvant. Immunological adjuvants can modulate the humoral (i.e., stimulation of antibody quantity, avidity, affinity, persistence, and/or isotype switching) and/or cellular [(i.e., stimulation of delayed-type hypersensitivity and cytotoxic T lymphocytes (CTL)] immune responses to vaccine antigens. QS-21 has been shown to stimulate both humoral and cell-mediated immunity.

4.1.1. Modulation of Humoral Immune Response

4.1.1.1. Antibody Quantity and Isotypes

QS-21 has been shown to stimulate antibody responses to various vaccine antigens in mice, guinea pigs, rats, rhesus monkeys, and baboons.[4, 6-19] The IgG response to a QS-21-adjuvanted antigen is typically increased 10- to 1000-fold compared to that induced by unadjuvanted antigen. Whereas 5-20 µg of QS-21 has adjuvant activity in mice, doses of 50 to 100 µg are effective in nonhuman primates.

QS-21 modulates IgG subclasses in mice. This adjuvant was shown to stimulate higher levels of murine IgG2a and IgG2b compared to antigen alone or compared to antigen/aluminum hydroxide.[4, 6, 14, 15]

4.1.1.2. Functional Antibody Responses

QS-21 improves functional antibody responses (viral neutralizing and bactericidal antibody) in animals. QS-21 was shown to stimulate a substantially higher serum neutralizing antibody titer to HIV-1 after immunization of baboons with HIV glycoprotein gp120 in comparison to the viral neutralizing response raised by gp120/aluminum hydroxide.[12]

A respiratory syncytial virus (RSV) fusion protein adjuvanted with QS-21 also induced higher levels of neutralizing antibody to RSV in mice than a vaccine adjuvanted with aluminum hydroxide.[21] QS-21/glycoprotein (gB) antigen enhanced serum neutralizing titers to human cytomegalovirus (CMV) in mice by 3- to 9-fold, respectively, compared to gB given in complete Freund's adjuvant or aluminum hydroxide.[14] QS-21 also enhances functional antibody to bacterial antigens. Murine antisera generated by immunization with recombinant *Borrelia burgdorferi* OspA plus QS-21 was shown to have a borreliacidal activity that was enhanced by 16-fold compared to antisera generated by OspA/aluminum hydroxide immunization or OspA/saline immunization.[15]

4.1.1.3. Antigen Dose-Sparing

Two biweekly SC doses of 5 µg of ovalbumin, adjuvanted with 10 µg QS-21, induced titers in C57BL/6 mice that were over 100-fold higher than the titers induced by 125 µg of unadjuvanted ovalbumin.[6] A similar dose-sparing effect was noted with HIV-1 gp120 in guinea pigs[11] and in baboons.[11] These findings suggest that QS-21 adjuvant could be used to decrease the minimum immunogenic dose of antigen (antigen dose-sparing).

4.1.1.4. Mucosal Route and responses

QS-21 adjuvant has also been shown to be useful via intranasal routes or via oral routes in mice for the induction of both serum antibody as well as mucosal antibody.[22]

4.1.1.5. Persistence of Response/Immunological Memory

Adjuvants have also been shown to affect the duration of the antibody response. This was evaluated with various HIV-1 gp120 formulations with or without QS-21 in guinea pigs.[23] After a single immunization, a peak serum antibody titer was observed, followed by a pseudo first-order decay of antibody. This was followed by a plateau of low level antibody titers that were approximately 10-fold lower than the peak. This plateau of low level antibody after the decay phase is called antibody persistence. Various antigen formulations, including QS-21, MF-59, aluminum hydroxide, and no adjuvant, yielded similar antibody decay kinetics. However, considerable differences were observed between adjuvants for peak titers and plateau titers. A strong correlation between peak titers and plateau titers was observed. Of formulations tested, QS-21 induced the highest peak titers and the highest plateau titers.

Recombinant subunit vaccines containing QS-21 have also been shown to evoke immunological memory, i.e. a rapid antibody recall response or delayed-type hypersensitivity upon exposure to native antigen. This is illustrated by an experiment carried out with CMVgB.[14] Balb/c mice received two SC immunizations with recombinant CMV gB in complete Freund's adjuvant (CFA), QS-21, or aluminum hydroxide and were then allowed to rest for two months. They were then infected with recombinant vaccinia virus encoding the gB protein. Antibody titers were measured 6 days later to look at the recall response (rather than the primary response to gB vaccinia). All mice receiving QS-21 or CFA, but not aluminum hydroxide, responded with enhanced titers to gB.

4.1.2. Modulation of Cell-Mediated Immune Response

QS-21 has been shown to induce $CD8^+$ cytotoxic T-lymphocyte (CTL) responses to subunit antigens in mice. Induction of antigen-specific $CD8^+$ CTL is believed to play a critical role for protection against and clearance of infection with intracellular organisms such as viruses or some bacteria and parasites. Other examples of QS-21 induction of CTLs to subunit antigens in mice include HIV-1 gp160D,[8] rec-CMV gB,[14] and RSV fusion protein.[21] The levels of CTLs induced by RSV fusion protein/QS-21 were

comparable to those achieved by experimental infection with RSV. SIV envelope and gag vaccines formulated with QS-21 have also induced CTLs in rhesus monkeys.[10]

4.1.3. Pharmacokinetics and Drug Metabolism in Animals

A study was conducted to investigate the biodistribution and basic pharmacokinetics of ^{3}H-QS-21 following IM administration in NZW rabbits (Study No. 2-R8). A 100 µg dose of ^{3}H-QS-21 was administered by IM injection to 4 rabbits (2 males/2 females) on study day 1. One male and female were euthanized at 24 hours post-dose for collection of tissues. Blood samples were collected from the other male and female prior to dosing and at 5, 10, 20, 40, 60 min, 2, 4, 6, 8, 12, 24, 30, and 48 hours post-dose. Tissues were collected at 48 hours post-dose for this set of rabbits. Tritium was quantitated in plasma, tissues and voided urine and feces. Total tritium recoveries over the 48 hour period were 81 and 86% of the administered dose, respectively. Terminal elimination half-lives of ^{3}H-QS-21 of 25 and 24 hours were measured for male and female, respectively. The mean residence time values were 37 hours. Between 45 to 50% of the tritium was excreted in urine and approximately 1% in feces. The liver showed the highest total uptake of absolute tritium whereas the iliac lymph nodes showed the highest cpm/g value (increasing from 24 h to 48 h). Analysis of the liver showed only intact QS-21, within the limit of detection of the assay. Analysis of the urine showed QS-21 and the hydrolysis product QS-21H (predominant product in urine). The level of QS-21 in the urine decreased with time.

4.2. Toxicology

4.2.1. Toxicology Studies of QS-21 in Absence of Antigen

Table 3 summarizes toxicology studies of QS-21 in absence of antigens.

Table 3: Toxicology Studies of QS-21 in Absence of Antigens

Study No.	Study Type and Route of Administration	Species, No. Per Sex Per Group	Absolute QS-21 Dose (µg)	Dose Interval	Duration of In-life Phase (Days)	Results
2-E23[a]	Repeat Dose, IM	NZW Rabbits, 4M, 4F	0, 10, 50, 100, 200	Days 1, 5, 8, 12	14	No clinical observations suggestive of a compound effect. Body weight changes were comparable across groups. Transient increase in polymorphonuclear leukocyte counts noted at day 2, 6, 9, and 13 in males and females receiving the 100 and 200 µg dose, returning to baseline at day 14. Histopathological examination of the injection site showed evidence of chronic inflammatory reaction in groups 1 (0 µg), 3 (50 µg), 4 (100 µg), and 5 (200 µg). This inflammatory reaction consisted of mononuclear cell infiltrates (macrophages and lymphocytes), varying degrees of fibrosis, and/or hemorrhage (attributed to physical trauma elicited by IM administration). Under the conditions of this study, the no-observable effect dose was 50 µg QS-21.
2-T25[b]	Single Dose, IM (Buffer comparison)	NZW Rabbits, 12M	0 (2 buffer control groups), 100 (5 groups, various buffer/vials)	Day 1	3 (6 animals per group) 7 (6 animals per group)	No clinical observations. Body weight changes were comparable across groups. No biologically relevant differences were noted in the hematology values when comparing treated groups to control groups. Elevation in creatine kinase levels were observed at day 3 in the QS-21 groups, with a return to baseline at day 8 (indicating a duration less than 5 days). Microscopic lesions, consisting of granulomatous inflammation, necrosis, and hemorrhage, ranging from mild to moderate were noted at injection site. QS-21 in succinate-buffered saline caused the most histopathological changes at injection site. QS-21 in saline was generally less irritating to tissues.

[a] Study designed to detect any potential toxicity elicited by QS-21

[b] Study designed to characterize the local acute toxicity elicited by a single IM administration of QS-21 in a variety of vials and vehicles: unbuffered saline in untreated glass vials and siliconized glass vials, PBS (pH 7.4) in untreated glass vials and siliconized vials, and succinate-buffered saline (pH 5.5) in untreated glass vials.

4.2.2. Toxicology Studies of QS-21 in Presence of Antigens

Table 4 summarizes toxicology studies of QS-21 and antigen mixtures.

Table 4: Toxicology Studies of QS-21 in Presence of Antigens

Study No.	Study Type and Route of Administration	Species, No. Per Sex Per Group	Absolute QS-21 Dose (µg)	Dose Interval	Duration of In-life Phase (Days)	Results
2-E24[a]	Repeated Dose, GM2-KLH antigen, IM	NZW Rabbits, 2 M, 2 F	0, 10, 50, 100, 200	Days 1, 7, 14, 21	28	Doses of ≥ 50 µg were associated with transient increases in total white blood cell counts at days 2, 8, 15, and 22 (1 day after dosing), with a return to baseline at day 28, indicating that the duration of white blood cell elevation was less than a week. Doses ≥ 10 µg were associated with apparent increases in absolute spleen weight, determined at day 28. No earlier or later time points were evaluated, hence, the onset and duration of spleen weight increase were not established. There were no remarkable differences in body weights, gross observations, microscopic lesions, or compound-related clinical observations or abnormalities.
2-E76[b]	USP Pyrogenicity, GM2-KLH antigen, IV	NZW Rabbits, 3M	2.86 µg /kg	Day 1	3 hours	Under the conditions of this test, GM2-KLH and QS-21 were not pyrogenic.
2-402[c]	Repeated Dose, rec-HIV gp160 antigen, SC	Rhesus monkeys, 3M	0, 50	Days 14, 42, 72	170	Although there were no significant clinical, physical, or clinical pathological abnormalities attributed to administration of the test vaccines, there was an increased incidence of neutropenia of unknown origin in group 3 (50 µg QS-21) on multiple blood collections.[g]
M96A-H19.2-M33[d]	Repeated Dose, pneumococcal polysaccharides, IM	NZW Rabbits, 6M, 6F	0, 25, 50, 100, 200	Days 1, 15	29	Abnormal clinical observations were limited to irritation at the site of injection. Body temperatures of group 4 and 5 rabbits (receiving 100 and 200 ug QS-21, respectively) were significantly increased on the day after the initial injection when compared to the vaccine control group. These were not different on the second day after injection. Statistical analysis of organ weights showed statistically significant decreases in liver weights in males in groups 2 (25 µg QS-21) , 4 (100 µg QS-21), and 5 (200 µg QS-21). However, histopathological evaluation of the liver and other selected tissues did not indicate any test-article related lesions. The toxicology laboratory concluded that the test articles were nontoxic when administered as two IM injections, two weeks apart, to NZW rabbits at the dose levels tested.
3-H49[e]	Repeated Dose, Malaria SPf66 peptide antigen, SC	NZW Rabbits, 6M, 6F	0, 100, 200	Days 1, 15, 29	56-59	Clinical observations revealed minor effects, such as edema, erythema, or SC thickening at or around the injection sites with no irritation above a grade 2 (well defined) erythema or grade 3 (moderate) edema. There was an increase in % polymorphonuclear leukocytes one day after the first injection in males (all QS-21 groups) and females (200 ug QS-21 plus aluminum hydroxide) and after the third injection in the males (200 ug QS-21 +/- aluminum hydroxide). This was not apparent at day 42, indicating duration less than 12 days. There were no local gross alterations associated with test article. There were microscopic observations consisting of macrophage accumulation, macrophage necrosis, chronic and subacute inflammation, and hemorrhage in groups 2, 5, and 6, which were the groups that received aluminum hydroxide. The toxicology laboratory concluded that the test articles appeared to be safe in NZW rabbits under the circumstances of the study.

Study No.	Study Type and Route of Administration	Species, No. Per Sex Per Group	Absolute QS-21 Dose (µg)	Dose Interval	Duration of In-life Phase (Days)	Results
7500-100[f]	Repeated Dose, AG-707 (rh-Hsc70/49) antigen, SC	(SD)IGS BR Rats, 50M, 50F	0, 50	Days 1, 15, and 29	43	Administration of either dose level of AG-707 (rh-Hsc70 complexed with 49 peptides) with QS-21 and QS-21 in PBS alone was associated with an increased incidence of edema around the dose site. Because the edema was noted in the animals given QS-21 in PBS without rh-Hsc70/49 but not in animals given PBS alone or rh-Hsc70/49 without QS-21, this was considered to be, in part, an adjuvant effect that may have been exacerbated by the rh-Hsc70/49. Body temperatures generally increased in all groups from 1 hour prior to dosing to 4 hours post on Day 1. Animals given QS-21 in PBS alone, rh-Hsc70/49 with QS-21 or rh-Hsc70/49 alone had changes in their hematology suggestive of inflammation. Microscopically, all groups given rh-Hsc70/49 with or without QS-21 showed an indication of chronic inflammation at the injection site. In animals given rh-Hsc70/49 without QS-21 this was minimal in severity whereas in animals given both doses of rh-Hsc70/49 with QS-21 the severity of the chronic inflammation ranged from minimal to slight. Although the clinical pathology indications all showed reversibility by the terminal sacrifice, the microscopic changes had not resolved by that time in animals given rh-Hsc70/49 and the spleen weights for the male animals given rh-Hsc70/49 with QS-21 were also elevated. The toxicology laboratory concluded that male and female rats tolerated rh-Hsc70/49 with and without QS-21 at all dose levels for up to three subcutaneous doses.

[a] Study designed to detect any potential toxicity of GM2-KLH vaccine with QS-21 added.
[b] Study designed to detect any potential pyrogenic effect of GM2-KLH vaccine with QS-21 added.
[c] Study designed for the collection of immunological data to assess the effect of QS-21 on immune responses to a co-administered antigen (HIV-1 recombinant gp160D antigen adsorbed to aluminum hydroside) and a previously administered antigen (tetanus toxoid) in juvenile rhesus monkeys. A secondary objective was the collection of toxicology data for QS-21 in rhesus monkeys.
[d] Study designed to evaluate the toxicity of a vaccine consisting of a commercial 23-valent pneumococcal polysaccharide vaccine in combination with QS-21.
[e] Study designed to evaluate the potential toxicity of malaria vaccine peptide SPf66 administered with QS-21 with or without aluminum hydroxide.
[f] Study designed to evaluate the toxicity of recombinant human heat shock protein (constitutive form rh-Hsc70) AG-707 (rh-Hsc70/49) complexed with 49 synthetic peptides when administered with or without QS-21.
[g] Data appears to be inconsistent with results from other studies with different species.

4.2.3. Genetic Toxicology Studies of QS-21

Table 5 summarizes genetic toxicology studies carried out on QS-21.

Table 5: Genetic Toxicology Studies of QS-21

Study Type	Site Study No.	QS-21 Dose Range
Bacterial Reverse Mutation Assay	AA26FP.503.BTL	75-5000 µg/plate
In Vitro Mammalian Chromosome Aberration Test	AA26FP.331.BTL	0.5, 1.5, 5.0, 7.5, 10.0, 12.5, 15.0 µg/ml
Bone Marrow Erythrocyte Micronucleus Test in Mice by Intraperitoneal Injection	AA38CA.123.BTL	0.0875, 0.175, 0.35 mg/kg

Bacterial Reverse Mutation Assay of QS-21 Adjuvant

Study # AA26FP.503.BTL was designed to evaluate the mutagenic potential of QS-21 by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* WP2 uvrA in the presence and absence of S9 activation. QS-21 was tested in the bacterial reverse mutation assay using *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *E. coli* tester strain WP2 uvrA in the presence and absence of Aroclor-induced rat liver S9. Doses of QS-21 tested were 75 µg/plate (low dose) and 5000 µg/plate (high dose). Under the conditions of the study, QS-21 adjuvant was concluded to be negative in the bacterial reverse mutation assay.

In Vitro Mammalian Chromosome Aberration Test of QS-21 Adjuvant

Study # AA26FP.331.BTL was designed to evaluate the clastogenic potential of QS-21 based upon its ability to induce chromosome aberrations in Chinese hamster ovary (CHO) cells. QS-21 was evaluated in both the presence and absence of an Aroclor-induced S9 activation system. It was tested in non-activation assays at doses from 0.5 to 15.0 µg/ml and in activation assays from 0.4 to 10 µg/ml. Under the conditions of the study, QS-21 adjuvant was concluded to be negative for inducing structural and numerical chromosome aberrations in the presence and absence of S9 metabolic activation in this study.

QS-21: Bone Marrow Erythrocyte Micronucleus Test in Mice by Intraperitoneal Injection

Study # AA38CA.123.BTL was designed to evaluate the clastogenic potential of QS-21 by measuring its ability to induce micronucleated polychromatic erythrocytes in mouse bone marrow. Male and female mice were dosed with 0.0875, 0.175, or 0.35 mg test article/kg of body weight (five/gender/dose at 0.0875 and 0.175 mg/kg doses and fifteen/gender/dose at 0.35 mg/kg dose) by intraperitoneal injection at a constant volume of 10 ml/kg body weight. No mortality or clinical signs were observed in any male or female mice in the micronucleus study. Bone marrow cells were collected at 24 and 48 hours after treatment and examined microscopically for micronucleated polychromatic erythrocytes. No significant increase in micronucleated polychromatic erythrocytes in QS-21 treated groups compared to the vehicle control group was observed in male or female mice at 24 or 48 hours after dose administration. Under the conditions of the study, QS-21 was concluded to be negative in the mouse micronucleus assay.

5. EFFECTS IN HUMANS

5.1. Pharmacokinetics

Not applicable. Biodistribution and pharmacokinetic studies of vaccine components (antigen and adjuvant) are not typically conducted in vaccine studies. Pharmacokinetic studies of QS-21 in humans have not been carried out.

5.2. Summary of Completed and On-Going Clinical Studies (Phases I-III)

This Brochure contains safety data from completed and ongoing domestic and international clinical studies primarily not sponsored by Antigenics Inc. The completeness of the data presented herein is dependent upon the willingness of the conducting sponsor to share clinical study data. Clinical studies containing QS-21 to date have focused primarily on its use as an adjuvant to enhance immune response evoked by preventative as well as therapeutic vaccines against infectious agents and cancers. Studies were and continue to be conducted by many sponsors with a wide variety of antigens and in varying indications.

To date, QS-21-containing vaccines have been evaluated in over 120 human clinical studies. QS-21 preparations were formulated with different antigens, in different vehicles, used alone or with other adjuvants, and at different pH levels.

5.2.1. Summary of Completed and On-Going Cancer Vaccine Studies

This section summarizes completed and ongoing cancer immunotherapy studies containing QS-21 as an adjuvant. Primarily these studies have not been sponsored by Antigenics. Therefore, the information presented is dependent upon the willingness of the conducting sponsor to share clinical study data. Where noted, the data has not been audited by Antigenics. In some of these studies, QS-21 has been mixed with other adjuvants and/or excipients in formulations that are proprietary to the conducting sponsor.

Table 6: Completed Cancer Vaccine Studies

Disease	Phase of Study	Antigen	Subjects receiving QS-21 (QS-21 Dose)	Route	Immunization Schedule
Melanoma	I	GM2-KLH (2 studies)[24-26]	4 (10 µg), 6 (50 µg), 30 (100 µg), 6 (200 µg)	SC	0, 2, 4, 6, 14, 22 wks
	I	GM2-KLH+CycloP	6		
	I	GD3-KLH	6		
	UNK	IMEL-pg1[27]	6 (100 µg)	SC	0, 2, 4, 6, 14, 36 wks
	UNK	GD3, GD3-lactone-KLH[28]	15 (100 µg)	SC	0, 1, 2, 3, 7, 19 wks
	II	BEC2[29]	6 (100 µg)	ID	0, 2, 4, 6, 10 wks
	UNK	GD2L-KLH[30]	18 (100 µg)	SC	0, 1, 2, 3, 10 ,24 wks
	UNK	TYROSINASE PEPTIDE YMDGTMSQV[31]	9 (100 µg)	SC	1, 4, 7 wks
	I	GM2-KLH+GD2-KLH[32, 33]	28 (100 µg)	SC	0, 1, 2, 3, 11, 23, 35 wks
	UNK	9-o-acetyl-GD3-KLH	5		
	UNK	BEC2/GD3L	24		
	UNK	Multiepitope Peptides	9		
	I	YLEPGPVTA[34]	12 (100 µg)	SC	0, 1, 2, 3, 6, 9, 12 mo
	Ib, II	Anti-ID (1A7) (2 studies)[35, 36]	61 (100 µg)	SC	1, 2, 3, 4, 8, 12, etc wks till progression
	UNK	Anti-ID (1A7)+/-IL-2	4		
	II	SPAN	9		
	II	GM2-KLH/IFN-ALFA2B[37]	107 (100 µg)	SC	0, 1, 2, 3, 12, 24, 36 wks
Breast Cancer	UNK	MUC1-KLH[38, 39]	43 (100 µg)	SC	1, 2, 3, 7, 19 wks
	UNK	Anti-ID (11D10)	11		
	I	GLOBOH-KLH[40]	27 (100 µg)	SC	1, 2, 3, 7, 19 wks
Prostate Cancer	UNK	GLOBOH-KLH[41, 42]	20 (100 µg)	SC	0, 1, 2, 6, 18 wks
	UNK	MUC1-KLH	20		
	UNK	GM2-KLH	18		
	UNK	MUC2-KLH	15		
	UNK	sTn(c)-KLH	25		
	UNK	Tn(c)-KLH; Tn(c)-PAM[43]	25 (100 µg)	SC	1, 2, 3, 7, 19, 50 wks
CML	I	Bcr/abl[44]	12 (100 ug)	SC	0, 2, 4, 6, 10 wks
	II	Bcr/abl	14		

Disease	Phase of Study	Antigen	Subjects receiving QS-21 (QS-21 Dose)	Route	Immunization Schedule
Ovarian Cancer	I	Lewis-Y-KLH[45]	25 (100 ug)	SC	0, 1, 2, 6, 18 wks
Adenocarcinoma (Lung, pancreas, and colon)	Ib	RAS oncoprotein	1		
Colorectal Cancer	Feasibility	Anti-ID (3H1)[46]	5 (100 ug)	SC	0, 2, 4, 6 wks, then monthly for 24 mos.
Pancreatic Cancer	I	RAS peptide	17		
B Cell Lymphoma	I/II	BCL-ID-KLH	24		
Small Cell Lung Cancer	UNK	Polysialic acid-KLH[47]	13 (100 ug)	SC	0, 1, 2, 3, 7, 15 wks
	I/II	Fucosyl-GM1-KLH[51]	13 (100 ug)	SC	1, 2, 3, 4, 8, 16 wks
Metastatic Cancer	I/II	MAGE-3 Protein[48]	54 (100 ug)	I.M.	0, 3, 6, 9, 12, 15 wks
Completed Cancer Subtotal			**733**		

[a] Data in this table is provided by collaborators. It is unaudited by Antigenics. If a study is published, the QS-21 dose, route, and schedule are listed.

Table 7: On-Going Cancer Vaccine Studies[a]

Disease	Phase of Study	Antigen	Subjects receiving QS-21 (QS-21 Dose, µg)	Route	Immunization Schedule
Melanoma	III	GM2-KLH[49]	440	S.C.	0, 1, 2, 3 wks, each 12 wks during wks 12-96
	III	GM2-KLH	650		
Breast Cancer					
	I	STNcluster-KLH	27		
	UNK	GLOBOH-KLH	26		
	UNK	MUC2-KLH	25		
	I	Her2Neu	45		
Prostate Cancer	UNK	TF(c)-KLH	15		
	UNK	Gly-MUC1-KLH	12		
CML	UNK	bcr/abl + GM-CSF[50]	16		
Ovarian Cancer	UNK	KSA, KSA-KLH	20		
Small Cell Lung Cancer					
	UNK	Anti-ID (GD2)	2		
Colorectal Cancer	UNK	sTN-KLH	5		
Pancreatic Cancer	UNK	Ab 6G6.c4	9		
B Cell Lymphoma	UNK	BCL-ID-KLH	7		
Neuroblastoma	UNK	Anti-ID (1A7)+/GM-CSF[b]	28		
Metastatic Cancer	IIB	MAGE-3	180		
Ongoing Cancer Subtotal			**1481**		

[a] Data in this table is provided by collaborators. It is unaudited by Antigenics. If a study is published, the QS-21 dose, route and immunization schedule are listed.

[b] Pediatric study.

5.2.2. Summary of Completed and On-Going Infectious Diseases, Alzheimer's and Excipient Studies

This section summarizes completed and on-going infectious diseases, Alzheimer's and excipient studies containing QS-21 as an adjuvant. Primarily these studies have not been sponsored by Antigenics. Therefore, the information presented below is dependent upon the willingness of the conducting sponsor to share clinical study data. In some of these studies, QS-21 has been mixed with other adjuvants and/or excipients in formulations that are proprietary to the conducting sponsor.

Table 8: Completed Infectious Diseases, Alzheimer's and Excipient Studies

Disease	Phase of Study	Antigen	Subjects receiving QS-21 (QS-21 dose, µg)	Route	Immunization Schedule
HIV	I	gp 120 (MN) (4 studies)[52-54]	252 (Various: 50, 100 µg)	I.M.	Various: 0, 1,2 mo 0, 1, 6 mo 0, 1, 10 mo
	I	CTLB36 peptide (p24/V3 MN)	3		
	I	Nef, gag, env, lipopeptides[55-57]	14 (dose undisclosed)	I.M.	0, 4, 16, 48 wks
	I	gp 120 (W61D)[58, 59]	24 (50 µg)	I.M.	0, 4, 28 wks
Malaria	I, II	RTS,S (15 studies)[b] [60-65]	746 (Various)	I.M.	Various
	I	FMP1	15		
	I	Circumsporozoite protein multiple antigen peptide[66-68]	16 (50 µg) 8 (100 µg)	S.C.	Various: 0, 28, 56 days 0, 28, 237 days
	I	SPF66[69]	33 (50 µg) 39 (100 µg)	I.M.	Various: 0, 30 days 0, 30, 180 days
Influenza	I, II	Split virion (7 studies)	441		
Hepatitis Prophylactic	I/II	Envelope (3 studies)	184		
Hepatitis Therapeutic	I/II	Envelope (8 studies)	284		
Hepatitis Therapeutic	I-II	Envelope[70]	10 (50 µg) 10 (100 µg)	I.M.	0, 2, 4, 10, 14 wks
Herpes Simplex Therapeutic	I/II	GD (4 studies)	229		
Human Papilloma Virus	I/II	HPV 6 L2E7 - (4 studies)	220		
RSV	I	PFP	40		
Excipient Studies[a]	I	NONE (3 studies)[71]	63 (50 µg)	I.M.	Various: 4 –5 injections, weekly or bi-weekly
Alzheimer's	I	AN1792	24		
	I	AN1792	80		
	IIa	AN1792[c] [72-75]	298 (50 µg)	I.M.	0, 1, 3, 6 9, 12 mo
Completed Infectious Diseases, Alzheimer's, and Excipient Studies Subtotal			**3033**		

[a] Antigenics sponsored studies. All data from Antigenics –sponsored studies is audited. Antigenics has not audited the other studies.

[b] Includes 2 studies in 150 pediatric subjects, ages 1-11.

[c] Study halted due to SAEs (see listing in Table 16)

Table 9: On-Going Infectious Disease Studies

Disease	Phase of Study	Antigen	Subjects receiving QS-21
VZV	I	Proprietary	24
Hepatitis	II	Envelope (2 studies)	181
On-Going Infectious Disease and Studies Subtotal			**205**

5.2.3. Summary of Completed Quilimmune-P™ Pneumococcal Vaccine Studies

This section summarizes completed Antigenics, Inc., sponsored Quilimmune-P pneumococcal vaccine studies in which QS-21 was evaluated as an adjuvant.

Table 10: Completed Quilimmune-P™ Pneumococcal Vaccine Studies

Disease	Phase of Study	Antigen	Subjects receiving QS-21 (QS-21 dose, µg)	Route	Immunization Schedule
S. pneumonia	I	Pneumococcal polysaccharide 7-valent conjugate of different serotypes of *S. pneumonia*[a]	10 (50 µg)	I.M.	0, 4 mo
	I	Pneumococcal polysaccharide from each of 23 different serotypes of *S. pneumonia* (2 studies)[a]	32 (25 µg) 30 (50 µg) 18 (100 µg)	Various: S.C. I.M.	Day 0
	II	Pneumococcal polysaccharide from each of 23 different serotypes of *S. pneumonia*[a]	107 (25 µg) 212 (50 µg)	I.M.	Day 0
	IIb	Pneumococcal polysaccharide from each of 23 different serotypes of *S. pneumonia* (re-immunization study)[a]	10 (50 µg)[b]	I.M.	0, 12 mo
Completed Quilimmune-P™ Pneumococcal Vaccine Studies Subtotal			**409**		

[a] Study sponsored by Antigenics. Data has been audited by Antigenics.
[b] Subset of subjects in phase II study.

5.3. Safety

This section contains safety data from completed and ongoing domestic and international clinical studies primarily not sponsored by Antigenics Inc. The completeness of the data presented herein is dependent upon the willingness of the conducting sponsor to share clinical study data. Clinical studies containing QS-21 to date have focused primarily on its use as an adjuvant to enhance immune response evoked by preventative as well as therapeutic vaccines against infectious agents and cancers. Studies were and continue to be conducted by many sponsors with a wide variety of antigens and in varying indications.

5.3.1. Adverse Events

The following table summarizes the adverse events reported to Antigenics and in the literature for patients enrolled in studies containing QS-21 as an adjuvant. Primarily these studies have not been sponsored by Antigenics. Therefore, the completeness of the data presented herein is dependent upon the willingness of the conducting sponsor to share clinical study data.

Table 11: Reported Adverse Events

Body System	Symptom		
Whole Body	Injection Site Pain	Leg Pain	Chills
	Injection Site Discomfort	Muscle Ache	Flu Syndrome
	Injection Site Tenderness	Muscle stiffness	General Edema
	Injection Site Inflammation	Malaise	Infection
	Injection Site Reaction	Headache	Bacterial Infection
	Arm Pain	Fever	Irritability
	Drowsiness		
Digestive	Nausea	Stomach Ache	Vomiting
	Sore Throat	Diarrhea	Esophageal spasm
	Constipation	Anorexia	
Respiratory	Runny Nose	Cough	
Nervous System	Dizziness	Worsening of chemotherapy induced sensory neuropathy	Vasovagal symptoms
	Neurosensory toxicity		
Metabolic	Proteinuria	Serum Glucose Elevation	Increase in amylase
Cardiovascular	Hypotension	Neutrpenia	Lymphopenia
	Leukopenia	Anemia	Thrombocytopenia
	Granulocytopenia		
Musculoskeletal	Fatigue	Myalgia	Arthralgias
	Neuromotor toxicity		
Skin	Rash	Erythema	Skin Discoloration
	Induration	Swelling	Skin Ulcer
	Pruritis/Itching	Burning Sensation	Necrosis
Special Senses	Difficulty Walking (following injection site swelling)		

One subject in an investigational HIV-1 DNA vaccine study using QS-21 reported cutaneous vasculitis. One day after receiving a protein boost injection, of which 50 μg of QS-21 is a component, the subject developed Grade 3 leukocytoclastic vasculitis that the investigator determined to have a probable relationship to the vaccine. A relationship to QS-21 was not ruled out. The subject reported a bilateral lower extremity rash, petechial-like and confluent. Two weeks after the vaccination, the rash had resolved leaving

residual mild erythema and mild purities. Urine analysis, neurological exam and stool card sampling for 3 days for occult blood were negative at 7-day follow-up.

5.3.2. Pain on Injection

Because some recipients of vaccines formulated with QS-21 complained of severe pain at the injection site, Antigenics, Inc. sponsored and designed several studies to evaluate the tolerance of antigen-free formulations containing QS-21. **Table 12** lists studies designed to evaluate the mechanism of pain on injection following administration of QS-21 preparations as well as the effects of several excipients on the pain. The formulations contained QS-21 alone, or were mixed with either aluminum hydroxide, polysorbate 80, hydroxypropyl-beta-cyclodextrin, or benzyl alcohol. All formulations were administered by the intramuscular route. The studies are described in more detail below.

Table 12: Studies Evaluating the Tolerance of Different Formulations of QS-21

Study Objective	Antigen	QS-21 Dose (µg)	Number of Subjects
Effect of pH on injection site pain[a]	None	50	33
Effect of aluminum hydroxide and polysorbate 80 on injection site pain[a]	None	50	15
Effect of polysorbate 80, benzyl alcohol and hydroxypropyl-beta-cyclodextrin on injection site pain[a]	None	50	15
Completed Studies Evaluating the Tolerance of Different Formulations of QS-21			63

[a] Study sponsored by Antigenics. Data has been audited by Antigenics.

5.3.2.1. Double-Blinded Randomized Trial Evaluating the Safety and Tolerance of Two Different pH Preparations of the Saponin Adjuvant QS-21 Following Intramuscular Administration to Normal Volunteers

This Phase I study was designed to assess the safety and tolerability of QS-21 and phosphate-buffered saline (PBS, placebo) solutions formulated at two different pH levels (6.0 and 7.2) in normal volunteers to determine the effect of pH on pain on injection. Thirty-three subjects were enrolled and analyzed. Each volunteer randomly received each of the four formulations, including 1) PBS at pH 6.0; 2) PBS, pH 7.2; 3) 50 µg of QS-21 at pH 6.0, and 4) 50 µg of QS-21 at pH 7.2 in 4 IM injections one week apart. Volunteers were also randomized to receive the first injection either in the right arm, or the left arm. Each of the 4 formulations was injected into the deltoid region in random order, with each subject acting as his/her own control. Volunteers assessed the severity of pain on a numerical scale where 0 = none, 1-3 = mild, 4-7 = moderate, and 8-10 = severe.

All participants completed the scheduled injections. Volunteers in all groups experienced pain at the injection site. However, QS-21 preparations were significantly more painful, and induced pain of greater severity than control formulations. Pain frequency and

severity did not differ between the two pH formulations of QS-21 (mean score of 4.9 for QS-21, pH 6.0, and of 4.7 for QS-21, pH 7.2). In comparison, mean pain scores were less than 1 for each of the 2 placebo formulations (PBS, pH 6.0 and pH 7.2). Severe pain episodes were infrequent, and were noted in less than 10% of volunteers following administration of QS-21 preparations, and in none following injections with placebo. Nearly half of the volunteers experienced mild tenderness at the injection site following QS-21 injection. Nine percent of the volunteers experienced mild local pain following injection with the placebo. There were 4 reports of systemic symptoms, of which none but 1 (severe headache) was felt to be QS-21-related. Five volunteers had transient elevation (>2-fold increase) in creatine phosphokinase levels compared to baseline. All values were within normal limits at the study's end.

The pH of the QS-21 formulation influenced neither the severity of pain, nor its character and duration.

Table 13: Effect of pH on Injection Site Pain

	Study Formulation			
	PBS, pH 6.0	**PBS, pH 7.2**	**QS-21, pH 6.0**	**QS-21, pH 7.2**
Total Number of Subjects[a]	33	33	33	33
Pain Score				
Mean	0.88	0.39	4.91	4.73
Range	0-5	0-3	0-9	0-8

[a] A total of 33 subjects were given each of the 4 formulations at random, into alternate deltoid areas.

5.3.2.2. Double-blinded, Randomized Trial Evaluating the Safety and Tolerance of Two Excipient Formulations of the Saponin Adjuvant QS-21 Following Intramuscular Administration to Normal Subjects

This study design was similar to the one mentioned in the previous section, except for the formulations used. Fifteen volunteers randomly received each of four IM injections at weekly intervals into alternating right and left deltoid muscles. One formulation contained PBS only (negative control). Three formulations contained QS-21 (50 µg); one in PBS (positive control), one with 4 mg/mL polysorbate 80, and one with 1 mg/mL of aluminum hydroxide. The main objective was to determine the effects of polysorbate 80 and aluminum hydroxide on the incidence and severity of immediate pain on injection following IM administration of QS-21. In addition, the study aimed at evaluating the overall safety and tolerance of polysorbate- and aluminum hydroxide-containing formulations compared to control formulations containing PBS with or without QS-21.

All volunteers (N=15) completed the scheduled four injections. The four formulations induced varying amounts of pain at the injection site. The QS-21 containing formulations had more frequent and severe pain than the PBS negative control formulation (**Table 14**). In addition, the QS-21 formulation containing polysorbate 80 was the closest in pain response levels to the negative control formulation, and induced significantly less pain than the positive control preparation (QS-21 in PBS) or QS-21/ aluminum hydroxide. All

formulations except the negative control induced mild tenderness. There were no serious adverse events reported. Polysorbate 80 appeared to reduce both the frequency and severity of pain on injection following IM administration of QS-21.

Table 14: Effect of Aluminum Hydroxide and Polysorbate 80 on Injection Site Pain

	Study Formulation			
	PBS	QS-21 4 mg/mL Polysorbate 80	QS-21 in PBS	QS-21 in Aluminum Hydroxide
Total Number of Subjects[a]	15	15	15	15
Pain Score				
Mean	0.3	2.1	3.7	3.8
Range	0-3	0-5	0-7	0-8

[a] A total of 15 subjects were given each of the 4 formulations at random, into alternate deltoid areas.

5.3.2.3. Double-Blinded, Randomized Trial Evaluating the Safety and Tolerance of Five Different Preparations of the Saponin Adjuvant, QS-21, Following Intramuscular Administration to Normal Volunteers

This study design was similar to the one mentioned in the previous section, except for the formulations used. Fifteen volunteers randomly received each of five I.M. injections at weekly intervals into alternating right and left deltoid muscles. The primary objective of this study was to determine whether the addition of polysorbate 80, hydroxypropyl-beta-cyclodextrin, or benzyl alcohol to a 50 µg dose of QS-21 influenced the incidence and severity of immediate pain following IM administration. The formulations are listed below.

The study also assessed the overall safety and tolerability of the QS-21 excipient formulations compared to a positive control containing 50 µg QS-21 and PBS and a negative control containing only aluminum hydroxide (500 µg) in saline.

The formulations tested in this study were as follows:

Aluminum hydroxide (500 µg) in saline – Negative Control

QS-21 (50 µg) + benzyl alcohol (0.72% in saline)

QS-21 (50 µg) + hydroxypropyl-beta-cyclodextrin (30 mg/mL in PBS)

QS-21 (50 µg) + polysorbate 80 (8 mg/mL in PBS)

QS-21 (50 µg) in PBS - Positive Control

Each subject received each of the 5 formulations in random order. One subject did not receive all injections due to relocation for a new job.

All three excipients tested in this study substantially reduced the immediate pain on injection sometimes associated with QS-21 (**Table 15**). QS-21 in hydroxypropyl-beta-

cyclodextrin was the least painful (mean pain score 0.9) and similar to the aluminum hydroxide group (mean score 0.3). QS-21 in 0.72% benzyl alcohol and 8 mg/ml polysorbate 80 averaged mean pain scores of approximately 2, while the QS-21 preparation in PBS had the highest mean pain score (4.3).

Overall, pain was mild in most volunteers, and no severe pain was recorded in any group. Furthermore, no serious adverse event occurred, and no significant laboratory abnormalities were encountered.

Table 15: Effect of Aluminum Hydroxide, Polysorbate 80, Hydroxypropyl-Beta-Cyclodextrin, and Benzyl Alcohol on Injection Site Pain

	Study Formulation				
	QS-21 in Hydroxypropyl-Beta-Cyclodextrin	Aluminum Hydroxide	QS-21 in 0.72% Benzyl Alcohol	QS-21 in 8 mg/mL Polysorbate 80	QS-21 in Phosphate-Buffered Saline
Total Number of Subjects[a]	14	15	15	14	15
Pain Score					
Mean	0.9	0.3	1.9	2.3	4.3
Range	0-5	0-2	0-6	0-6	1-7

[a] A total of 15 subjects were given each of the 5 formulations at random, into alternate deltoid areas.

The addition of all 3 excipients used in this study (benzyl alcohol, hydroxypropyl-beta-cyclodextrin and polysorbate 80) significantly reduced both the frequency and severity of immediate pain following IM injections of QS-21.

5.3.3. Serious Adverse Events

The following table summarizes the serious adverse events reported to Antigenics from patients enrolled in studies containing QS-21 as an adjuvant. To our knowledge, this includes all SAEs from trials reported in Tables 6-12. Primarily these studies have not been sponsored by Antigenics Inc. Therefore, the completeness of the data presented herein is dependent upon the willingness of the conducting sponsor to share clinical study data. Table 16 includes those serious adverse events listed as definite, related, or probable. Table 17 includes those serious adverse events listed as possible. An additional 142 serious adverse events listed as unknown (5), not related (113), and unlikely (24) have occurred in Antigenics' sponsored trials or been reported to Antigenics by the conducting sponsor.

Table 16: Serious Adverse Events Categorized as Definite, Related, or Probable

Event	Patient Identifier	Vaccine	Investigator Assessment	QS-21 Dose (μg)	Route	Schedule
Severe Cellulitis	16162	GM2-KLH plus QS-21	Definite	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Fever	278-DB	GM2-KLH plus QS-21	Definite	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Erythema	328-BC	GM2-KLH plus QS-21	Definite	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Injection site reaction	336-DAWE	GM2-KLH plus QS-21	Definite	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Elevated bilirubin (Grade 3)	16080	GM2-KLH plus QS-21	Related	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Syncope	16173	GM2-KLH plus QS-21	Probable	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Elevated bilirubin (Grade 3)	16251	GM2-KLH plus QS-21	Probable	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Skin desquamation at injection site (Grade 3)	16366	GM2-KLH plus QS-21	Probable	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Asthenia (Grade 3)	16258	GM2-KLH plus QS-21	Probable	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Diarrhea, dehydration, nausea & vomiting, fatigue, dizziness, fever/chills (Grade 4); two episodes	16456	GM2-KLH plus QS-21	Probable	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks

All SAEs in Table 16 reported by collaborator or licensee; Not audited by Antigenics.

Table 17: Serious Adverse Events Categorized as Possible

Event	Patient Identifier	Vaccine	Investigator Assessment	QS-21 Dose (μg)	Route	Schedule
Acute on chronic confusion, collapse	8011-MFC	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Cerebral infarction	0505-BG	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Confusional state; abnormal MRI	0301-JMD	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Convulsions NOS	6011-GC	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Depressed level of consciousness	0019-MSM	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Eczematous rash	6035-EMP	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis NOS[b]	8013-VD	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis NOS[b]	9166-JG	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis NOS[b]	1289-AC	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis NOS[b]	0038-MGW	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis NOS[b]	1093-LZ	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo

Event	Patient Identifier	Vaccine	Investigator Assessment	QS-21 Dose (μg)	Route	Schedule
Encephalitis NOS[b]	1256-FBE	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis NOS[b]	0299-EJG	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis NOS[b]	1279-VSC	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis NOS[b]	1502-MGC	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis NOS[b]	1092-LGV	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis NOS[b]	1325-VJU	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis NOS[b]	0033-HPP	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalopathy NOS	0007-HJG	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis post immunization	1332-JC	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis post immunization	1295-FT	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Encephalitis post immunization	1333-MA	AN1792[a] plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo

Event	Patient Identifier	Vaccine	Investigator Assessment	QS-21 Dose (μg)	Route	Schedule
Hemiparesis	0505-BG	AN1792® plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Hemorrhagic stroke	1285-MS	AN1792® plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Meningitis aseptic; cerebrovascular accident NOS	0503-GD	AN1792® plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Retinal vein thrombosis; confusion	0063-RE	AN1792® plus the adjuvant QS-21	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo
Hospitalized with cellulites/infection (Grade 2)	16099	GM2-KLH plus QS-21	Possible	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Elevated AST, bilirubin (Grade 3)	16104	GM2-KLH plus QS-21	Possible	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Elevated AST, ALT (Grade 3)	16292	GM2-KLH plus QS-21	Possible	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Elevated bilirubin (Grade 3)	17096	GM2-KLH plus QS-21	Possible	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Elevated total bilirubin (Grade 2)	17127	GM2-KLH plus QS-21	Possible	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Sleep interruption and hallucinations following vaccination	17047	GM2-KLH plus QS-21	Possible	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Elevated bilirubin (Grade 3)	17127	GM2-KLH plus QS-21	Possible	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Transaminitis (elevated AST, Grade 3)	17241	GM2-KLH plus QS-21	Possible	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks

Event	Patient Identifier	Vaccine	Investigator Assessment	QS-21 Dose (μg)	Route	Schedule
Elevated AST (Grade 3)	17241	GM2-KLH plus QS-21	Possible	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Neutropenia (Grade 3)	16491	GM2-KLH plus QS-21	Possible	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Fatigue, headache, dizziness, hot & cold	113-DJB	GM2-KLH plus QS-21	Possible	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Palpitations	371-JABR	GM2-KLH plus QS-21	Possible	100	S.C.	0, 1, 2, 3, 12, 24, 48, 60, 72, 84, 96 wks
Viral hepatitis[c]	undisclosed	Hepatitis envelope plus QS-21[d]	Possible	100	I.M.	0, 2, 4, 6, 14, 16, 18, 20, 28, 36, 44, 52 weeks
Flu syndrome[c]	undisclosed	Hepatitis envelope plus QS-21[d]	Possible	100	I.M.	0, 2, 4, 6, 14, 16, 18, 20, 28, 36, 44, 52 weeks
Anaphylactic reaction[c]	undisclosed	Hepatitis envelope plus QS-21[d]	Possible	100	I.M.	0, 2, 4, 6, 14, 16, 18, 20, 28, 36, 44, 52 weeks
Otitis media[c]	undisclosed	HIV antigen plus QS-21[d]	Possible	50	I.M.	0, 1, 6 mo
Syncope with a reflex anoxic seizure[c]	undisclosed	HIV antigen plus QS-21[d]	Possible	50	I.M.	0, 1, 6 mo
Flu like syndrome[c]	undisclosed	HPV antigen plus QS-21[d]	Possible	50	I.M.	0, 1, 2 mo
Flu like syndrome; elevated liver enzymes[c]	undisclosed	HPV antigen plus QS-21[d]	Possible	50	I.M.	0, 1, 2 mo

Event	Patient Identifier	Vaccine	Investigator Assessment	QS-21 Dose (μg)	Route	Schedule
Flu syndrome; back pain; difficulty in walking; oedema; fever[c]	undisclosed	HSV antigen plus QS-21[d]	Possible	50	I.M.	0, 2, 4, 6 mo
Flu like syndrome[c]	undisclosed	HSV antigen plus QS-21[d]	Possible	50	I.M.	0, 2, 4, 6 mo
Uveitis[c]	undisclosed	HSV antigen plus QS-21[d]	Possible	25 or 50 (undisclosed)	I.M.	0, 1, 6 mo
Tonic-clonic seizure 5 min post-vaccination[c]	undisclosed	HSV antigen plus QS-21[d]	Possible	25, 50, 75, or 100 (undisclosed)	I.M.	0, 1 mo
Flu syndrome	undisclosed	HSV antigen plus QS-21[d]	Possible	25, 50, 75, or 100 (undisclosed)	I.M.	0, 1 mo
Flu syndrome	undisclosed	HSV antigen plus QS-21[d]	Possible	25, 50, 75, or 100 (undisclosed)	I.M.	0, 1 mo
Increased ALT[c]	undisclosed	Malaria antigen plus QS-21[d]	Possible	50	I.M.	0, 1, 5, 19 mo
Worsening of symptoms (Alzheimer's)	8014-HGR	QS-21 alone	Possible	50	I.M.	0, 1, 3, 6, 9, 12 mo

[a]AN1792 is a synthetic form of the 42 amino acid beta amyloidal peptide
[b]Encephalitis events have not been reported in the QS-21-alone control-arm of this study. Additionally, no events of encephalitis have been reported to Antigenics from any other studies containing adjuvant QS-21.
[c]AE reported by collaborator or licensee; Not audited by Antigenics.
[d] QS-21 used in proprietary formulation containing other adjuvants/excipients.

5.4. Marketing Experience

QS-21 is an investigational product and is not currently marketed in either the United States or the rest of the world. Marketing approval for QS-21 has not been sought to date.

6. SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

6.1. Toxicity and Immunogenicity in Laboratory Animals

QS-21 has been shown to be a potent adjuvant for induction of antigen-specific immune responses both in laboratory animals and humans. Preclinical studies to date have shown this adjuvant to have an acceptable tolerance in mice, rabbits and primates administered QS-21-containing preparations by the SC and IM routes. Rabbits administered vaccines adjuvanted with QS-21 at the adjuvant doses as high as twice the recommended human dose (200 μg) often develop mild to moderate local side-effects, and minor biochemical and hematological alterations that are nonprognostic and brief. Laboratory analyses of immune responses evoked by QS-21-adjuvanted vaccines have established its immunoenhancing effects on the quality and magnitude of both the antibody and cellular responses against a variety of antigens. Studies performed in mice have demonstrated the ability of this adjuvant in the induction of antigen-specific CTL, an immune response that may be required for clearing infections caused by intracellular organisms, and for destroying tumor cells *in vivo*. Thus far, it is unclear how these effects are mediated. Activation of antigen-presenting cells (APC),[76] facilitation and/or enhancement of intracellular trafficking, lymph node homing of adjuvant-expanded APC are all possible mechanisms whereby adjuvants can affect antigen-specific immune responses.

6.2. Safety Evaluation in Humans

6.2.1. Anticipated Risks and Adverse Events

Vaccines containing QS-21 at doses of 50 and 100 μg induce local and systemic side-effects that tend to be more frequent, and of greater intensity compared to the same vaccines in an aluminum hydroxide formulation. However, aluminum hydroxide-induced tissue reactions such as granuloma, necrosis and nodules have never been reported in volunteers administered QS-21 alone, or combined with vaccine antigens. Nearly 90% of volunteers injected with vaccines mixed with 50 or 100 μg of QS-21 experience injection site pain of variable intensity and onset, mild to moderate erythema, induration and some arm soreness. Some volunteers have reported immediate and severe pain on injection following administration of QS-21 preparations. In most studies, this effect occurs in a small percentage of subjects. Data from completed clinical studies indicate these side-effects to be transient, resolving within 7 days without sequelae. Systemic side effects have also been reported following the administration of vaccines combined with 50 μg, 100 μg, and 200 μg of QS-21. The overall incidence and severity of these reactions are generally similar to those of other vaccines, except for a marked increase in reactogenicity among subjects given the 200 μg QS-21 dose.[24] Systemic events reported in recipients of

QS-21 formulations include low-grade fever, flu-like symptoms with body aches, malaise, chills, myalgia, headache, and dizziness. These symptoms are transient, resolving within 48 hours in most subjects. Severe adverse events (vaso-vagal reactions with hypotension and fainting) have also been reported, but they are extremely rare, occurring in less than 1% of QS-21 recipients. Very few volunteers have complained of non-pruritic rash, and hives following administration of GM2-KLH conjugate vaccines. These manifestations were mild, and resolved completely.

Severe allergic reactions have been noted among recipients of QS-21-containing malaria synthetic peptide vaccines in two trials. Two out of ninety volunteers administered 2 mg of the SPf66 antigen mixed with 50 µg of QS-21 developed generalized pruritus with a few pruritic hives and minor bronchospasm (one volunteer), facial erythema, palpebral edema and dysphonia (one volunteer). These manifestations occurred 5 to 10 minutes after administration of the third vaccine dose, and were accompanied by hypotension. The reactions resolved completely within 60 minutes following systemic therapy with epinephrine, hydrocortisone and anti-allergic drugs. Follow up exams performed 24 hours and 48 hours after the incidents were normal.

Allergic reactions have occasionally been reported among recipients of aluminum hydroxide-formulated SPf66 vaccines in previous trials in Latin America (R. Amador, personal communication). The reactions were described as generalized pruritus and minor bronchospasm that resolved after administration of corticosteroids and anti-allergic drugs. Contralateral reactions were also noted in volunteers vaccinated with the SPf66/aluminum hydroxide formulations in Latin America, Africa and Thailand. These reactions were noted in a very small number of volunteers, and resolved spontaneously.

Generalized pruritus and bronchospasm suggest IgE-mediated systemic hypersensitivity induced by the vaccine. Because aluminum hydroxide is reported to increase IgE titers, it is possible that aluminum hydroxide may have contributed to the allergic reactions noted earlier. The contributory role of SPf66, multiple antigen peptide or QS-21 to vaccine-induced allergy is unclear at present.

Meningoencephalitis events were observed in a Phase II study of an Alzheimer's Disease vaccine in Alzheimer's disease patients. This vaccine consisted of aggregated peptide Abeta42 (termed AN1792) plus QS-21 adjuvant. Subjects were originally scheduled to receive intramuscular immunizations of this vaccine at months 0, 1, 3, 6, 9, and 12. The dosing part of the study was halted after meningoencephalitis was observed in four subjects; follow-up continued. In all, the event was observed in 18 of 298 immunized subjects (in 1 subject receiving 1 dose, in 16 subjects receiving 2 doses, and in 1 subject receiving 3 doses, with all occuring within 6 months of the first immunization). The safety results of this study are described in Orgogozo et al[75]. The authors indicate that studies are underway to determine whether T-cell or microglial activation by the AN1792 vaccine, possibly associated with a T-cell activating domain in Abeta42, are responsible for the meningoencephalitis. To our knowledge, this event has not been observed with other QS-21 adjuvanted vaccines.

Data collected from Phase I and Phase II clinical trials of QS-21 containing vaccines indicate that the vast majority of adverse events are transient reactions confined to the site of injection. The potential health risks associated with these vaccines will depend mainly upon the type of antigen being used, and are likely to be a greater concern if the vaccine antigen has homology with normal tissue constituents. Tumor vaccines admixed with QS-21 should not be administered to persons who have a documented history of auto-immune diseases, unless skin testing or lab results have unequivocally excluded the antigen as a potential source of tissue reaction.

Clinical trials of QS-21 formulations containing the excipients benzyl alcohol, hydroxypropyl-beta-cyclodextrin, and polysorbate 80 have shown these excipients to have a substantial reductive effect on injection site pain. In contrast, the presence of Triton X-100 in QS-21 containing formulations, even in trace amounts, may contribute to pain on injection.

6.2.2. Pregnancy and Lactation

There are no data available on the reproductive effects of QS-21. Therefore, the potential risks posed by QS-21 alone or combined with vaccine antigens are unknown. For this reason, QS-21 formulations should not be given to pregnant or lactating women.

6.2.3. Pediatric Applications

Pediatric studies containing the adjuvant QS-21 involving 60 children age 6-11 years and 90 children age 1-5 years have been completed. Twenty-eight pediatric neuroblastoma patients have also been immunized with a 100 μg dose of QS-21. Vaccine studies targeting pediatric patients should be very carefully designed to include a dose-escalation scheme, and close monitoring of local side-effects.

6.2.4. Treatment of Over Dose

No information is available regarding the potential toxicity resulting from an overdose of QS-21 in humans.

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APPENDIX E – VACCINE GMP AND STABILITY TESTING

SUMMARY PROVIDED IN SUPPORT OF THE MANUFACTURE OF THE DRUG SUBSTANCE

Manufacturing facilities:

Manufacturing of the peptide (drug substance) will be performed at NeoMPS Inc. in facilities licensed by the California Department of Health Services, Food and Drug Division (Drug / Device manufacturing license # 63358) and registered with the Food and Drug Administration (FDA registration # 2028155).

I. PEPTIDE DESCRIPTION:

The sequence of the peptide (drug substance) to be manufactured for University of Arkansas for Medical Sciences is listed below using the single-letter code with the corresponding molecular weights, empirical formula and associated counter ion.

Peptide Sequence	Molecular Weight	Empirical formula	Counter ion	Peptide Content (Calculated)	Acetate Content (Calculated)
H-WRYTAPVHLGDG-aK-Cha-VAAWTLKAAa-NH_2	2706.2 amu.	$C_{128}H_{197}N_{35}O_{30}$	Acetate	90 %	10 %

Peptide Characterization QC testing and Specifications:

	QC Testing	Method	Specification
1	Appearance	Visual Observation	White to off-white powder
2	Identity	Mass Spectral Analysis	Correct MW ± 1 amu.
3	Sequencing	MS/MS for sequence confirmation	Correct Sequence
4	Identity	Amino Acid Analysis (AAA) for Identity	Correct Composition
5	Net peptide content (NPC)	Quantitative AAA for peptide content	≥75 %
6	Purity	RP-HPLC USP <621>	≥ 95% purity
7	Water Content	Karl Fischer, USP <921>	≤15 %
8	Residual solvents	Gas Chromatography, USP <467>	Report results
9	Quantification of Counter Ion, acetate	RP-HPLC, USP <621>	≤15 %
10	Total Fluorine	Combustion / Ion Selective Electrode USP <471>	<0.5%
11	Bacterial Endotoxin	Kinetic chromogenic, USP <85>	Report results
12	Specific rotation	Polarimetry, USP <781>	Report result

Notes: Purity: Spontaneous pyroglutamic acid formation for peptides starting with glutamic acid or glutamine residues is not considered an impurity.
Spontaneous cysteine and methionine oxidation for peptides that have those residues is not considered an impurity.
Tests 1-6 are performed in-house per applicable SOP's
Tests 7-12 may be subcontracted per applicable SOP (K0001)
See Appendix I for sample Certificate of Analysis of an exemplary product manufactured at NeoMPS for similar application

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II. SYNTHESIS / METHOD OF MANUFACTURE:

Chain assembly will be performed by solid phase peptide synthesis using Fmoc chemistry starting with the appropriate Fmoc Rink resin[2].

A. Starting materials:

1. Amino acids and derivatives:

   The protected amino acid used for the synthesis of these peptides will be of non-animal origin. The following natural amino acid derivatives, obtained from qualified vendors, will be used, as applicable, for the manufacture of the peptides: The amino acids will be received tested and released per applicable standard operating procedure (SOP).

Amino acid derivative	Approved supplier (selected)
Fmoc-Alanine	Senn Chemicals, Nova Biochem
Fmoc-D-Alanine	Senn Chemicals, Nova Biochem
Fmoc-Aspartic acid (tertiobutyl)	Senn Chemicals, Nova Biochem
Fmoc- N^{g} 2,2,4,6,7 pentamethyldihydrobenzofuran-5-sulfonyl-Arginine	Senn Chemicals, Nova Biochem
Fmoc-Cyclohexylalanine	Senn Chemicals, Nova Biochem
Fmoc-Glycine	Senn Chemicals, Nova Biochem
Fmoc-Histidine(trityl)	Senn Chemicals, Nova Biochem
Fmoc Isoleucine	Senn Chemicals, Nova Biochem
Fmoc Leucine	Senn Chemicals, Nova Biochem
Fmoc-N^{e}-Benzyloxycarbonyl-Lysine	Senn Chemicals, Nova Biochem
Fmoc-Proline	Senn Chemicals, Nova Biochem
Fmoc-O-tertiobutyl-Threonine	Senn Chemicals, Nova Biochem
Fmoc-Tryptophan(benzyloxycarbonyl)	Senn Chemicals, Nova Biochem
Fmoc-O-tertiobutyl-Tyrosine	Senn Chemicals, Nova Biochem

[1]Fmoc: fluorenylmethyloxycarbonyl. Name of the temporary protecting group of the N-α-amino group of the protected amino acids used during the synthesis. Also used to characterize the type of chemistry used for the peptide chain assembly.

[2]Merrifield, R.B. 1963. Solid phase peptide synthesis I: The synthesis of a tetrapeptide. J. Am. Chem. Soc. 85: 2149-2004.

2. Resins Used for Peptide Synthesis:

The following Fmoc derivatized amino acid resin, obtained from qualified vendors, were used for the manufacture of the peptide:

Resin	Selected Approved Supplier
Fmoc Rink Resin	Senn Chemicals, Nova Biochem

3. Other Chemicals

Chemical Name	Abbrev.	Grade	Selected Suppliers	Step Used in Manufacture	Minimum Acceptable Purity
2-Propanol	IPA	HPLC	EMD Chemical	Synthesis	99.5%
Acetic Acid	HOAc	ACS	Fisher/VWR	Cleavage / Purification	99.5%
Acetonitrile	ACN	HPLC	EMD Chemical	Purification	99.5%
Argon	Ar	Industrial	West Air	Packaging	99.99%
Diisopropylcarbodiimide	DIC	N/A	Albatross	Synthesis	94%
Diisopropylethylamine	DIEA	N/A	Albatross	Synthesis	98%
Dimethylformamide	DMF	ACS	EMD Chemical	Synthesis	99.5%
Hydroxybenzotriazole	HOBt	99%	Albatross	Synthesis	95%
Methanol	MeOH	HPLC	EMD Chemical	Column Cleaning	99.5%
Methylbenzhydrylamine resin	N/A	N/A	Sem Chemicals	Synthesis	N/A
Methylene Chloride	DCM	ACS	EMD Chemical	Synthesis	99.5%
Nitrogen	N_2	NF	West Air	Synthesis	99.99%
Piperidine		N/A	ChemImpex/Matrix	Synthesis	98%
Trifluoroacetic Acid	TFA	Biograde	Halocarbon	Cleavage / Purification	98%
tert-Butyl Methyl Ether	MTBE	HPLC	Fisher	Cleavage	99%
Water	H_2O	USP Purified	NeoMPS	All, except Synthesis	NLT 15 MΩ

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B. Flow Chart of Synthesis Cycle:

Figure 1 below describes the anticipated cycle for solid phase "Fmoc" chemistry of the peptide H-WRYTAPVHLGDG-aK-Cha-VAAWTLKAAa-NH_2. The starting resin is Fmoc Rink resin. The cycle is repeated for adding the remainder amino acids in the sequence listed below.

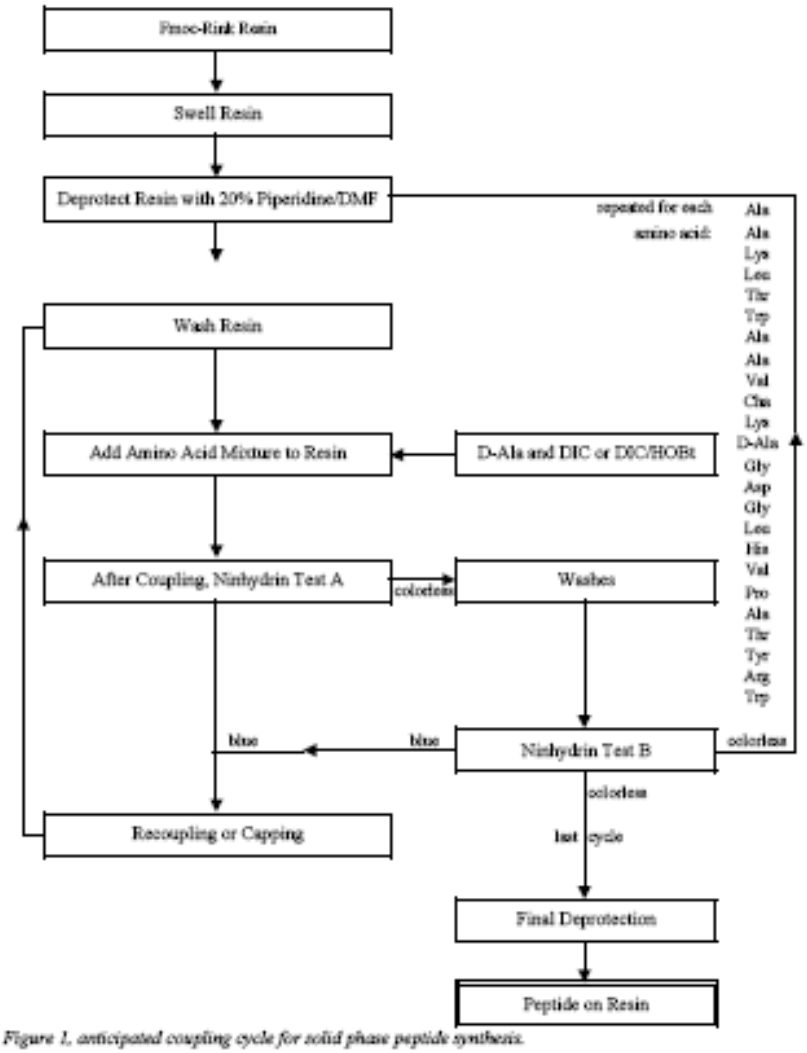

Figure 1, anticipated coupling cycle for solid phase peptide synthesis.

C. Detailed Description of Synthesis:

The general process of preparation of a peptide by solid phase synthesis can be broken down into 6 elemental steps:

1) Synthesis or assembly of the peptide on the resin; 2) side chain deprotection and cleavage of the peptide from the resin; 3) single or multiple step purification; 4) counter anion exchange, if needed; 5) packaging and labeling; and 6) QC testing and release.

1. Synthesis:

   The procedure used for the preparation of the peptide on resin is the general procedure described in the original paper of Merrifield[1] in 1963, with minor modifications.

   Synthesis will be performed for the peptide at room temperature, in an appropriate reaction vessel equipped with a fritted filter at the bottom for easy solvent wash and filtration of the solid support. Synthesis will be performed at an appropriate scale on the appropriate FmocRink resin[2]. Fmoc chemistry will be used throughout the peptide chain assembly. Calculation of solvent wash volumes, with the exception of the deprotection step, will be based on 8 ml/g of starting resin. Deprotection steps with 20% piperidine in dimethylformamide (DMF) will be performed with 12 ml per gram of starting resin, as a precaution to ensure complete Fmoc removal. Refer to page 5 for coupling cycle.

   The extent of coupling of each amino acid will be monitored by the ninhydrin test[3] and recoupling will be performed if a positive ninhydrin test result is obtained.

   After completion of the last coupling step (first amino acid in the peptide sequence), the resin will be treated with 20% piperidine/DMF to remove the N-terminal α-amino Fmoc protecting group.

   The deprotected resin will be washed with DCM and IPA, dried overnight under a nitrogen stream in the reaction vessel. The final yield of dry peptide resin will be determined and compared to the theoretically calculated amount as an in process check.

2. Cleavage of peptide:

   Each peptide will be cleaved from the resin using trifluoroacetic acid (TFA) and a number of appropriate cation scavengers to minimize side reactions. The cleaved peptide will be extracted in TFA and precipitated in ether. The precipitate will be filtered and dried under vacuum.

   In process checks includes a comparison of the crude yield versus theoretical yield, purity check by RP-HPLC and mass analysis for identity confirmation.

[1]Merrifield, R.B. 1963. Solid phase peptide synthesis I: The synthesis of a tetrapeptide. J. Am. Chem. Soc. 85: 2149-2004.

[2]Fmoc: fluorenylmethyloxycarbonyl. Name of the temporary protecting group of the N-α-amino group of the protected amino acids used during the synthesis. Also used to characterize the type of chemistry used for the peptide chain assembly.

[3]Sarin, V.K., et al. 1981. Quantitative monitoring of solid-phase peptide synthesis by the ninhydrin reaction. Anal. Biochemistry. 177: 147-157.

3. Purification:

The crude peptide obtained from the cleavage procedure will be purified using a "Waters" preparative HPLC system, using reverse phase chromatography.

A C_{18} derivatized silica column will serve as the stationary phase. Column size will be 5 cm in diameter and 30 cm in length. The mobile phase will be buffered water and acetonitrile. The gradient used will be tailored to achieve the purity specification. Care will be taken to clean the column after each purification run, as demonstrated with achievement of a flat baseline following methanol and acetonitrile wash of the column.

A single step purification should be sufficient to achieve the desired purity, although a second purification step may be required to achieve the required minimal purity specification by analytical HPLC. The column separation performance will be assessed with the analysis of the collected samples described below.

Analysis of the collected samples from purification will be accomplished using an analytical Beckman HPLC system and a Vydac (or equivalent) C_{18} 4.6 mm x 250 mm, 5 μm, 300 Å analytical column. The fractions containing pure peptide will be pooled and lyophilized to dryness in preparation for the exchange step or the second purification step. Lyophilization will be performed on a Virtis 25EL lyophilizer.

The purification step may yield the peptide as the proper counter ion in which case the exchange step to the final counter ion may not be necessary.

4. Ion Exchange Chromatography:

If necessary, ion exchange will be performed on an ion exchange resin, to convert the peptide to the acetate counter ion.

Lyophilization of the peptide to dryness will be performed on a Virtis 25EL lyophilizer.

5. Packaging and labeling:

Packaging of the peptide will be performed under argon in polyethylene containers with polypropylene closures. Alternatively glass amber vials with teflon lining with plastic closures.

6. QC testing / Release:

Product will be released for distribution after the analytical testing (see page 2) is completed, reviewed and reported on the Certificate of Analysis (see attached example Appendix I).

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III. FLOW CHART OF MANUFACTURING:

The anticipated flow chart of manufacturing should be similar to the sample outlined below *(figure 2)*, assuming a single step purification followed by a counter ion exchange from the trifluoroacetate to the acetate salt.

As indicated in the manufacturing details, a two-step purification may be adopted in order to achieve the desired purity level. In this case the ion exchange step may not be necessary.

Figure 2. Sample flow chart of manufacture.

IV. PROCESS CONTROLS:

Summary of In-Process Controls:

Process Step	Parameter	Acceptance Criteria
Synthesis	Ninhydrin test	Yellow color
	Final weight of peptide-resin	85 - 110 % of theoretical weight
TFA Cleavage	Theoretical Yield	State result
	Purity check by HPLC	For information only
	Mass Spectral analysis	Confirm identity
Purification	Purity check by HPLC	> 95 %
Pooling/Exchange (If necessary)	Purity check by HPLC	> 95 %
	Final weight of peptide acetate	85 - 100% of theoretical weight

V. REFERENCE STANDARD:

Approximately 500-1000 milligrams of the peptide manufactured in this campaign will be retained for the purpose of maintaining a reference standard.

VI. CONTAINERS AND CLOSURES:

Packaging of the peptide will be performed under argon in polyethylene containers with polypropylene closures. Alternatively glass amber vials with Teflon lining with plastic closures.

VII. PRODUCT RELEASE:

Product will be released for distribution after the analytical testing (see page 2) is completed, reviewed and reported on the Certificate of Analysis (see attached example Appendix I).

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APPENDIX I
Sample Certificate of Analysis

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APPENDIX F – UAMS INVESTIGATIONAL AGENT ACCOUNTABILITY RECORD

Investigational Agent Accountability Record

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PAGE NO. __
CONTROL RECORD
X
SATELLITE RECORD
☐

Name of Institution: **UAMS**	Protocol Number.:
Agent Name:	Dose Form and Strength:
Protocol Title:	Dispensing Area:
Investigator Name: **DR.**	Other Information:

Line No.	Date	Patient's Initials	Patient's ID No.	Dose	Quantity Dispensed or Received	Balance Forward / Balance	Lot #	Recorder's Initials
1.								
2.								
3.								
4.								
5.								
6.								
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15.								

APPENDIX G – UAMS RECORD DESTRUCTION POLICY

SCOPE: Health Information Management

POLICY: It is the policy of Health Information Department to ensure that medical records remain confidential throughout the destruction process.

PROCEDURE:

Planned destruction of records/data shall be carried out according to the approved record retention policy ML 2.02 in the Administrative Guide. No further approval for the destruction of records/data shall be required when such destruction is in accordance with the approved record retention schedule. Medical records are considered in original format when microfilmed or scanned into the Electronic Medical Record.

Medical record paper is destroyed at present when paper medical records are microfilmed and when paper medical records have been scanned into the electronic medical record system.

1. Method of Destruction

   Medical record data shall be destroyed in such a way that there is no possibility of reconstructing any of the information. One of the following methods shall be used: shredding, incineration or pulping. The use of contractual arrangements with a commercial record destruction company and/or microfilming company shall be permitted provided that appropriate guarantees of the confidentiality of the data are included in the contractual agreement. The confidentiality of the data shall be maintained throughout all stages of the destruction process.

2. Record of Destruction

   When a commercial record destruction company carries out the destruction process, the designated official of the company shall complete the record destruction certificate and provide it to the facility. All certificates of destruction shall be maintained indefinitely in a central file in Health Information Management. Certificates of destruction refer to box numbers not individual patient numbers.

REFERENCES: Medical Legal Policy 2.2

APPENDIX H - UAMS ADVERSE EVENTS STANDARD OPERATING PROCEDURE (SOP)

ARKANSAS CANCER RESEARCH CENTER — **Clinical Research Data Management**

SOP Number: 510.00	Title: Submission of Serious Adverse Event Reports	
Rev. No.:	Effective Date: Sep 1, 2008	Page 1 of 4
Supersedes: Date:	REQUIRED APPROVALS BELOW	
Regulatory Supervisor:	Sandy Annis Sandy Annis	Date: Aug. 28, 2008
Data Management Supervisor:	Kristen Hildebrand	Date: AUG 28, 2008
CRDM Administrator:	Wake Young Wake Young	Date: Aug 28, 2008
Clinical Research Administrator:	Phil Morgan Phil Morgan	Date: Aug 28, 2008

STANDARD OPERATING PROCEDURE

1.0		**Purpose**
		The purpose of this standard operating procedure is to describe the process/procedures used by the Clinical Research Data Management (CRDM) office when submitting serious adverse event (SAE) reports.
2.0		**Scope**
		This SOP applies to any/all CRDM staff members responsible for submitting and/or tracking SAE reports.
3.0		Responsibilities
	3.1	3.1.1 The clinical research associates are responsible for initial submission and processing of SAE reports, once they are reported to CRDM by the PI or research nurse. 3.1.2 The regulatory specialists are responsible for tracking data that has been submitted in ARIA, as defined in this SOP.
4.0		**References**
	4.1	Adherence to these standards will foster compliance of these studies with the Good Clinical Practice (GCP) guidelines that have been put into place by the International Conference of Harmonization (ICH) and applicable governing regulatory bodies according to 45 CFR 46 & 21 CFR 312.32.
5.0		**Definitions**
	5.1	5.1.1 **Adverse Event (AE)** - Any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure. 5.1.2 **Serious Adverse Event (SAE)** - Any adverse drug experience occurring at any dose that results in any of the following outcomes: death, a life-

threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

5.1.3 **Related** - An event is "related" if it is likely to have been caused by the research drug or treatment activity.

5.1.4 **Expected** - An event that is listed in the protocol, package insert, device manual and/or Investigator's Brochure (IB) as "expected."

5.1.5 **Unexpected** - An event is "unexpected" when its specificity, nature, severity or incidence are not accurately specified in or not consistent with the risk information in the protocol, IB or device manual information previously reviewed and approved by the IRB.

5.1.6 **Anticipated** – An event that is foreseeable at the beginning of the study.

5.1.7 **Unanticipated** - An event is "unanticipated" when it was unforeseen at the time it occurred.

5.1.8 **Life-Threatening** Diseases or conditions where the likelihood of death is high unless the course of the disease is interrupted and diseases or conditions with potentially fatal outcomes, where the end point of clinical trial analysis is survival.

5.1.9 **Clinical Research Associate (CRA)** - Manages the clinical aspects of a trial, including all clinical research data. The CRA is the primary contact for subject registration, determines subject eligibility, is responsible for AE/SAE reporting and subject data collection for both on- and off-sites, orders the study drug, coordinates on-site monitoring visits and works with the PI and Medical Monitor for prompt reporting of AEs/SAEs.

5.1.10 **CRDM Regulatory Specialist/Personnel** - Primary contact for all protocol-related regulatory submissions on- and off-site. The CRDM Regulatory Specialist assists the PI in updating protocols, maintains regulatory binders and a log of all protocol related activities, and maintains necessary documents.

5.1.11 **Office of Research Compliance (ORC)** - The ORC supports those activities that protect human research subjects and elevates the general level of research through systematic evaluation of research activities. The ORC functions as the auditing and compliance body for the UAMS Institutional Review Board. Functions include Auditing, Monitoring, Regulatory Affairs, Education and Advisory Consultation efforts that promote research compliance and integrity.

5.1.12 **Sponsor** - A person who takes responsibility for and initiates a clinical investigation. The Sponsor may be an individual or pharmaceutical company, governmental agency, academic institution, private organization, or other organization.

5.1.13 **Investigator** - An individual who actually conducts a clinical investigation (i.e., under whose immediate direction the drug is administered or dispensed to a subject). In the event an investigation is conducted by a team of individuals, the investigator is the responsible leader of the team. "Subinvestigator" includes any other individual member of that team.

5.1.14 **Medical Monitor** - The person responsible for monitoring the conduct and

		progress of the clinical investigation. This person should be a physician familiar with the disease for which the drug is under investigation. Their responsibilities include but are not limited to, assess and resolve clinical scenarios as they occur through the life-cycle of a trial regarding patient eligibility, concomitant medication, evaluability, SAE evaluation, etc.
6.0		**Procedures**
	6.1	6.1.1 **Serious Adverse Event Reporting:** Upon receipt of notification of an SAE, the CRA will complete either a "Serious Adverse Event Reporting Form" or "Death Reporting Form" in ARIA. 6.1.1.1 The CRA will discuss the clinical information with the treating physician to confirm accurate reporting. 6.1.1.2 If the study is an investigator-initiated study, the CRA will forward the forms to the medical monitor for review. 6.1.1.3 For **investigator-initiated studies only**, the medical monitor reviews the information and updates the report as he/she deems appropriate. 6.1.1.3.1 The medical monitor makes the final determination on the following classifications and signs the form in ARIA: serious/non-serious, expected/unexpected; relationship to study drug; affect on consent form; requirement for expedited FDA and IRB reporting; and requirement for follow-up reporting. 6.1.1.4 For all studies, the PI reviews and signs all required forms in ARIA. 6.1.1.4.1 For **investigator-initiated studies only**, data/form disagreements are resolved with the medical monitor. 6.1.1.4.2 For **investigator-initiated studies only**, the medical monitor can only upgrade an event following PI review. 6.1.1.5 The CRA will collect all forms from the PI, any additional forms required by the sponsor, and any required source documents, and will then finalize the SAE or Death Report forms. 6.1.1.5.1 The CRA will submit sponsor-specific forms to the sponsor. 6.1.1.6 For **investigator-initiated studies only**, the CRA will complete and submit the MedWatch form to the Research Support Center. 6.1.1.7 CRDM will make and maintain copies of all signed forms. 6.1.1.8 The regulatory specialist for the applicable study will track SAE-related data in ARIA to ensure that it is approved. 6.1.2 **Non-Serious Adverse Event Reporting (Investigator-initiated studies only):** Upon receipt of notification of an AE, the CRA will complete an "Adverse Event Reporting Form." This information will be maintained for the annual report. 6.1.3 **Reporting Deadlines.** The following are required reporting deadlines: 6.1.3.1 Related deaths will be reported immediately to the IRB. 6.1.3.2 Unexpected life-threatening or fatal SAEs will be reported to the FDA within 7 days. 6.1.3.3 All other SAEs will be reported to the IRB within 10 days. 6.1.3.4 Unexpected SAEs will be reported to the FDA within 15 days.

7.0		Attachments :
8.0		Revision History
Revision	Date	Description of Change
0		Initial SOP

APPENDIX J – UAMS MEDIAL MONITOR SOP

Winthrop P. Rockefeller Cancer Institute

Division of Hematology/Oncology and Clinical Research Data Management

SOP Number: 511.00	**Title: Medical Monitors**	
Rev. No.:	**Effective Date: Nov 1, 2008**	**Page 1 of 2**
Supersedes: **Date:**	**REQUIRED APPROVALS BELOW**	
Data Management Supervisor:	**Kristen Hildebrand**	**Date:** 10/27/2008
CRDM Administrator:	**Wake Young**	**Date:** 10/28/08
Hematology/Oncology Division Chair:	**Laura Hutchins, M.D.**	**Date:** 10/27/08

STANDARD OPERATING PROCEDURE

1.0	**Purpose**
	The purpose of this standard operating procedure is to define the credentialing requirements for and responsibilities of the medical monitor of an oncology investigational drug study.
2.0	**Scope**
	This SOP applies to all Hematology/Oncology physicians who serve as a medical monitor.
3.0	**Responsibilities**
	Medical monitors are responsible for the following: 3.1 Ensuring that they maintain proper credentials required to perform duties as a medical monitor. 3.2 Reviewing adverse events (AEs) and serious adverse events (SAEs). 3.3 Reviewing monitoring reports. 3.4 Working with the principal investigator (PI) in assessing and resolving various clinical scenarios related to subjects participating in a clinical trial.
4.0	**Procedures**
	4.1 Credentialing and Selection Criteria 4.1.1 The medical monitor must maintain current licensure as an MD. 4.1.2 The medical monitor should be a physician in Hematology/ Oncology or the field that usually cares for subjects with the disease being studied or the agent being studied. 4.1.3 The medical monitor must have the knowledge and ability required to provide medical care to the subjects participating in the study; however, the medical monitor should be the physician least likely to provide care to the subjects participating in the study and should not be supervised by the PI. 4.2 Review of AEs/SAEs 4.2.1 Upon receipt of a Serious Adverse Event Reporting Form or a Death Reporting Form, the medical monitor will review the circumstances of the event and the decisions of the principal investigator pertaining to the following, to validate

whether or not the event was:

4.2.1.1 An adverse event or a serious adverse event.

4.2.1.2 Expected or unexpected.

4.2.1.3 Related or unrelated to the investigational new drug.

4.2.2 If the medical monitor agrees with the decisions of the principal investigator, no further action is required.

4.2.3 If the medical monitor does not agree with the decisions of the principal investigator, he/she may upgrade, but not downgrade, the severity of the incident by annotating the applicable Serious Adverse Event Reporting Form or the Death Reporting Form and returning the document(s) to the study's clinical research associate.

4.3 Reviewing monitoring reports.

4.3.1 The medical monitor will review monitoring reports at times indicated in the study protocol.

4.4 Assessing and Resolving Clinical Scenarios

4.4.1 During the course of a clinical trial, it is possible that questions may arise regarding the eligibility of a potential study subject, the use of concomitant medications, the evaluability of a subject, or other clinical scenario.

4.4.2 Questions on any of these types of clinical scenarios may be surfaced to the medical monitor by the principal investigator, co-investigators, research nurses, or clinical research associates.

4.4.3 Feedback from the medical monitor will be forwarded to the principal investigator, who will then determine the final course of action.

4.4.4 The principal investigator will then notify the clinical research associate so that proper reporting can be accomplished.

5.0 Attachments

6.0 Revision History

Date	Description of Change
	None; this is the initial SOP

Eligibility Checklist

Subject ID: _____

Consent Date: ___ (Day) ___ (Month) ___ (Year)

Subject Initials: ___

Instructions: *Check the appropriate box for each Inclusion and Exclusion Criterion below. Each criterion must be marked and all protocol criteria have to be met prior to enrolling the subject.*

Inclusion Criteria

Each criterion must be addressed and documented in the subject's medical record or source. All inclusion criteria must be checked 'yes' or a waiver must be obtained from the medical monitor.

Y	N	
☐	☐	1. Subjects must have histologically or cytologically confirmed diagnosis of stage IV female breast carcinoma (newly diagnosed metastatic or relapsed after primary or adjunctive therapy, which has not required a treatment change for 2 months).
☐	☐	2. Subjects has no other significant medical, surgical or psychiatric condition or require any medication or treatments which would interfere with protocol compliance.
☐	☐	3. Subjects must not have organic brain syndrome or dementia that would preclude consent or compliance with the protocol.
☐	☐	4. Subjects must be immunocompetent by a minimum of two recall antigens by skin testing.
☐	☐	5. Subjects must have recovered from prior surgical procedure.
☐	☐	6. Performance ECOG status of equal to or greater than 1.
☐	☐	7. Measurable or evaluable disease.
☐	☐	8. Adequate organ functions measured within two weeks of registration: - White blood cell count > 3000/mm3 - Platelet county >= 100000/mm3 - AST <= 2 X ULN - Serum creatinine <= 1.8 mg/dL
☐	☐	9. Life expactancy of >= 3 months
☐	☐	10. Subjects must be able to sign informed consent and be agreeing to comply with therapy and follow-up
☐	☐	11. Negative pregnancy test in women with childbearing potential, within 48 hrs prior to initiation of treatment
☐	☐	12. Fertile women must agree to use adquate contraception prior to study entry, for the duration of the study participation, and for a minimum of 18 months after therapy.
☐	☐	13. Age >= 18 years

Subject ID: | | | | | |

Consent Date: | | Day | | | | Month | | | | | Year

Subject Initials: | | | |

Exclusion Criteria

Each criterion must be addressed and documented in the subject's source record. All exclusion criteria must be checked 'no' or a waiver must be obtained from the medical monitor.

Y	N	
☐	☐	1. Subject is lactating.
☐	☐	2. Subject has autoimmune disorder(s).
☐	☐	3. Subject with active bacterial infection(s).
☐	☐	4. Subject with immunosupression or treatment with corticosteriods, oral or continuious topical.
☐	☐	5. Other co-existing malignancies diagnosed with the last 5 years with the exception of adequately treated basal cell (or squamous cell) skin cancer, in situ cervical cancer, lobular or dutal carcinoma in situe, Clark level 1 melanoma, and/or melanoma in
☐	☐	6. Subject has allergies to shellfish.
☐	☐	7. Inability to comply with study and/or follow-up procedures
☐	☐	8. Subject is HIV / AIDS negative.

Subject ID: ☐☐☐☐☐ **Consent Date:** ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Subject Initials: ☐☐☐

Date of Birth: ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Race:
(Mark all which apply)

- ☐ White
- ☐ Black or African American
- ☐ Native Hawaiian or other Pacific Islander
- ☐ Asian
- ☐ American Indian or Alaska Native
- ☐ Unknown

Ethnicity:
(Mark only 1)

- ☐ Hispanic or Latino
- ☐ Non-Hispanic
- ☐ Unknown

Subject ID: | | | | | |

Consent Date: | | Day | | | | Month | | | | Year

Subject Initials: | | | |

Date of initial diagnosis of breast carcinoma: | | Day | | | | Month | | | | Year

Stage at Initial diagnosis (TNM):

Date of diagnosis of metastatic breast carcinoma | | Day | | | | Month | | | | Year

Stage at metastatic diagnosis (TNM):

Stage Grouping (at study entry)

☐	Stage I	☐	Stage IIIA	☐	IIIC
☐	Stage II	☐	Stage IIIB	☐	Stage IV

Medical History

Subject ID: _____

Consent Date: ____ (Day) ____ (Month) ____ (Year)

Subject Initials: ____

Record any previous illnesses, surgeries or medical condtions in the space provided below

System	Normal	Abnormal	List Date, Diagnosis, and CTCAE Grade
Head/Ears/Eyes/Nose/Throat	☐	☐	
Respiratory	☐	☐	
Cardiovascular	☐	☐	
Gastrointestinal	☐	☐	
Hepatic/GallBladder	☐	☐	
Renal	☐	☐	
Genitourinary	☐	☐	
Musculoskeletal	☐	☐	
Dermatological	☐	☐	
Nervous System	☐	☐	
Hematological	☐	☐	
Lymphatic	☐	☐	
Endocrine	☐	☐	
Psychological/Psychiatric	☐	☐	
Other _____	☐	☐	

Prior Metastatic Cancer Treatment

Subject ID: _____ **Consent Date:** Day _____ Month _____ Year _____

Subject Initials: _____

Prior Surgery for Breast Cancer

Has the subject had any surgical procedure related to tretment of breast cancer?

☐ No ☐ Yes (specify below)

	Site code	Surgery Description	Year	Month	Day
1					
2					
3					

Prior Radiation Therapy for Metastatic Breast Cancer

(Subject must have measurable disease outside of the radiation therapy port and radiation must begin prior to randomization)

☐ No ☐ Yes (specify below)

	Specify Area	Total Dose (cGy)	Day	Month	Year
1					
2					
3					

Subject ID: _____ **Consent Date:** ___ (Day) ___ (Month) ____ (Year)

Subject Initials: ___

Prior Chemotherapy for Metastatic Breast Cancer

Has the subject had any chemotherapy related to the treatment of Metastatic Breast Cancer?

☐ No ☐ Yes (specify below)

Regimen or Medication

1 _____ ☐ check if adjuvant therapy

Start ___ (Day) ___ (Month) ____ (Year) ___ (Day) ___ (Month) ____ (Year)

2 _____ ☐ check if adjuvant therapy

Start ___ (Day) ___ (Month) ____ (Year) ___ (Day) ___ (Month) ____ (Year)

3 _____ ☐ check if adjuvant therapy

Start ___ (Day) ___ (Month) ____ (Year) ___ (Day) ___ (Month) ____ (Year)

Prior Local Therapy for Breast Cancer

Has the subject had any local therapy related to treatment of breast cancer?

☐ No ☐ Yes (specify below)

	Therapy code	Site	
1	_____	_____	___ (Day) ___ (Month) ____ (Year)
2	_____	_____	___ (Day) ___ (Month) ____ (Year)
3	_____	_____	___ (Day) ___ (Month) ____ (Year)

Local Therapy Codes

1 = Chemoembolization 2 = Radiofrequency ablation 3 = Local injection

Subject ID: | | | | | |

Consent Date: | | Day | | | | Month | | | | Year

Subject Initials: | | | |

Prior Hormonal Therapy for Breast Cancer

List the hormonal therapy the subject was taking when disease progression occurred

	Hormone Therapy	Start Date: Day	Start Date: Month	Start Date: Year	Stop Date: Day	Stop Date: Month	Stop Date: Year
1							

Physical Exam

Subject ID: _____

Consent Date: ____ (Day) ____ (Month) ____ (Year)

Subject Initials: ____

Date of this exam: ____ (Day) ____ (Month) ____ (Year)

	WNL	ABN	ND	Comment if Abnormal
General Appearance	☐	☐	☐	_____
Head	☐	☐	☐	_____
Ear/Eyes/Nose/Throat	☐	☐	☐	_____
Cardiovascular	☐	☐	☐	_____
Breasts	☐	☐	☐	_____
Pulmonary	☐	☐	☐	_____
Abdomen	☐	☐	☐	_____
Musculoskeletal	☐	☐	☐	_____
Skin	☐	☐	☐	_____
Neurological	☐	☐	☐	_____
Lymphatic	☐	☐	☐	_____
Extremities	☐	☐	☐	_____
Other_____	☐	☐	☐	_____

Subject ID: _____ **Consent Date:** ____ ____ ____ ____

Day Month Year

Subject Initials: ___ ___ ___

Date of this exam: ____ ____ ____ ____

Day Month Year

Height: ___ ___ ___ . ___ ___ cm ___ in ___ Not Done

Weight: ___ ___ ___ . ___ ___ kg ___ lbs ___ Not Done

Blood Pressure: ___ ___ ___ / ___ ___ ___ mmHg ___ Not Done

Pulse: ___ ___ ___ beats/min ___ Not Done

Temperature: ___ ___ ___ . ___ ___ °C ___ °F ___ Not Done

Performance Status: ___ Not Done

___ 0

___ 1

___ 2

___ 3

___ 4

Laboratory

Subject ID: _____ **Consent Date:** ____ ____ Day ____ ____ ____ Month ____ ____ ____ ____ Year

Subject Initials: _____

Laboratory Values:

Specimen Collection Date: ____ ____ Day ____ ____ ____ Month ____ ____ ____ ____ Year

		ND			ND
WBC (K/µL)	____ . ____			____ . ____	
ANC	_____		LDH (IU/L)	_____	
Platelets (K/µL)	____		Alk. phos. (IU/L)	_____	
Hemoglobin (g/dL)	____ . ____		Albumin (g/dL)	____ . ____	
Neutrophils (%)	____		SGOT/AST (IU/L)	____	
Sodium (mEq/L)	____		T4	____	
Potassium (mEq/L)	____ . ____		GGT (IU/L)	____	
Amylase	____		PT (sec)	____ . ____	
TSH	____		PTT (sec)	____ . ____	
Calcium	____		ANA	____ ____	
Creatinine (mg/dL)	____ . ____		HC	____	
Lymphocyte	____		Pregnancy results:	____ Positive ____ Negative	

Study Labs

Were study labs performed? ____ Yes ____ NO

if yes, then date and time: ____ ____ Day ____ ____ ____ Month ____ ____ ____ ____ Year ____ : ____ Time

Radiology

Week 0 . 0

Subject ID: _____

Consent Date: _____ _____ _____
Day Month Year

Subject Initials: _____

	Day	Month	Year	Normal	Evidence of Disease	Not Done
Chest X-ray	_____	_____	_____	_____	_____	_____
CT Chest	_____	_____	_____	_____	_____	_____
and/or						
MRI Chest	_____	_____	_____	_____	_____	_____
CT Abdomen	_____	_____	_____	_____	_____	_____
and/or						
MRI Abdomen	_____	_____	_____	_____	_____	_____
CT Pelvis	_____	_____	_____	_____	_____	_____
and/or						
MRI Pelvis	_____	_____	_____	_____	_____	_____
PET/ CT	_____	_____	_____	_____	_____	_____
Bone Scan	_____	_____	_____	_____	_____	_____

Skin Test

Week 0 . 0

Subject ID: | | | | | |

Consent Date: | | Day | | | | Month | | | | Year | | | |

Subject Initials: | | | |

Skin Test Administration

	Dose	**Day**	**Month**	**Year**	**Time** (24 hr clock)
Tetanus-Diaphtheria Toxoid Antigen Administration	_____				:
Candida Antigen Administration	_____				:

Skin Test Reading

(48-72 hrs post administration)

	Induration (mm)	**Day**	**Month**	**Year**	**Time** (24 hr clock)
Tetanus-Diaphtheria Toxoid Antigen Administration	_____				:
Candida Antigen Administration	_____				:

Physical Exam

Subject ID: _____

Date: ____ (Day) ____ (Month) ____ (Year)

Subject Initials: _____

☐ **Check here if physical exam not performed**

Date of this exam: ____ (Day) ____ (Month) ____ (Year)

	WNL	ABN	ND	Comment if Abnormal
General Appearance	☐	☐	☐	_____
HEENT	☐	☐	☐	_____
Pulmonary	☐	☐	☐	_____
Cardiovascular	☐	☐	☐	_____
Abdomen	☐	☐	☐	_____
Musculoskeletal	☐	☐	☐	_____
Extremities	☐	☐	☐	_____
Neurological	☐	☐	☐	_____
Skin	☐	☐	☐	_____
Lymphatic	☐	☐	☐	_____
Other_____	☐	☐	☐	_____

Vital Signs

Subject ID: ☐☐☐☐☐ **Date:** ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Subject Initials: ☐☐☐

☐ **Check here if vital signs not performed**

Date of this exam: ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Height: ☐☐☐ . ☐ ☐ cm ☐ In ☐ Not Done

Weight: ☐☐☐ . ☐ ☐ kg ☐ lbs ☐ Not Done

Blood Pressure: ☐☐☐ / ☐☐☐ mmHg ☐ Not Done

Pulse: ☐☐☐ beats/min ☐ Not Done

Temperature: ☐☐☐ . ☐ ☐ °C ☐ °F ☐ Not Done

Performance Status: ☐ Not Done

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5

Laboratory

Subject ID: _____

Date: ___ (Day) ___ (Month) _____ (Year)

Subject Initials: ___

☐ **Check here if Labs not performed**

Laboratory Values:

Specimen Collection Date: ___ (Day) ___ (Month) _____ (Year)

		ND			ND
WBC (K/µL)	__.__	☐		__.__	☐ ☐
ANC	_____	☐	LDH (IU/L)	____	☐ ☐
Platelets (K/µL)	___	☐	Alk. phos. (IU/L)	____	☐ ☐
Hemoglobin (g/dL)	___.__	☐	Albumin (g/dL)	__.__	☐
Neutrophils (%)	___		SGOT/AST (IU/L)	___	☐
Sodium (mEq/L)	___	☐	T4	___	☐
Potassium (mEq/L)	_.__	☐	GGT (IU/L)	___	☐
Amylase	___	☐	PT (sec)	__.__	☐
TSH	___	☐	PTT (sec)	__.__	☐
Calcium	___	☐	ANA	___ ☐	☐
Creatinine (mg/dL)	___.__	☐	HC	___	☐
Lymphocyte	___	☐			

Skin Test

Week 1 . 0

Subject ID: | | | | | |

Date: | | (Day) | | | | (Month) | | | | (Year)

Subject Initials: | | | |

Skin Test Administration

	Dose	Day	Month	Year	Time
Tetanus-Diaphtheria Toxoid Antigen Administration	_____				:
Candida Antigen Administration	_____				:

Skin Test Reading

	Induration (mm)	Day	Month	Year	Time
Tetanus-Diaphtheria Toxoid Antigen Administration	_____				:
Candida Antigen Administration	_____				:

Physical Exam

Subject ID: _____

Date: ____ (Day) ____ (Month) ____ (Year)

Subject Initials: _____

☐ **Check here if physical exam not performed**

Date of this exam: ____ (Day) ____ (Month) ____ (Year)

	WNL	ABN	ND	Comment if Abnormal
General Appearance	☐	☐	☐	_____
HEENT	☐	☐	☐	_____
Pulmonary	☐	☐	☐	_____
Cardiovascular	☐	☐	☐	_____
Abdomen	☐	☐	☐	_____
Musculoskeletal	☐	☐	☐	_____
Extremities	☐	☐	☐	_____
Neurological	☐	☐	☐	_____
Skin	☐	☐	☐	_____
Lymphatic	☐	☐	☐	_____
Other_____	☐	☐	☐	_____

Subject ID: ☐☐☐☐☐ **Date:** ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Subject Initials: ☐☐☐

☐ **Check here if vital signs not performed**

Date of this exam: ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Height: ☐☐☐.☐ ☐ cm ☐ In ☐ Not Done

Weight: ☐☐☐.☐ ☐ kg ☐ lbs ☐ Not Done

Blood Pressure: ☐☐☐ / ☐☐☐ mmHg ☐ Not Done

Pulse: ☐☐☐ beats/min ☐ Not Done

Temperature: ☐☐☐.☐ ☐ °C ☐ °F ☐ Not Done

Performance Status: ☐ Not Done

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5

Laboratory

Subject ID: _____

Date: ____ (Day) ____ (Month) ____ (Year)

Subject Initials: ____

☐ **Check here if Labs not performed**

Laboratory Values:

Specimen Collection Date: ____ (Day) ____ (Month) ____ (Year)

		ND			ND
WBC (K/µL)	____ . ____	☐	Total bilirubin (mg/dL)	____ . ____	☐
ANC	_____	☐	LDH (IU/L)	____	☐
Platelets (K/µL)	____	☐	Alk. phos. (IU/L)	____	☐
Hemoglobin (g/dL)	____ . ____	☐	Albumin (g/dL)	____ . ____	☐
Neutrophils (%)	____		SGOT/AST (IU/L)	____	☐
Sodium (mEq/L)	____	☐	T4	____	☐
Potassium (mEq/L)	____ . ____	☐	GGT (IU/L)	____	☐
Amylase	____	☐	PT (sec)	____ . ____	☐
TSH	____	☐	PTT (sec)	____ . ____	☐
Calcium	____	☐	ANA	____ ____	☐
Creatinine (mg/dL)	____ . ____	☐	HC	____	☐
Lymphocyte	____	☐			

Study Labs

Were study labs performed? ☐ Yes ☐ NO

if yes, then date and time: ____ (Day) ____ (Month) ____ (Year) ____ : ____ (Time)

Study Drug Administration

Subject ID: | | | | | |

Date: | | Day | | | | Month | | | | Year | | | |

Subject Initials: | | | |

Administration **Subcutaneously in rotating sites**

_____ ug _____ Site | | Day | | | | Month | | | | Year | : Time

Additional Comments:

Physical Exam

Subject ID: _____

Date: ____ (Day) ____ (Month) ____ (Year)

Subject Initials: _____

☐ **Check here if physical exam not performed**

Date of this exam: ____ (Day) ____ (Month) ____ (Year)

	WNL	ABN	ND	Comment if Abnormal
General Appearance	☐	☐	☐	_____
HEENT	☐	☐	☐	_____
Pulmonary	☐	☐	☐	_____
Cardiovascular	☐	☐	☐	_____
Abdomen	☐	☐	☐	_____
Musculoskeletal	☐	☐	☐	_____
Extremities	☐	☐	☐	_____
Neurological	☐	☐	☐	_____
Skin	☐	☐	☐	_____
Lymphatic	☐	☐	☐	_____
Other_____	☐	☐	☐	_____

Subject ID: _____

Date: ___ (Day) ___ (Month) _____ (Year)

Subject Initials: ___

☐ **Check here if vital signs not performed**

Date of this exam: ___ (Day) ___ (Month) _____ (Year)

Height: ___ . ___ ☐ cm ☐ In ☐ Not Done

Weight: ___ . ___ ☐ kg ☐ lbs ☐ Not Done

Blood Pressure: ___ / ___ mmHg ☐ Not Done

Pulse: ___ beats/min ☐ Not Done

Temperature: ___ . ___ ☐ °C ☐ °F ☐ Not Done

Performance Status: ☐ Not Done

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5

Laboratory

Subject ID: _____

Date: ___ (Day) ___ (Month) _____ (Year)

Subject Initials: ___

☐ **Check here if Labs not performed**

Laboratory Values:

Specimen Collection Date: ___ (Day) ___ (Month) _____ (Year)

		ND			ND
WBC (K/µL)	___ . ___	☐	Total bilirubin (mg/dL)	___ . ___	☐
ANC	_____	☐	LDH (IU/L)	____	☐
Platelets (K/µL)	___	☐	Alk. phos. (IU/L)	____	☐
Hemoglobin (g/dL)	___ . ___	☐	Albumin (g/dL)	___ . ___	☐
Neutrophils (%)	___		SGOT/AST (IU/L)	___	☐
Sodium (mEq/L)	___	☐	T4	___	☐
Potassium (mEq/L)	___ . ___	☐	GGT (IU/L)	___	☐
Amylase	___	☐	PT (sec)	___ . ___	☐
TSH	___	☐	PTT (sec)	___ . ___	☐
Calcium	___	☐	ANA	___ ☐	☐
Creatinine (mg/dL)	___ . ___	☐	HC	___	☐
Lymphocyte	___	☐			

Study Labs

Were study labs performed? ☐ Yes ☐ NO

if yes, then date and time: ___ (Day) ___ (Month) _____ (Year) _____ (Time)

Study Drug Administration

Subject ID: | | | | | |

Date: | | | | | | | | | | | | | | | |

Day Month Year

Subject Initials: | | | |

Administration **Subcutaneously in rotating sites**

_____ ug _____ | | | | | | | | | | | | | | | | : | |

Site Day Month Year Time

Additional Comments:

Physical Exam

Subject ID: | | | | | |

Date: | | Day | | | | Month | | | | Year

Subject Initials: | | | |

☐ **Check here if physical exam not performed**

Date of this exam: | | Day | | | | Month | | | | Year

	WNL	ABN	ND	Comment if Abnormal
General Appearance	☐	☐	☐	_____
HEENT	☐	☐	☐	_____
Pulmonary	☐	☐	☐	_____
Cardiovascular	☐	☐	☐	_____
Abdomen	☐	☐	☐	_____
Musculoskeletal	☐	☐	☐	_____
Extremities	☐	☐	☐	_____
Neurological	☐	☐	☐	_____
Skin	☐	☐	☐	_____
Lymphatic	☐	☐	☐	_____
Other_____	☐	☐	☐	_____

Subject ID: _____

Date: __ (Day) ___ (Month) ____ (Year)

Subject Initials: ___

☐ **Check here if vital signs not performed**

Date of this exam: __ (Day) ___ (Month) ____ (Year)

Height: ___ . _ ☐ cm ☐ In ☐ Not Done

Weight: ___ . _ ☐ kg ☐ lbs ☐ Not Done

Blood Pressure: ___ / ___ mmHg ☐ Not Done

Pulse: ___ beats/min ☐ Not Done

Temperature: ___ . _ ☐ °C ☐ °F ☐ Not Done

Performance Status: ☐ Not Done

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5

Laboratory

Subject ID: _____

Date: __ (Day) ___ (Month) ____ (Year)

Subject Initials: ___

☐ **Check here if Labs not performed**

Laboratory Values:

Specimen Collection Date: __ (Day) ___ (Month) ____ (Year)

		ND			ND
WBC (K/µL)	__.__	☐	Total bilirubin (mg/dL)	__.__	☐
ANC	_____	☐	LDH (IU/L)	____	☐
Platelets (K/µL)	___	☐	Alk. phos. (IU/L)	____	☐
Hemoglobin (g/dL)	___.__	☐	Albumin (g/dL)	__.__	☐
Neutrophils (%)	___		SGOT/AST (IU/L)	___	☐
Sodium (mEq/L)	___	☐	T4	___	☐
Potassium (mEq/L)	_.__	☐	GGT (IU/L)	___	☐
Amylase	___	☐	PT (sec)	__.__	☐
TSH	___	☐	PTT (sec)	__.__	☐
Calcium	___	☐	ANA	___ ☐	☐
Creatinine (mg/dL)	___.__	☐	HC	___	☐
Lymphocyte	___	☐			

Study Labs

Were study labs performed? ☐ Yes ☐ NO

if yes, then date and time: __ (Day) ___ (Month) ____ (Year) ____ (Time)

Physical Exam

Subject ID: ☐☐☐☐☐ **Date:** ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Subject Initials: ☐☐☐

☐ **Check here if physical exam not performed**

Date of this exam: ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

	WNL	ABN	ND	Comment if Abnormal
General Appearance	☐	☐	☐	_____
HEENT	☐	☐	☐	_____
Pulmonary	☐	☐	☐	_____
Cardiovascular	☐	☐	☐	_____
Abdomen	☐	☐	☐	_____
Musculoskeletal	☐	☐	☐	_____
Extremities	☐	☐	☐	_____
Neurological	☐	☐	☐	_____
Skin	☐	☐	☐	_____
Lymphatic	☐	☐	☐	_____
Other_____	☐	☐	☐	_____

Subject ID: _____ **Date:** __ (Day) ___ (Month) ____ (Year)

Subject Initials: ___

☐ **Check here if vital signs not performed**

Date of this exam: __ (Day) ___ (Month) ____ (Year)

Height: ___ . ___ ☐ cm ☐ In ☐ Not Done

Weight: ___ . ___ ☐ kg ☐ lbs ☐ Not Done

Blood Pressure: ___ / ___ mmHg ☐ Not Done

Pulse: ___ beats/min ☐ Not Done

Temperature: ___ . ___ ☐ °C ☐ °F ☐ Not Done

Performance Status: ☐ Not Done

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5

Laboratory

Subject ID: _____

Date: ____ (Day) ____ (Month) ____ (Year)

Subject Initials: _____

☐ **Check here if Labs not performed**

Laboratory Values:

Specimen Collection Date: ____ (Day) ____ (Month) ____ (Year)

		ND			ND
WBC (K/µL)	____ . ____	☐	Total bilirubin (mg/dL)	____ . ____	☐
ANC	_____	☐	LDH (IU/L)	_____	☐
Platelets (K/µL)	____	☐	Alk. phos. (IU/L)	_____	☐
Hemoglobin (g/dL)	____ . ____	☐	Albumin (g/dL)	____ . ____	☐
Neutrophils (%)	____		SGOT/AST (IU/L)	____	☐
Sodium (mEq/L)	____	☐	T4	____	☐
Potassium (mEq/L)	____ . ____	☐	GGT (IU/L)	____	☐
Amylase	____	☐	PT (sec)	____ . ____	☐
TSH	____	☐	PTT (sec)	____ . ____	☐
Calcium	____	☐	ANA	____ ☐	☐
Creatinine (mg/dL)	____ . ____	☐	HC	____	☐
Lymphocyte	____	☐			

Skin Test

Week 5 . 0

Subject ID: | | | | | |

Date: | | (Day) | | | | (Month) | | | | (Year)

Subject Initials: | | | |

Skin Test Administration

	Dose	Day	Month	Year	Time
Mimotope P10s-PADRE Administration	_____				:
Tetanus-Diaphtheria Toxoid Antigen Administration	_____				:
Candida Antigen Administration	_____				:

Skin Test Reading
(48-72 hrs post administration)

	Induration (mm)	Day	Month	Year	Time
Mimotope P10s-PADRE Administration	_____				:
Tetanus-Diaphtheria Toxoid Antigen Administration	_____				:
Candida Antigen Administration	_____				:

Physical Exam

Subject ID: _____

Date: ____ (Day) ____ (Month) ____ (Year)

Subject Initials: _____

☐ **Check here if physical exam not performed**

Date of this exam: ____ (Day) ____ (Month) ____ (Year)

	WNL	ABN	ND	Comment if Abnormal
General Appearance	☐	☐	☐	_____
HEENT	☐	☐	☐	_____
Pulmonary	☐	☐	☐	_____
Cardiovascular	☐	☐	☐	_____
Abdomen	☐	☐	☐	_____
Musculoskeletal	☐	☐	☐	_____
Extremities	☐	☐	☐	_____
Neurological	☐	☐	☐	_____
Skin	☐	☐	☐	_____
Lymphatic	☐	☐	☐	_____
Other_____	☐	☐	☐	_____

Vital Signs

Subject ID: ☐☐☐☐☐ **Date:** ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Subject Initials: ☐☐☐

☐ **Check here if vital signs not performed**

Date of this exam: ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Height: ☐☐☐ . ☐ ☐ cm ☐ In ☐ Not Done

Weight: ☐☐☐ . ☐ ☐ kg ☐ lbs ☐ Not Done

Blood Pressure: ☐☐☐ / ☐☐☐ mmHg ☐ Not Done

Pulse: ☐☐☐ beats/min ☐ Not Done

Temperature: ☐☐☐ . ☐ ☐ °C ☐ °F ☐ Not Done

Performance Status: ☐ Not Done

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5

Laboratory

Subject ID: _____

Date: __ (Day) ___ (Month) ____ (Year)

Subject Initials: ___

☐ **Check here if Labs not performed**

Laboratory Values:

Specimen Collection Date: __ (Day) ___ (Month) ____ (Year)

		ND			ND
WBC (K/µL)	___ . ___	☐	Total bilirubin (mg/dL)	___ . ___	☐
ANC	_____	☐	LDH (IU/L)	____	☐
Platelets (K/µL)	___	☐	Alk. phos. (IU/L)	____	☐
Hemoglobin (g/dL)	___ . ___	☐	Albumin (g/dL)	___ . ___	☐
Neutrophils (%)	___		SGOT/AST (IU/L)	___	☐
Sodium (mEq/L)	___	☐	T4	___	☐
Potassium (mEq/L)	_ . ___	☐	GGT (IU/L)	___	☐
Amylase	___	☐	PT (sec)	___ . ___	☐
TSH	___	☐	PTT (sec)	___ . ___	☐
Calcium	___	☐	ANA	___ _	☐
Creatinine (mg/dL)	___ . ___	☐	HC	___	☐
Lymphocyte	___	☐			

Physical Exam

Subject ID: _____

Date: ____ (Day) ____ (Month) ____ (Year)

Subject Initials: _____

☐ **Check here if physical exam not performed**

Date of this exam: ____ (Day) ____ (Month) ____ (Year)

	WNL	ABN	ND	Comment if Abnormal
General Appearance	☐	☐	☐	_____
HEENT	☐	☐	☐	_____
Pulmonary	☐	☐	☐	_____
Cardiovascular	☐	☐	☐	_____
Abdomen	☐	☐	☐	_____
Musculoskeletal	☐	☐	☐	_____
Extremities	☐	☐	☐	_____
Neurological	☐	☐	☐	_____
Skin	☐	☐	☐	_____
Lymphatic	☐	☐	☐	_____
Other_____	☐	☐	☐	_____

Subject ID: _____

Date: ___ (Day) ___ (Month) _____ (Year)

Subject Initials: ___

☐ **Check here if vital signs not performed**

Date of this exam: ___ (Day) ___ (Month) _____ (Year)

Height: ___ . ___ ☐ cm ☐ In ☐ Not Done

Weight: ___ . ___ ☐ kg ☐ lbs ☐ Not Done

Blood Pressure: ___ / ___ mmHg ☐ Not Done

Pulse: ___ beats/min ☐ Not Done

Temperature: ___ . ___ ☐ °C ☐ °F ☐ Not Done

Performance Status: ☐ Not Done

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5

Laboratory

Subject ID: _____

Date: ___ (Day) ___ (Month) _____ (Year)

Subject Initials: ___

☐ **Check here if Labs not performed**

Laboratory Values:

Specimen Collection Date: ___ (Day) ___ (Month) _____ (Year)

		ND			ND
WBC (K/µL)	___.___	☐	Total bilirubin (mg/dL)	___.___	☐
ANC	_____	☐	LDH (IU/L)	_____	☐
Platelets (K/µL)	___	☐	Alk. phos. (IU/L)	_____	☐
Hemoglobin (g/dL)	___.___	☐	Albumin (g/dL)	___.___	☐
Neutrophils (%)	___		SGOT/AST (IU/L)	___	☐
Sodium (mEq/L)	___	☐	T4	___	☐
Potassium (mEq/L)	___.___	☐	GGT (IU/L)	___	☐
Amylase	___	☐	PT (sec)	___.___	☐
TSH	___	☐	PTT (sec)	___.___	☐
Calcium	___	☐	ANA	___ ___	☐
Creatinine (mg/dL)	___.___	☐	HC	___	☐
Lymphocyte	___	☐			

Study Labs

Were study labs performed? ☐ Yes ☐ NO

if yes, then date and time: ___ (Day) ___ (Month) _____ (Year) _____ (Time)

Study Drug Administration

Subject ID: _____

Date: ____ (Day) ____ (Month) _____ (Year)

Subject Initials: _____

Administration **Subcutaneously in rotating sites**

_____ ug _____ (Site) ____ (Day) ____ (Month) _____ (Year) : (Time)

Additional Comments:

Physical Exam

Subject ID: | | | | | |

Date: | | (Day) | | | | (Month) | | | | | (Year)

Subject Initials: | | | |

☐ **Check here if physical exam not performed**

Date of this exam: | | (Day) | | | | (Month) | | | | | (Year)

	WNL	ABN	ND	Comment if Abnormal
General Appearance	☐	☐	☐	_____
HEENT	☐	☐	☐	_____
Pulmonary	☐	☐	☐	_____
Cardiovascular	☐	☐	☐	_____
Abdomen	☐	☐	☐	_____
Musculoskeletal	☐	☐	☐	_____
Extremities	☐	☐	☐	_____
Neurological	☐	☐	☐	_____
Skin	☐	☐	☐	_____
Lymphatic	☐	☐	☐	_____
Other_____	☐	☐	☐	_____

Subject ID: ☐☐☐☐☐ **Date:** ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Subject Initials: ☐☐☐

☐ **Check here if vital signs not performed**

Date of this exam: ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Height: ☐☐☐.☐ ☐ cm ☐ In ☐ Not Done

Weight: ☐☐☐.☐ ☐ kg ☐ lbs ☐ Not Done

Blood Pressure: ☐☐☐ / ☐☐☐ mmHg ☐ Not Done

Pulse: ☐☐☐ beats/min ☐ Not Done

Temperature: ☐☐☐.☐ ☐ °C ☐ °F ☐ Not Done

Performance Status: ☐ Not Done

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5

Laboratory

Subject ID: _____

Date: ____ (Day) ____ (Month) ____ (Year)

Subject Initials: _____

☐ **Check here if Labs not performed**

Laboratory Values:

Specimen Collection Date: ____ (Day) ____ (Month) ____ (Year)

		ND			ND
WBC (K/µL)	____ . ____	☐	Total bilirubin (mg/dL)	____ . ____	☐
ANC	_____	☐	LDH (IU/L)	_____	☐
Platelets (K/µL)	____	☐	Alk. phos. (IU/L)	_____	☐
Hemoglobin (g/dL)	____ . ____	☐	Albumin (g/dL)	____ . ____	☐
Neutrophils (%)	____		SGOT/AST (IU/L)	____	☐
Sodium (mEq/L)	____	☐	T4	____	☐
Potassium (mEq/L)	____ . ____	☐	GGT (IU/L)	____	☐
Amylase	____	☐	PT (sec)	____ . ____	☐
TSH	____	☐	PTT (sec)	____ . ____	☐
Calcium	____	☐	ANA	____ ____	☐
Creatinine (mg/dL)	____ . ____	☐	HC	____	☐
Lymphocyte	____	☐			

Physical Exam

Subject ID: _____

Date: ____ (Day) ____ (Month) ____ (Year)

Subject Initials: _____

☐ **Check here if physical exam not performed**

Date of this exam: ____ (Day) ____ (Month) ____ (Year)

	WNL	ABN	ND	Comment if Abnormal
General Appearance	☐	☐	☐	_____
HEENT	☐	☐	☐	_____
Pulmonary	☐	☐	☐	_____
Cardiovascular	☐	☐	☐	_____
Abdomen	☐	☐	☐	_____
Musculoskeletal	☐	☐	☐	_____
Extremities	☐	☐	☐	_____
Neurological	☐	☐	☐	_____
Skin	☐	☐	☐	_____
Lymphatic	☐	☐	☐	_____
Other_____	☐	☐	☐	_____

Subject ID: ☐☐☐☐☐ **Date:** ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Subject Initials: ☐☐☐

☐ **Check here if vital signs not performed**

Date of this exam: ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Height: ☐☐☐.☐ ☐ cm ☐ In ☐ Not Done

Weight: ☐☐☐.☐ ☐ kg ☐ lbs ☐ Not Done

Blood Pressure: ☐☐☐ / ☐☐☐ mmHg ☐ Not Done

Pulse: ☐☐☐ beats/min ☐ Not Done

Temperature: ☐☐☐.☐ ☐ °C ☐ °F ☐ Not Done

Performance Status: ☐ Not Done

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5

Laboratory

Subject ID: _____

Date: ___ (Day) ___ (Month) _____ (Year)

Subject Initials: ___

☐ **Check here if Labs not performed**

Laboratory Values:

Specimen Collection Date: ___ (Day) ___ (Month) _____ (Year)

		ND			ND
WBC (K/µL)	___.___	☐	Total bilirubin (mg/dL)	___.___	☐
ANC	_____	☐	LDH (IU/L)	_____	☐
Platelets (K/µL)	___	☐	Alk. phos. (IU/L)	_____	☐
Hemoglobin (g/dL)	___.___	☐	Albumin (g/dL)	___.___	☐
Neutrophils (%)	___		SGOT/AST (IU/L)	___	☐
Sodium (mEq/L)	___	☐	T4	___	☐
Potassium (mEq/L)	___.___	☐	GGT (IU/L)	___	☐
Amylase	___	☐	PT (sec)	___.___	☐
TSH	___	☐	PTT (sec)	___.___	☐
Calcium	___	☐	ANA	___ ☐	☐
Creatinine (mg/dL)	___.___	☐	HC	___	☐
Lymphocyte	___	☐			

Study Labs

Were study labs performed? ☐ Yes ☐ NO

if yes, then date and time: ___ (Day) ___ (Month) _____ (Year) _____ (Time)

Skin Test

Week 9 . 0

Subject ID: _____

Date: ____ (Day) ____ (Month) ____ (Year)

Subject Initials: _____

Skin Test Administration

	Dose	Day	Month	Year	Time
Mimotope P10s-PADRE Administration	_____	____	____	____	:
Tetanus-Diaphtheria Toxoid Antigen Administration	_____	____	____	____	:
Candida Antigen Administration	_____	____	____	____	:

Skin Test Reading
(48-72 hrs post administration)

	Induration (mm)	Day	Month	Year	Time
Mimotope P10s-PADRE Administration	_____	____	____	____	:
Tetanus-Diaphtheria Toxoid Antigen Administration	_____	____	____	____	:
Candida Antigen Administration	_____	____	____	____	:

Physical Exam

Subject ID: | | | | | |

Date: | | Day | | | | Month | | | | Year

Subject Initials: | | | |

☐ **Check here if physical exam not performed**

Date of this exam: | | Day | | | Month | | | | Year

	WNL	ABN	ND	Comment if Abnormal
General Appearance	☐	☐	☐	_____
HEENT	☐	☐	☐	_____
Pulmonary	☐	☐	☐	_____
Cardiovascular	☐	☐	☐	_____
Abdomen	☐	☐	☐	_____
Musculoskeletal	☐	☐	☐	_____
Extremities	☐	☐	☐	_____
Neurological	☐	☐	☐	_____
Skin	☐	☐	☐	_____
Lymphatic	☐	☐	☐	_____
Other_____	☐	☐	☐	_____

Subject ID: _____ **Date:** __ (Day) ___ (Month) ____ (Year)

Subject Initials: ___

☐ **Check here if vital signs not performed**

Date of this exam: __ (Day) ___ (Month) ____ (Year)

Height: ___ . _ ☐ cm ☐ In ☐ Not Done

Weight: ___ . _ ☐ kg ☐ lbs ☐ Not Done

Blood Pressure: ___ / ___ mmHg ☐ Not Done

Pulse: ___ beats/min ☐ Not Done

Temperature: ___ . _ ☐ °C ☐ °F ☐ Not Done

Performance Status: ☐ Not Done

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5

Laboratory

Subject ID: _____

Date: __ (Day) ___ (Month) _____ (Year)

Subject Initials: ___

☐ **Check here if Labs not performed**

Laboratory Values:

Specimen Collection Date: __ (Day) ___ (Month) _____ (Year)

		ND			ND
WBC (K/µL)	__ . __	☐	Total bilirubin (mg/dL)	__ . __	☐
ANC	_____	☐	LDH (IU/L)	____	☐
Platelets (K/µL)	___	☐	Alk. phos. (IU/L)	____	☐
Hemoglobin (g/dL)	___ . __	☐	Albumin (g/dL)	__ . __	☐
Neutrophils (%)	___		SGOT/AST (IU/L)	___	☐
Sodium (mEq/L)	___	☐	T4	___	☐
Potassium (mEq/L)	_ . __	☐	GGT (IU/L)	___	☐
Amylase	___	☐	PT (sec)	__ . __	☐
TSH	___	☐	PTT (sec)	__ . __	☐
Calcium	___	☐	ANA	___ _	☐
Creatinine (mg/dL)	___ . __	☐	HC	___	☐
Lymphocyte	___	☐			

Study Labs

Were study labs performed? ☐ Yes ☐ NO

if yes, then date and time: __ (Day) ___ (Month) ____ (Year) ____ : ____ (Time)

Study Drug Administration

Week 19 . 0

Subject ID: _____

Date: ___ (Day) ___ (Month) _____ (Year)

Subject Initials: ___

Administration **Subcutaneously in rotating sites**

_____ ug _____ Site ___ (Day) ___ (Month) _____ (Year) : (Time)

Additional Comments:

Physical Exam

Subject ID: | | | | | |

Week Date: | | Day | | | | Month | | | | Year

Subject Initials: | | | |

☐ **Check here if physical exam not performed**

Date of this exam: | | Day | | | | Month | | | | | Year

	WNL	ABN	ND	Comment if Abnormal
General Appearance	☐	☐	☐	_____
HEENT	☐	☐	☐	_____
Pulmonary	☐	☐	☐	_____
Cardiovascular	☐	☐	☐	_____
Abdomen	☐	☐	☐	_____
Musculoskeletal	☐	☐	☐	_____
Extremities	☐	☐	☐	_____
Neurological	☐	☐	☐	_____
Skin	☐	☐	☐	_____
Lymphatic	☐	☐	☐	_____
Other_____	☐	☐	☐	_____

Subject ID: ☐☐☐☐☐

Week Date: ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Subject Initials: ☐☐☐

☐ **Check here if vital signs not performed**

Date of this exam: ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year

Height: ☐☐☐ . ☐ ☐ cm ☐ In ☐ Not Done

Weight: ☐☐☐ . ☐ ☐ kg ☐ lbs ☐ Not Done

Blood Pressure: ☐☐☐ / ☐☐☐ mmHg ☐ Not Done

Pulse: ☐☐☐ beats/min ☐ Not Done

Temperature: ☐☐☐ . ☐ ☐ °C ☐ °F ☐ Not Done

Performance Status: ☐ Not Done

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5

Laboratory

Subject ID: _____

Consent Date: ___ (Day) ___ (Month) _____ (Year)

Subject Initials: ___

☐ **Check here if Labs not performed**

Laboratory Values:

Specimen Collection Date: ___ (Day) ___ (Month) _____ (Year)

		ND			ND
WBC (K/µL)	__ . __	☐	Total bilirubin (mg/dL)	__ . __	☐
ANC	_____	☐	LDH (IU/L)	____	☐
Platelets (K/µL)	___	☐	Alk. phos. (IU/L)	____	☐
Hemoglobin (g/dL)	___ . __	☐	Albumin (g/dL)	__ . __	☐
Neutrophils (%)	___		SGOT/AST (IU/L)	___	☐
Sodium (mEq/L)	___	☐	T4	___	☐
Potassium (mEq/L)	_ . __	☐	GGT (IU/L)	___	☐
Amylase	___	☐	PT (sec)	__ . __	☐
TSH	___	☐	PTT (sec)	__ . __	☐
Calcium	___	☐	ANA	___ _	☐
Creatinine (mg/dL)	___ . __	☐	HC	___	☐
Lymphocyte	___	☐			

Physical Exam

Subject ID: | | | | | |

Week Date: | | Day | | | Month | | | | Year

Subject Initials: | | | |

☐ **Check here if physical exam not performed**

Date of this exam: | | Day | | | Month | | | | Year

	WNL	ABN	ND	Comment if Abnormal
General Appearance	☐	☐	☐	_____
HEENT	☐	☐	☐	_____
Pulmonary	☐	☐	☐	_____
Cardiovascular	☐	☐	☐	_____
Abdomen	☐	☐	☐	_____
Musculoskeletal	☐	☐	☐	_____
Extremities	☐	☐	☐	_____
Neurological	☐	☐	☐	_____
Skin	☐	☐	☐	_____
Lymphatic	☐	☐	☐	_____
Other_____	☐	☐	☐	_____

Subject ID: _____

Week Date: ____ (Day) ____ (Month) ____ (Year)

Subject Initials: ___

☐ **Check here if vital signs not performed**

Date of this exam: ____ (Day) ____ (Month) ____ (Year)

Height: ____ . ____ ☐ cm ☐ In ☐ Not Done

Weight: ____ . ____ ☐ kg ☐ lbs ☐ Not Done

Blood Pressure: ____ / ____ mmHg ☐ Not Done

Pulse: ____ beats/min ☐ Not Done

Temperature: ____ . ____ ☐ °C ☐ °F ☐ Not Done

Performance Status: ☐ Not Done

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5

Laboratory

Subject ID: _____

Consent Date: ___ (Day) ___ (Month) _____ (Year)

Subject Initials: ___

☐ **Check here if Labs not performed**

Laboratory Values:

Specimen Collection Date: ___ (Day) ___ (Month) _____ (Year)

		ND			ND
WBC (K/µL)	___.___	☐	Total bilirubin (mg/dL)	___.___	☐
ANC	_____	☐	LDH (IU/L)	_____	☐
Platelets (K/µL)	___	☐	Alk. phos. (IU/L)	_____	☐
Hemoglobin (g/dL)	___.___	☐	Albumin (g/dL)	___.___	☐
Neutrophils (%)	___		SGOT/AST (IU/L)	___	☐
Sodium (mEq/L)	___	☐	T4	___	☐
Potassium (mEq/L)	___.___	☐	GGT (IU/L)	___	☐
Amylase	___	☐	PT (sec)	___.___	☐
TSH	___	☐	PTT (sec)	___.___	☐
Calcium	___	☐	ANA	___ ___	☐
Creatinine (mg/dL)	___.___	☐	HC	___	☐
Lymphocyte	___	☐			

Study Labs

Were study labs performed? ☐ Yes ☐ NO

if yes, then date and time: ___ (Day) ___ (Month) _____ (Year) _____ : _____ (Time)

Skin Test

Week 21 . 0

Subject ID: _____

Consent Date: ____ ____ ____ ____ ____
Day Month Year

Subject Initials: _____

Skin Test Administration
(48-72 hrs post administration)

	Dose	**Day**	**Month**	**Year**	**Time**
Mimotope P10s-PADRE Administration	_____	____	____	____	____ : ____
Tetanus-Diaphtheria Toxoid Antigen Administration	_____	____	____	____	____ : ____
Candida Antigen Administration	_____	____	____	____	____ : ____

Skin Test Reading

	Induration (mm)	**Day**	**Month**	**Year**	**Time**
Mimotope P10s-PADRE Administration	_____	____	____	____	____ : ____
Tetanus-Diaphtheria Toxoid Antigen Administration	_____	____	____	____	____ : ____
Candida Antigen Administration	_____	____	____	____	____ : ____

Subject Discontinuation

Visit End of Treatment

Subject ID: ☐☐☐☐☐

Discontinuation Date: ☐☐ (Day) ☐☐☐ (Month) ☐☐☐☐ (Year)

Subject Initials: ☐☐☐

Specific reason for discontinuing this subject:

- ☐ Disease progression
- ☐ Subject not willing to continue treatment on study
- ☐ Grade 4 Autoimmune disease
- ☐ Investigators decision based on:
  - ☐ clinically significant deterioration of subject's condition
  - ☐ persistent (≥3 wks) NCI-CTCAE Version 3.0 Grade 3 or Grade 4 adverse event or any significant adverse event that does not resolve to baseline with appropriate medical management, that compromises the subject's ability to continue in the study
  - ☐ investigators determination that it is not in the subject's best interest to continue participation
  - ☐ pregnancy
  - ☐ unacceptable toxicity
  - ☐ deteriorating performance
- ☐ Death

Post Study Treatment(s)

Visit Post Study Treatment

Subject ID: _____ **Discontinuation Date:** _____ _____ _____
Day Month Year

Subject Initials: _____

Is the subject receiving therapy after termination from Vaccine Protocol? ☐ Yes ☐ No
If Yes, see below

Therapy Agents

1) _____

Start Date: ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year Stop Date: ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year ☐ Ongoing √

2) _____

Start Date: ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year Stop Date: ☐☐ Day ☐☐☐ Month ☐☐☐☐ Year ☐ Ongoing √

Survival

Visit Survival

Subject ID: ☐☐☐☐☐

Discontinuation Date: ☐☐ (Day) ☐☐☐ (Month) ☐☐☐☐ (Year)

Subject Initials: ☐☐☐

Vital Status

☐ Alive ☐ Dead (Complete End of Study CRF)

Date of last contact or death: ☐☐ (Day) ☐☐☐ (Month) ☐☐☐☐ (Year)

Performance Status:

☐ 0

☐ 1

☐ 2

☐ 3

☐ 4

☐ 5

☐ Unknown

Adverse Events

Subject ID: ☐☐☐☐☐

Subject Initials: ☐☐☐

☐ **Mark here if no adverse events occurred**

Adverse Event	Grade	Drug Relationship: Fulvestrant	Drug Relationship: Erlotinib	Action taken	Outcome	Serious?
1 _____	☐ 1	☐	☐ Not related	☐ None	☐ Persisted	☐ Yes
	☐ 2	☐	☐ Unlikely	☐ Erlotinib reduced	☐ Resolved	☐ No
	☐ 3	☐	☐ Possible	☐ Drug stopped temporarily	☐ Unknown	
Start Date ☐☐ ☐☐☐ ☐☐☐☐	☐ 4	☐	☐ Definite		☐ Death	
Stop Date ☐☐ ☐☐☐ ☐☐☐☐ ☐	☐ 5			☐ Drug stopped permanently		
Day Month Year Ongoing?						
2 _____	☐ 1	☐	☐ Not related	☐ None	☐ Persisted	☐ Yes
	☐ 2	☐	☐ Unlikely	☐ Erlotinib reduced	☐ Resolved	☐ No
	☐ 3	☐	☐ Possible	☐ Drug stopped temporarily	☐ Unknown	
Start Date ☐☐ ☐☐☐ ☐☐☐☐	☐ 4	☐	☐ Definite		☐ Death	
Stop Date ☐☐ ☐☐☐ ☐☐☐☐ ☐	☐ 5			☐ Drug stopped permanently		
Day Month Year Ongoing?						
3 _____	☐ 1	☐	☐ Not related	☐ None	☐ Persisted	☐ Yes
	☐ 2	☐	☐ Unlikely	☐ Erlotinib reduced	☐ Resolved	☐ No
	☐ 3	☐	☐ Possible	☐ Drug stopped temporarily	☐ Unknown	
Start Date ☐☐ ☐☐☐ ☐☐☐☐	☐ 4	☐	☐ Definite		☐ Death	
Stop Date ☐☐ ☐☐☐ ☐☐☐☐ ☐	☐ 5			☐ Drug stopped permanently		
Day Month Year Ongoing?						

Concomitant Medications

Subject ID: ____

Subject Initials: ____ ____ ____

	Medication Name	Indication	Dose/Unit/Route/Frequency
1)			
2)			
3)			
4)			
5)			
6)			
7)			

Start Date: Day ____ ____ Month ____ ____ ____ Year ____ ____ ____ ____

Stop Date: Day ____ ____ Month ____ ____ ____ Year ____ ____ ____ ____ Ongoing √ ____

CONSENT FORM
AND
INFORMATION ABOUT

Vaccination of High Risk Breast Cancer Patients

TO BE CONDUCTED AT

UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES

(Study Doctor: Laura Hutchins, MD)

SUBJECT NAME _____ HOSPITAL I.D. NUMBER _____

INTRODUCTION

This is a clinical trial, a type of research study being conducted at the University of Arkansas for Medical Sciences (UAMS) by Dr. Thomas Kieber-Emmons, Dr. Laura Hutchins and Dr. Issam Makhoul. This research is being paid for by the United States Department of Defense and is being sponsored by UAMS. Your research doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part. Please take your time to make your decision about taking part. You may discuss your decision with your friends and family. You can also discuss it with your health care team. If you have any questions, you can ask your research doctor for more information. The vaccine that will be used in this study is not approved by the Food and Drug Administration (FDA) for general public use; however, the FDA has allowed it to be used in this research study.

You are being asked to take part in this research study because you have breast cancer that has spread. You may continue on your current treatment if you choose to be involved in this study.

Why is this research being done?

The purpose of this research is to determine whether an experimental breast cancer vaccine, the Mimotope P10s-PADRE, in combination with QS-21 (used to stimulate immune cell production) is safe and able to be tolerated by people who have breast cancer. This study will also assess your immune response to the vaccine. There are no commercially available breast cancer vaccines at this time. There are other breast cancer vaccine trials at other institutions but no other breast cancer vaccine trials are now available in Arkansas.

Mimotope P10s-PADRE Vaccine:

The experimental vaccine (Mimotope P10s-PADRE) is a small protein that looks like the carbohydrate antigens on breast cancer cells. Because Mimotope P10s-PADRE looks like a breast cancer antigen, but has a different chemical make-up, if the vaccine is successful, it will increase your body's immune response against breast cancer. This is the first time this vaccine will be used in humans.

Stimulon® QS-21: QS-21 is an investigational agent (not commercially available to the general public) that is mixed with the vaccine to make it stronger. Adding QS-21 to Mimotope P10s-PADRE should stimulate your immune system even more than Mimotope P10s-PADRE would if it were given by itself.

How many people will take part in research?

We plan to consent approximately 24 subjects with hope of reaching our enrollment goal of 6-12 women, ages 18 and older. At the beginning of the research, 3 research participants will be treated with a dose of the vaccine. If this does not cause any side effects, the dose will be made higher as new research participants take part in the study. If the vaccine instead causes bad side effects, the vaccine dose will be lowered, and new research participants will receive the lower dose. All research participants will receive the same amount of QS-21 with the vaccine.

What is involved in this research?

Your participation in this research is voluntary. If you refuse to participate in this research, you will not be penalized in any way and you will not lose any benefits to which you are otherwise entitled. If you choose to participate in this research, but then change your mind, you can stop participating in the research and you will not be penalized or lose any benefits.

If you volunteer to participate in this research we will ask you to do the following things:

Before you begin the research...

You will need to have the following exams, tests or procedures to find out if you can participate in the research. Most of these exams, tests or procedures are part of regular cancer care and will be done even if you do not join the research. If you have had some of them recently, they may not need to be repeated. This will be up to your research doctor.

You will need to meet certain criteria listed here:

1) You must be female with proven breast cancer that has spread outside the breast and axillary (under the arm) lymph nodes. You must be stable having bee n on the same treatment for at least 2 months.

2) You must be 18-years-old or older.

3) You must be fit eno ugh to take care of yourself but you ma y ha ve sympto ms from your disease or treatment.

4) You must not have an active infection requiring treatment with injectable antibiotics.

5) You must not have other significant medical, surgical or psychiatric condition s or require any medication or treatment which may interfere with participation in the study.

6) You must not have a diagnosis o r evidence of organic brain syndrome or significant impairment of basal co gnitive function (like Alzheimer's d isease) or any psychiatric disorder that might prevent you from fully participating in the study.

7) You must have no ot her current malignancie s except certain skin ca ncers unless you have been in remission for more than 5 years.

8) You must not have a utoimmune disorders, like rheumatoid arthriti s or systemic lupus erythematosus. You must not have conditions of immunosuppression such as HIV, being the recipient of an organ transplant or having been treated with systemic corticosteroids, including or al steroids (i.e. prednisone, dexamethasone), continuous use of topical steroid creams or ointments, or any steroid containing inhalers.

9) If you are of childb earing pote ntial, you must not be pregnant (negative serum pregnancy test within 2 weeks of registering on the study) or breast-feeding.

10) If you are of childbearing potential you will be counseled to use an accepte d and effective method of contraception (includ ing abstinen ce) while par ticipating in this research and for a p eriod of 18 months af ter completing or discontinuing your participation in this research.

11) You must have acceptable b lood counts measured within 2 weeks prior to registration.

12) You mu st have acceptable liver and kidney function checked by blood work wit hin 2 weeks prior to registration.

13) You must react to a minimum of 2 recall antigens by skin testing;

14) You must sign this informed consent approved by the UAMS Institutional Review Board.

Summary of tests to be done prior to the study: research

Standard medical care for cancer treatments:

- History and physical exam
- Blood work for blood counts, liver and kidney function, and calcium level. This takes about one and one-half teaspoons of blood.
- Urine for a pregnancy test if you are able to have children.

Test being done only for the research:

- Skin tests. These are shallow injections of a small amount of liquid into the skin just below the surface to see if your immune system is working. You will be skin tested for 2 things prior to participating in the study.
- Blood work to check your body for an overactive immune system. This will take one teaspoon of blood.
- Blood tests for special immune system measurements which will take 2 teaspoons of blood.

The total amount of blood that will be taken to see if you meet the criteria to participate in the study is 4 and one-half teaspoons.

Summary of tests to be done during the clinical trial:

If the exams, tests and procedures show that you can be in the research, and you choose to take part, then you will need the following tests and procedures that are standard for patients undergoing cancer treatments.

- Complete blood count
- Chemistry profile of your blood and organ function
- Medical history and physical exams
- Notations of side effects

You will need the following tests and procedures that are either being tested in this research or are being done to see how the research is affecting your body:

- Skin test
- Serology (blood tests to check the reaction of the immune system)

What happens when I am finished taking the vaccine?

The research lasts for about 21 weeks, and you will continue your standard treatments and follow-ups after completing the study.

You should avoid becoming pregnant for at least 18 months after participation in the study. To avoid becoming pregnant, you should either abstain form sexual relations or practice a method of birth control. Except for surgical removal of the uterus, birth control methods such as the use of condoms, a diaphragm or cervical cap, birth control pills, IUD, or sperm killing products are not totally effective in preventing pregnancy.

During this study you will be asked to provide blood samples. These samples will be used to determine the effect of the vaccine on your immune system. Any left over samples will be discarded. There will be no commercial development from these samples. You may agree to participate in the research protocol, but refuse to provide the additional sample(s) discussed above.

Research Chart

You will receive a shot of breast cancer vaccine at weeks 1, 2, 3, 7 and 19 for a total of 5 doses. These shots will be given to you at different places on your body including your arm, thigh, and abdomen. You will need skin tests at weeks 5, 9 and 21. The chart below shows what will happen to you while you are participating in this research study and when everything will happen.

Day	What you do
Prior to study	Standard Care : • Clinic visit for history and physical exam • Get routine blood tests • Urine for pregnancy test if indicated For the Research: • Skin tests • Blood work for research Total blood draw: 4 ½ teaspoons
Week 1	Standard care: • Clinic visit for history and physical exam

	• Get routine blood tests For the research: • Vaccination with Mimotope P10s-PADRE and QS-21 • Toxicity notation
Week 2	Standard care: • Clinic visit for history and physical exam • Get routine blood tests For the Research: • Vaccination with QS-21 and breast cancer vaccine • Toxicity notation • Blood work for research Total blood draw: 2 teaspoons
Week 3	Standard Care: • Clinic visit for history and physical exam • Toxicity notation • Routine blood test For Research**:** • Vaccination with QS-21 and breast cancer vaccine • Blood tests for research Total blood draw: 4 ½ teaspoons
Week 4	For the Research**:** • Skin test (DTH assay) • Blood test for research Total blood draw: 2 teaspoons
Week 7	Standard Care: • Clinic visit for history and physical exam

	• Routine blood test For the Research: • Vaccination with QS-21 and breast cancer vaccine • Blood tests for study Total blood draw: 4 ½ teaspoons
Week 9	Standard: • Clinic visit for history and physical exam • Routine blood test For the Research: • Skin test • Blood tests for research Total blood draw: 4 ½ teaspoons

Research Plan

Another way to find out what will happen to you during th e study is t o read the chart below.

	Week						
	Prestudy	1	2	3 4		7 9	
(Vaccine) Vaccination (GM-CSF)		r[5]	R	R		R	
(Side effects) Toxicity Notation		I[6]	I	I		I I	
History/PE	I	I		I		I I	
(Blood Work) CBC with Diff	I			I	I		I
SGOT	I			I	I		I
Alk Phos	I			I	I		I
LDH	I			I	I		I
GGT	I			I	I		I
Creat	I			I	I		I
Calcium	I			I	I		I
Albumin	R			R	R		R
Amylase	R			R	R		R
TSH	R			R	R		R
T_4	R			R	R		R
ANA	R			R	R		R
(Urine) Pregnancy test[1]	I						
(Blood Work) Study Lab[2]	R		R	R R		R R	
Skin Test[3] R					R[4]		R[4]

1. For women of child bearing potential.
2. 10 ml serum samples in red top tubes for LeY ELISA and CC assays. Call Dr. Kieber-Emmons lab to pick-up specimen: 526-5930
3. Skin Tests to include the following: Candida Antigen, Trichophyton Antigen.
4. DTH assay with mimotope and Diphtheria-tetanus antigen as control.
5. **"R" means paid for by the sponsor.**

6. **"I" means billed to insurance.**

How long will I be in the research?

You will be treated with breast cancer vaccine for 5 doses within 5 months. If you have significant side effects or problems related to the vaccine and QS-21 , the remaining doses will n ot be given. After you are finished t aking vacci ne, the rese arch doctor will follow you up for about 2 weeks for research tests and then ask you to visit the office for follow-up exams as otherwise indicated for your regular care.

Can I stop being in the research?

Yes. You can decide to stop at any time. Tel l the research doctor if you are thi nking about stopping or decide to stop. He or she will tell you how to stop safely.

It is important to tell the research d octor if you are thinking about stop ping so any risks from the vaccine can be evaluated by your doctor. Another reason to tell your doctor that you are thin king about stopping is t o discuss w hat follow up care and testing could be most helpful for you.

The research doctor may stop you from taking part in this study at any time if he/she believes it is in your be st interest; if you do not follow the st udy rules; o r if the st udy is stopped. This may hap pen without your conse nt. If there is new information from the research co ncerning sid e effects or new information about alternate tre atments which may benefit you, you will be informed by your research doctor.

If blood samples are coll ected and you withdraw from the research, we will discard your samples.

What side effects or risks can I expect from being in the research?

You may have side effe cts while on the research. Everyone taking part in the study will be watched carefully for any side effects. However, doct ors don't know all the side effects tha t may happe n. Side effe cts may be mild or very serious. Y our health care team ma y give you me dicines to h elp lessen side effect s. Many side effects go a way once you stop taking t he vaccine . In some cases, side effects can be serious, long lasting, or may never go away.

You should talk to your research doctor about any side effects that you have while taking part in the research. In general th e risks and side effect s are lower than stand ard chemotherapy treatments and the blood draws are similar in the quantity of blood t aken and the frequency of the tests.

Risks and side effects related to the vaccine include those which are:

Likely

- Localized itching, redness, stinging at the site of injection of the vaccine or the skin tests
- A decrease in energy
- Temporary swelling, tenderness of the glands (lymph nodes)
- Discomfort from needles used for the skin tests and blood work.

Less Likely

- Generalized flu-like symptoms including muscle ache, joint aches, fever, chills, nausea and diarrhea

Rare but serious

- Severe allergic reactions can result in shock and even death
- Over activation of your immune system.

Reproductive Risks

You should not become pregnant or have a baby while on this study because the vaccine in this study can affect an unborn baby. Women should not breastfeed a baby while on this study. It is important you understand that you need to use birth control while on this study. Check with your study doctor about what kind of birth control methods to use and how long to use them. It is not known how this vaccine would affect an unborn baby.

Procedure	Risks	Measures to Minimize Risks
Complete History and Physical Exam, Including blood chemistries	Identification of previously unknown condition	Qualified Health Care provider to evaluate potential subject Research records are kept in a locked area with access to study personnel only.
Administration of Study Vaccine Mimotope 106-KLH	Experimental agent may be toxic or harmful. First time use in humans	Careful monitoring by clinic visits and 24 hour, 7 days per week physicians on call for unexpected problems. Only non-pregnant, non-breast feeding females may participate. The use of contraception during the study and the use of contraception for 18 months post completion of the trial is required. Frequent laboratory test including CBC with differential, liver function tests, etc.

	Risks of Local reaction (i.e. swelling, redness, tenderness, itching, extravasations) Potential for side effects ranging from hemotologic toxicitites and hypersensitivity reactions to anaphylaxis. Unanticipated risks Unknown risks	Close and frequent monitoring of participant by qualified staff. Test dose/ prescreen skin tests to monitor for hypersensitivity reactions Emergency equipment at site (including crash carts); advanced cardiac life support (ACLS) certified staff; rescue medications such as Benadryl, epinephrine, high dose steroids, etc. also on site The Medical Monitor will review all side effects on a regular basis and will be available to aid research participants as needed. The study drug may be discontinued. This is an experienced clinical research center. There is a reporting and monitoring mechanism in place for side effects or unanticipated problems
Administration of QS 21 100 µg subcut	Dermatology/Skin: local erythema, rash, pruritis; Gastrointestinal: Diarrhea, anorexia, nausea, vomiting, abnormal taste; Hepatic: Elevated hepatic enzymes, hypo-albuminemia with prolonged treatment; Neurology:Confusion, neuropathies;	Careful monitoring by clinic visits and 24 hour, 7 days per week physicians on call for unexpected problems. Only non-pregnant, non-breast feeding females may participate. The use of contraception during the study and the use of contraception for 18 months post completion of the trial are required. Frequent blood work to monitor health Close and frequent monitoring of participant by qualified staff.

	Pulmonary: Dyspnea (due to fluid retention and capillary leak syndrome), pleuritis; Cardiovascular: HTN Cardiac arrhythmias, atrial fibrillation, pericarditis; Pain: Headache, arthralgias, bone pain, abdominal pain, chest pain, myalgia; Coagulation: partial thromboplastin time (PTT), Prothrombin time (PT), thrombo-embolic phenomena; Fever, flu-like syndrome (chills, rigors, myalgias), fatigue, headache, abnormal labs includind BUN and albumin.	Test dose/ prescreen skin tests to monitor for hypersensitivity reactions Emergency equipment at site (including crash carts); ACLS certified staff; rescue medications such as Benadryl, epinephrine, high dose steroids, etc. also on site The Medical Monitor will review all side effects on a regular basis and will be available to aid research participants as needed. The study drug may be discontinued. This is an experienced clinical research center. There is a reporting and monitoring mechanism in place for side effects or Unanticipated problems
Collection of Blood Samples	Pain, bruising at the injection site, and rarely infection. Discovery of previously unknown conditions Possible breach of confidentiality	Experienced personnel will perform the needle sticks using approved techniques. Pressure and dressings will be used to minimize pain, bruising and infection. Research records are kept in a locked area with access to study personnel only. Participant study numbers will be used for identification of samples so that may be retained for future research and confidentiality is ensured.
Serum Pregnancy Testing	Discovery of previously unknown conditions Possible breach of confidentiality	Research records are kept in a locked area with access to study personnel only. Participants will be identified by study

		numbers only on all research documents.
Serum for immunologic evaluation	Discovery of previously unknown conditions Possible breach of confidentiality	Research records are kept in a locked area with access to study personnel only. Participants will be identified by study numbers only on all research documents.
Skin Test at various locations on participants backs	Pain, bruising at the injection site, and rarely infection. Potential for allergic reaction and anaphylaxis Discovery of previously unknown conditions	Experienced personnel will perform the injections using approved techniques. Pressure and dressings will be used to minimize pain, bruising and infection. Emergency equipment at site (including crash carts); ACLS certified staff; rescue medications such as Benadryl, epinephrine, high dose steroids, etc. also on site This is an experienced clinical research center. There is a reporting and monitoring mechanism in place for side effects or unanticipated problems Research records are kept in a locked area with access to study personnel only. Participants will be identified by study numbers only on all research documents.
Collection of data	Breach of Patient privacy and confidentiality	Research records are kept in a locked area with access to study personnel only. Participants will be identified by study numbers only on all research documents. Investigators to provide certification of completion of human subjects protections training course UAMS shall retain the records and reports for 2 years after a marketing application is approved for the drug; or, if an application is

		not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has been so notified. After such time all study records will be destroyed as well as the links between identifiers of the research participants and their research study numbers according to UAMS' record destruction policy.

For more information about risks and side effects, ask your research doctor.

Are there benefits to taking part in the research?

Taking part in this research may or may not directly benefit your health. While doctors hope a breast cancer vaccine will be more useful against breast cancer as compared to the usual treatment, there is no proof of this yet. We do know that the information from this research will help doctors learn more about breast cancer treatment. This information could help future breast cancer patients. You may benefit from the increased monitoring during the research.

UAMS cannot and does not guarantee you will benefit if you take part in this study. The research drug you receive may even be harmful. You have the right to refuse to participate in this study.

Will I be paid to take part in this research?

You will not receive any payment for your participation in this research study.

Will I receive any compensation? There are no plans to reimburse persons who participate in this study.

Sponsor Statement: In the event of injury, illness, or an adverse event resulting from your participation in this research, appropriate acute medical care will be provided to you. However, the study doctor and UAMS have made no provision to reimburse you for the cost of medical care beyond emergency medical treatment or to pay for any lost wages, pain and suffering, hospitalization, or other expenses you may incur as a result of any such complication or injury.

Any suspected toxicity-related symptoms must be brought to the attention of your physician immediately. Treatment will be provided but this will be billed to you and/or your insurance company. There is no guarantee that your insurance company will agree to cover those charges.

Since this research is sponsored by the Department of Defense, if you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have any questions about this medical care, talk to the principal investigator for this study, Dr. Laura Hutchins at 501-686-8530. If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221.

What other choices do I have if I do not take part in this research?

Your other choices may include:

- Getting chemotherapy or hormonal therapy as standard treatment or care for your cancer without the vaccine.
- Taking part in another study
- Getting no treatment

Talk to your doctor about your choices before you decide if you will take part in this research.

Will my medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used. Participants will be identified by study numbers only on all research documents. The connection with the participants name will be kept by research personnel in a locked facility. UAMS shall retain the records and reports for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has been so notified. After such time all study records will be destroyed as well as the links between identifiers of the research participants and their research study numbers

Many organizations are eligible to review your medical records as part of their responsibilities to protect human participants in research, and to conduct quality assurance, safety and data analysis. These organizations include:

- Winthrop P. Rockefeller Cancer Institute
- Food and Drug Administration (FDA)
- National Cancer Institute (NCI)

- Office of Human Research Protections
- University of Arkansas for Medical Science's Institutional Review Board (IRB)
- U.S. Army Medical Research And Materiel Command
- UAMS Office of Research Support
- UAMS Office of Research Compliance

Authorized representatives from these agencies may need to review your records and may see your name. They are bound by rules of confidentiality not to reveal your identity to others.

What are the costs of taking part in this research ?

Sponsor Statement: You and/or your health plan/ insurance company will need to pay for some or all of the costs of treating your cancer in this research. Any routine, non-research testing or medical care will be billed to your insurance company. Taking part in this research may or may not cost your insurance company more than the cost of getting regular cancer treatment. The investigational skin and blood testing involved in the study will not be charged to you. The breast cancer vaccine and QS-21 will be provided free of charge. All items in the research are designated in the chart above by an "I" for those items billed to insurance and an "R" for those items paid for by the funding agency which is the U.S. Army Medical Research and Material Command.

UAMS Statement: The research will include tests and procedures that are conducted solely for the research study and not as part of routine care for your condition. Your health insurance company will usually only pay for the routine care. In addition, your physician will discuss with you any additional tests and procedure that may be required due to changes of your condition during your research participation which may or may not be standard of treatment. You have the right to refuse to have any additional tests or procedures done. If you feel that you have been billed in error, please contact the person who is responsible for conducting this study. His or her name and telephone number is included on this consent form.

What are my rights if I take part in this research?

Taking part in this research is your choice. You may choose either to take part or not to take part in the research. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Your relationship with your doctor or with UAMS will not be affected by not participating or leaving the research. Leaving the research will not affect your medical care. You can still get your medical care from our institution.

We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study. We will tell you of any new findings (good or bad) in the research or new alternatives to participation that may cause you to

change your mind about continuing in the research. If new information is provided to you, your consent will be re-obtained. You may also request the results of the research from your research doctor after all the analyses are completed.

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

Who can answer my questions about the research?

If you have questions during the study about the research, you should contact Dr. Laura Hutchins at (501) 686-8530. After hours you may reach Dr. Hutchins by digital pager at (501) 688-6061 or the medical oncology physician on call at UAMS at (501) 686-8530.

For questions about your rights while taking part in this research, call the UAMS Institutional Review Board (a group of people who review the research to protect your rights) at 501-686-5667.

Signature

I have been given a copy of all eighteen pages of this form. I have read it or it has been read to me. I understand the information and have had my questions answered. I agree to take part in this study.

_____	_____	_____
Research Participant Signature	**Date**	**Time**

_____

Printed Name of Research Participant

_____	_____	_____
Principle Investigator Signature	**Date**	**Time**

_____	_____	_____
Witness Signature	**Date**	**Time**

_____	_____	_____
Person Obtaining Consent Signature	**Date**	**Time**

INVESTIGATOR'S BROCHURE

For

Mimotope P10S-PADRE

Edition – 3.0 Dated 05/20/09

Sponsored by:

University of Arkansas for Medical Sciences
4301 W. Markham
Little Rock, AR 72205

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Tumor Associated Carbohydrate Antigens - TACA
Human Leukocyte Antigen –HLA
Major Histocompatibility Complex - MHC
Food and Drug Administration - FDA,
Good Laboratory Practice - GLP
Current Good Manufacturing Practice -cGMP
Complement-Dependent Cytotoxicity - CDC
Cytotoxic T-lymphocyte- CTL
Phosphate Buffered Saline - PBS
Keyhole Limpet Hemocyanin - KLH
Natural Killer - NK
Neutrophils - NE
Monocytes – MO
Investigational New Drug - IND
Guillain-Barre Syndrome - GBS.

SUMMARY –The P10s-PADRE vaccine is comprised of a peptide mimetic of Tumor Associated Carbohydrate Antigens (TACAs) with the amino acid sequence WRYTAPVHLGDG that is synthesized in tandem with a non-natural pan-HLA-DR-binding peptide (PADRE) that binds with high or intermediate affinity to 15 of 16 of the most common HLA-DR types tested to date. In preclinical studies the P10s mimetic induces antibodies cross-reactive with TACAS expressed on both human and murine breast cancer cells lines. The PADRE peptide (aK-Cha-VAAWTLKAAa) was specifically engineered to be immunogenic in humans so that it may be used as a carrier to induce T-cell "help" in vaccine constructs designed for human use. The safety and immunogenicity of the PADRE peptide, manufactured under current good manufacturing practice (cGMP) regulations, has been tested in good laboratory practice (GLP) animal studies and in a limited number of Phase 1 clinical trials.

GLP studies of P10s-PADRE in mice indicate a safety profile suitable for human clinical studies. Characterization of P10s-PADRE in humans remains to be determined. These characteristics include mechanisms of action, toxicology, optimum storage and stability conditions, risks and safety assessments. Preclinical studies required by the Food and Drug Administration (FDA), included evaluations of safety, and immunopathology in a mouse model that expresses the tumor antigens targeted by P10s-PADRE. The candidate vaccine was considered safe after the following endpoint criteria were met: (i) mortality < 5% with no vaccine-attributable deaths, (ii) incidence of morbidity < 10% with no early sacrifice, (iii) absence of severe injection-site reactions (e.g. skin ulceration and/or severe myocyte necrosis) and (iv) immunopathology was absent or mild in non-targeted organs.

2. INTRODUCTION –P10s-PADRE is a synthetically derived immunogenic peptide designed to induce a multi-faceted immune response against tumor associated carbohydrate antigens (TACA) on human breast cancer cells. P10s-PADRE will be used clinically as an immunogen, in addition to other established therapies for breast cancer to increase the therapeutic benefits of breast cancer treatments. Our study will investigate the safety, risk, toxicity, dosage of and immune response of breast cancer patients to the P10s-PADRE vaccine. Subjects will receive the P10s-PADRE vaccine along with an adjuvant and be assessed for endpoints associated with toxicity.

2.1 Rationale – Previous clinical trials investigating TACA demonstrated that patient survival significantly correlates with carbohydrate-reactive IgM levels (1). Such results suggest that TACA-targeting vaccines might have a beneficial effect on the course of malignant disease. A unique advantage in targeting TACA is that multiple proteins and lipids on the cancer cell can be modified with the same carbohydrate structure. Thus, targeting the carbohydrate antigen broadens the spectrum of antigens recognized by the immune response, which is crucial for mechanisms of mimotope immunotherapy dependent on epitope spreading (2). In addition, antibodies that recognize glycolipids are more apt to mediate complement-dependent cytotoxicity (CDC) and may, therefore, be more cytotoxic to tumor cells than antibodies that recognize protein antigens (3).

Tumors over express TACA which are reactive with B cells, but TACA usage as immunogens is restricted by a limited cooperation between TACA reactive B cell

and T cells (4). To circumvent this draw back we have developed carbohydrate mimetic peptides with overlapping B and T cell epitopes to link TACA reactive humoral responses with anti-tumor cellular responses. In preclinical prophylactic and therapeutic vaccination studies, peptide mimics of TACA (peptide mimotopes) were efficacious in eliciting immune responses that reduced tumor burden and inhibited metastatic outgrowth (5-7). Thus, peptide mimotopes of TACA represent a new and very promising tool to overcome T-cell independence and to increase the efficiency of the immune response to glycan antigens.

There are several benefits to vaccination strategies that employ peptide mimotopes of TACA. First, peptide mimotopes function as xenoantigens and, consequently, provide an advantage to overcome tolerance to carbohydrate self-antigens. Antibodies induced by peptide mimotopes are thought to have low affinities for TACA. Specific targeting of tumor cells is due in part to over-expression of the carbohydrate antigen on tumor cells, which compensates for the low affinity of the carbohydrate cross-reactive antibodies (8). In addition, mimotope-induced antibodies preferentially recognize the terminal residues of the TACA oligosaccharides, which are often structurally distinct from those found on normal cells (9). Thus, potential immunopathology due to destruction of normal tissue is minimized. Second, peptide mimotopes have the potential to overcome immune deficiencies that prevent vaccine-induced carbohydrate-directed responses (10). Unlike carbohydrate antigens and carbohydrate-conjugate vaccines, peptide mimotopes also prime B and T cells for subsequent memory of carbohydrate antigens, facilitating long-term surveillance through recall of carbohydrate immune responses (11). This effect may minimize the need for constant boosting. In addition, they can functionally emulate conserved structures of TACA, inducing antibodies that recognize multiple TACA, and therefore function like a TACA multivalent vaccine (10,12,13). Third, peptide mimotopes can be manipulated in ways that TACA cannot. Peptide mimotopes can be engineered to induce $CD8^+$ T cells cross-reactive with tumor-associated glycopeptides and/or to induce $CD4^+$ T cells that benefit the further expansion of $CD8^+$ T cells and B cells (7,10).

P10s, and the homologous mimotope P10 **(GVVWRYTAPVHLGDG)**, is capable of inducing delayed type hypersensitivity to GD2 expressing tumor cells (14) and the T cell responses to the Lewis Y mimotope p106 are skewed to the Th1 phenotype (15) indicting a potential for a broad range of cellular cooperation phenomena initiated by mimotope immunization. The ability to induce a humoral carbohydrate cross-reactive response, a $CD4^+$ T helper (Th) response, and a $CD8^+$ cytotoxic T-lymphocyte (CTL) response with one simple inoculation is a novel approach to vaccination. Therefore, peptide mimotopes of TACA hold the potential to generate a multifaceted TACA-reactive immune response.

Finally, tumors expressing high levels of certain types of TACA exhibit greater metastasis than those expressing low levels of these antigens, and this negatively impacts prognosis (16-18). Our in vitro studies demonstrate that peptide

mimotopes of the Lewis Y antigen and gangliosides induce serum antibodies in mice that recognize the appropriate carbohydrate antigens on human and murine breast cancer cell lines (5,19). Our in vivo studies demonstrate that the peptide mimotopes induce sustained immunity to these antigens (5-7). Collectively, these data provide the experimental foundation for evaluating peptide mimotopes of TACA as potential cancer vaccines in subjects with breast cancer.

2.2 Clinical Plan –The P10s-PADRE vaccine will be administered with the adjuvant QS-21 to 6-12 subjects at rotating sites including the arm, thigh, or abdomen. The test material will be given on weeks 1, 2, 3, 7 and 19. P10s-PADRE will be administered initially at a dose of 300 µg, and then will be accelerated to 500 µg or decelerated to 100 µg based on subjects' response to the vaccine. Vaccine toxicity and immune responses elicited by subjects in response to the immunization will be observed and assayed throughout the study. Detailed discussion of the treatment plan can be found in the study protocol.

3.0 PHYSICAL, CHEMICAL, AND PHARMACEUTICAL PROPERTIES AND FORMULATION

3.1 Physical and Formulation Properties

3.1.1 Vaccine (P10s-PADRE)

The vaccine is a sterile preparation of a synthetic 25-amino-acid peptide synthesized by NeoMPS, Inc. under GMP conditions. The vaccine is supplied in vials as a sterile lyophilized powder. The PADRE peptide sequence is as follows: WRYTAPVHLGDG-aK-Cha-VAAWTLKAAa, with capital letters indicating L-amino acids, lowercase letters indication D-amino acids and Cha indicating cyclohesylalanine. PADRE is a synthetic, non-natural pan-HLA-DR-binding peptide that binds with high or intermediate affinity to 15 of 16 of the most common HLA-DR types tested to date. Because of its binding promiscuity, PADRE should overcome the problems posed by the extreme polymorphism of HLA-DR molecules in the human population. Furthermore, the PADRE peptide was specifically engineered to be immunogenic in humans. This property represents another significant feature of PADRE, suggesting its potential utility as a carrier to induce T-cell "help" in vaccine constructs designed for human use. The safety and immunogenicity of the PADRE peptide, manufactured under current good manufacturing practice (cGMP) regulations, has been tested in good laboratory practice (GLP) animal studies and in a limited number of Phase 1 clinical trials.

NeoMPS Inc. (San Diego, CA 92126 USA) will synthesize Mimotope P10S covalently linked with PADRE under GMP conditions. Mimotope P10s-PADRE will be prepared in facilities licensed by the California Department of Health Services, Food and Drug Division (Drug / Device

manufacturing license # 63358) and registered with the FDA (FDA registration # 2028155). Chain assembly will be performed by solid phase peptide synthesis using Fmoc chemistry starting with the appropriate Fmoc resin.

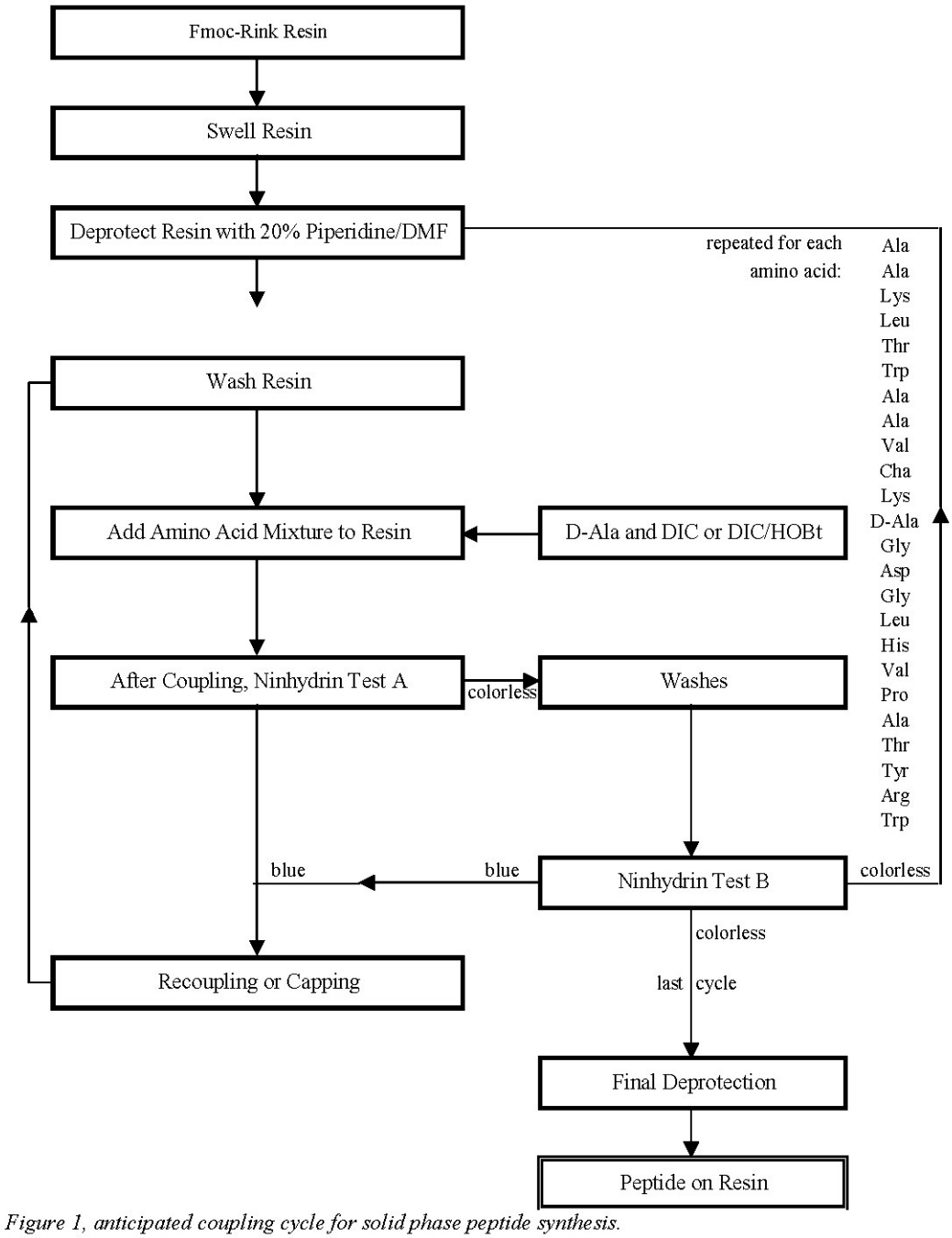

Figure 1, anticipated coupling cycle for solid phase peptide synthesis.

3.1.2 Stimulon® (common name QS-21)

The optimal adjuvants for vaccinating with peptides is not as yet defined in humans. The P10s-PADRE vaccine will be admixed with Stimulon® (common name QS-21) as an adjuvant prior to administration. QS-21 is an immunological adjuvant available from Antigenics, Inc under the name Stimulon®. QS-21 is a naturally occurring saponin molecule purified from the South American tree *Quillaja saponaria* Molina. It is a triterpene glycoside with the general structure of a quillaic acid 3, 28-O-bis glycoside with the formula $C_{92}H_{148}O_{46}$, and a molecular weight of 1990 Kd. It is a mixture of two structural isomers: 3-*O*-β-D-galactopyranosyl-(1→2) -[β-D-xylopyranosyl-(1→3)]- β -D-glucuronopyranosyl-quillaic acid 28-O-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-3-[5-O-α-L-arabinofuranosyl 3,5-dihydroxy-6-methyl-octanoyl]-3,5-di-hydroxy-6-methyl-octanoyl]-β-D-fucopyranoside [CAS 141256-04-4] and 3-O-β-D-galactopyranosyl-(1→2) -[β -D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-quillaic acid 28-O-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-3-[5-O-α-L-arabinofuranosyl 3,5-dihydroxy-6-methyl-octanoyl]-3,5-di-hydroxy-6-methyl-octanoyl]-β-D-fucopyranoside [CAS 145633-52-9]. These isomers are not separable by reversed-phase HPLC, but they can be separated by hydrophilic interaction chromatography or by capillary electrophoresis.

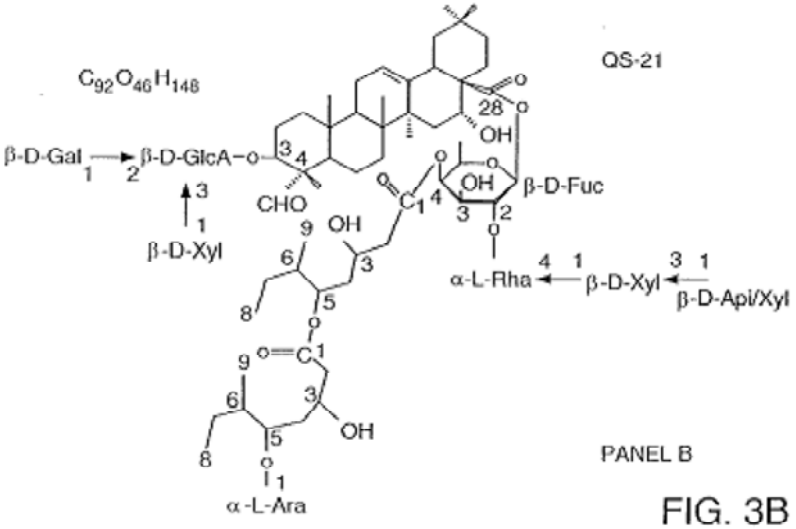

FIG. 3B

Figure 2: Structure of QS-21 from obtained from US patent #6231859

QS-21 is available as an odorless white powder, which goes readily into solution in buffered saline at a pH ranging between 5 and 7. QS-21 is soluble in aqueous solutions with a solubility limit of 17 mg/mL in

buffered saline at pH 7.0. It is also soluble in methanol and mixed methanol/water solutions. It is practically insoluble in chloroform.

A mixed antigen/adjuvant formulation should be used within 8 hours of mixing. Stability of the QS-21 in the final antigen/adjuvant formulation will be dependent upon the particular antigen, buffer, final QS-21 concentration, pH, and excipients included. Stock solutions of P10s-PADRE and QS-21 will be made with sterile phosphate buffered saline (PBS) and mixed for injection

3.1.3 Formulation

Mimotope P10s-PADRE will be administered SC in a volume of up to 1.5 mls in the rotating sites on the abdomen and extremities. 10 mg of QS-21 powder will be solubilized directly in an amber glass vial with the appropriate volume of sterile PBS to obtain a stock solution of 2mg QS-21/ml. The P10s-PADRE vaccine will be received in powder condition and will be stored frozen at ≤ -20° C for maximum stability.

Example of final vaccine solution preparation protocol (Table 1):
Vaccine stock solution (Ci) = 10mg/ml (or to be determined)
QS-21 stock solution = 2 mg/ml (or to be determined)
Volume needed for 1 mice injection (Vf) = 0.500ml (or to be determined)

Table 1. Concentrations of Test Article

Vaccine concentration (Cf)	2.5 mg/ml (500 µg/dose)	1.5 mg/ml (300 µg/dose)	0.5 mg/ml (100 µg/dose)
Vaccine volume (Vi)	0.125 ml	0.075 ml	0.025 ml
QS-21 volume (100 µg/mouse injection)	0.050 ml	0.050 ml	0.050 ml
Sterile buffered saline volume	0.325 ml	0.375 ml	0.425 ml

Formula for vaccine preparation Vi = (Cf x Vf) / Ci

Add the appropriate amount of sterile buffered saline into the appropriate tubes.
Add the appropriate vaccine volume calculated into the sterile buffered saline.
Mix by vortexing for about 15 seconds.
Add the appropriate volume of QS-21 calculated into the diluted vaccine solution.
Mix by gently vortexing the final vaccine solution for about 15 seconds.
Keep on ice until loading a disposable syringe.

3.2 Packaging, Storage, and Handeling

3.2.1 Vaccine (P10s-PADRE)

The vaccine supply will be labeled with the following message:
"Caution: New Drug – Limited by Federal Law to Investigational Use" P10s-

PADRE will be ordered by the Winthrop P. Rockefeller Cancer Institute Research Pharmacy staff from NeoMPS located at 9395 Cabot Drive, San Diego, CA 92126, The agent will be shipped directly to the Cancer Institute Pharmacy and will be stored in the Cancer Institute pharmacy under the supervision of the research pharmacist who will be responsible for maintaining the supply according to the manufacturer's specifications, dispensing the drug for administration and maintaining all accountability logs. Once received, the P10s-PADRE vaccine will be stored frozen at ≤ -20º C for maximum stability. Drug disposition (drug receipt, dispensing, transfer or return) will be maintained on the UAMS Investigational Agent Accountability Record. The vaccine supply will be kept in a secure, limited access storage area under the recommended storage conditions in the research pharmacy in the Winthrop P. Rockefeller Cancer Institute under the direction of the research pharmacist. During the course of the study, the following information will be noted on the Investigational Agent Accountability Record; the study number, the research subject's initials, the research subject's assigned number, the dose of drug, the date(s) and quantity of drug dispensed to the subject, the balance forward, the lot number and the recorder's initials.

3.2.2 Stimulon® (common name QS-21)

QS-21 is soluble in aqueous solutions with a solubility limit of 17 mg/ml in buffered saline at pH 7.0. It is also soluble in methanol and mixed methanol/water solutions. It is practically insoluble in chloroform. The QS-21 to be used in this study will be supplied in a vialed sterile aqueous form in phosphate buffered saline (PBS) to be mixed in the clinic with separate vials of antigen. The QS-21 vial will be resuspended by gentle inversion to assure mixing prior to withdrawal. A volume of 0.2 ml of the QS-21 formulation should be withdrawn with a sterile 1 ml tuberculin syringe and needle, and mixed with the antigen. Although the QS-21 in the vial may have a turbid appearance, it is expected to clarify after dilution with antigen. The QS-21 vials do not contain a preservative, are single-use only, and should not be reentered. Aqueous QS-21 should be stored at ≤ -20ºC; the shelf life at this storage condition is 3 years. The expiration date is listed on the vials and the certificate of analysis.

4.0 NONCLINICAL STUDIES –

4.1 Nonclinical Studies

4.1.1 In Vitro Studies

Prominent amongst TACA are the gangliosides GM2, GD2, GD3, TF and sTn, Lewis Y, GM2, GD3, polysialic acid, T, Tn, sTn, GloboH and sialyl Lea. There is now sufficient experience from clinical trials with vaccine-induced antibody responses against GM2, GD2, TF and sTn antigens, and

passive administration of mAb against GD2, GD3, Lex and sTn to draw conclusions about the consequences of antigen distribution on various normal tissues (20,21). Against this background, GM2, GD3, polysialic acid, TF, Tn, sTn, GloboH and LeY all appear to be good targets for active immunotherapy with vaccines.

The use of peptide mimics (peptide mimotopes) of TACA provides an alternative approach to generating responses against TACA because, unlike TACA, protein surrogates are T-cell-dependent antigens. Short peptides encoding an epitope capable of binding an anti-TACA antibody, which mimic an unrelated structure, are termed mimetics. Mimetics contain key chemical groups spatially arranged in a conformation that allows cross-reactivity with the anti-TACA antibody. Mimetics that induce cross-reactive responses to TACA are called mimotopes. These immunogenic mimotopes function by selecting in vivo for antibodies, which have similar binding properties to TACA as TACA (6,14,22,23). Not all peptides capable of binding to the variable region of an antibody are mimotopes (24,25). To truly be considered a mimotope, the peptide must be capable of generating antibodies in vivo that recognize the original antigen. Peptides, which simply bind the antibody, but do not generate an appropriate immune response are termed mimetics.

P10s has been shown to bind to monoclonal antibodies reactive with the LeY antigen, and to monoclonal antibodies reactive with the gangliosides GD2 and GD3 (22). P10s has been shown to compete with the LeY antigen for anti-LeY antibody binding and has shown to compete with the GD2 for binding to anti-GD2 antibody binding to GD2. P10s reacts with antibodies that are also cross-reactive with TF and Tn antigens (2). Consequently, P10s cross-reacts with several different classes of TACA reactive monoclonal antibodies suggesting that P10s is a broad spectrum mimetic.

4.1.2 In Vivo Studies

P10s is a WRY containing peptide analog of several mimotopes shown to induce antitumor responses (2,22). Mimotopes with this motif display an ability to induce antibodies cross-reactive with tumor cells, induce cellular responses to tumor cells and induce or activate natural killer (NK) cells with anti-tumor activity. Preclinical studies in mice with vaccines containing P10s or P10 (a longer CMP that contains the P10s sequence but is three amino acids longer) have demonstrated these mimotopes induce a robust immunogenic response that includes cross-reactivity with breast cancer cell lines, stimulation of tumor cell reactive cellular responses and/or stimulation of tumor targeting NK cells. Although the mechanism of action appears to vary depending upon the peptide (P10s or P10), coupling agent and adjuvant (KLH vs. PADRE and QS-21) employed, all

vaccines tested in mice to date that contain P10s or P10 have consistently inhibit metastatic outgrowth of murine tumor cells expressing TACA structural homologues. Antibodies raised against our P10s-PADRE vaccine are tumor specific in that along with NK activation they contribute to immune surveillance reminiscent of anti-pathogen vaccines. NK cells recognize many tumor cells but not normal cells, and they are thought to aid in the elimination of nascent tumors. The major function of NK cells in fighting cancer is likely to be in surveillance and elimination of cells that become malignant before they can cause a tumor (26). Collectively, these data provide the experimental foundation for evaluating P10s-PADRE as a potential cancer vaccine in patients at high for breast cancer recurrance.

4.1.3 General Pharmacology

The preferred animal model for toxicity testing is an animal expressing the relevant tumor antigen. The neolactoseries antigen LeY is not expressed in mice, but gangliosides, also mimicked by peptide P10s, are endogenously expressed on murine tumors from Balb/c mice. Therefore, a preclinical safety study was performed to provide a gross characterization of the nature, frequency and severity of adverse responses following vaccine administration in this tolerant mouse setting. The preclinical study provides an initial basis for determining whether the vaccine exhibits a safety profile appropriate for further study. Groups of animals were treated with fixed dose levels of 100, 300 or 500 µg of the vaccine admixed with 20 µg of QS-21 delivered by SC injections. Each treatment was administered at weeks 1, 2, 3, 7, and 19 for a total of five treatments at each dose, to closely mimic the proposed Phase 1 study. Animals were monitored three times weekly for injection-site reactions and changes in weight or general health status.

For evaluation of immunopathology and serological analyses, groups of 8 animals were sacrificed before vaccination and at weeks 3, 9, and 21 for each dose level. Thus, a total of 96 mice was required for the safety study (vaccine: 8 animals x 3 time points x 3 doses; QS-21: 8 animals x 3 time points). Necropsy was performed on each animal upon sacrifice or unscheduled death, with recording of organ weights and gross pathology, and preservation of a complete list of tissues at necropsy. All gross lesions and all tissues from the highest dose were evaluated and compared with saline-treated animals. Dr. Leah Hennings, DVM, an experienced, licensed veterinary pathologist, performed these studies at UAMS. The candidate vaccine was considered safe if the endpoints met the following criteria: (i) mortality < 5% with no vaccine-attributable deaths, (ii) incidence of morbidity < 10% with no early sacrifice, (iii) absence of severe injection-site reactions (e.g. skin ulceration and/or severe myocyte necrosis) and (iv) immunopathology was absent or mild in non-targeted organs. Conclusions will be submitted when the final data are analyzed and summarized. If the candidate vaccine exhibits an acceptable safety

profile in animals, we will proceed to submit an IND application to the FDA for the vaccine.

4.2 Pharmacokinetics and Metabolism in Animals

Both immunization and autoimmune pathology induced by a peptide vaccine have been found dependent on the pharmacokinetics of the peptides (27). In most of the cases the peptides would exhibit extremely high elimination constants in solution in the internal environment due to proteolysis. In the case they don't and their half-life is sufficient to achieve considerable distribution in tissues and organs distal from the injection site, the outcome of the immunization depends on the rate of this distribution. A fast rise of the peptide concentration in the peripheral tissues leads to a distinct immune consequences dependent on the preexisting immune response to this peptide or to a cross-reactive antigen. In naive individuals the CTL responses are suppressed due to tolerization of the naive $CD8^+$ T cells, which compromises the vaccine effect but poses no autoimmune threat. In individuals immunized with the peptide or a cross-reactive antigen the preexisting memory and effector CTL can cause prompt tissue destruction in the organs with high influx of the peptide (e.g. extensive lung destruction within 16 h in the case of Ad5 E1A (28)). The efficiency of the vaccine seems to depend on a very slow distribution (a good depot effect) and a steep gradient from the injection site providing mostly loading of antigen presenting cells locally with long living MHC/peptide complexes but accompanied by very little systemic distribution. In this case the steep gradient of distribution and the short half-life pose little threat for autoimmune pathology mediated directly by the peptide. Whatever the scenario for the particular peptide, the major risk of autoimmunity is associated with tissue necrosis and leukocyte infiltration, which was the main criterion in our safety studies. We interpret our results of no apparent pathology in any of the organs of the immunized mice as an indication that the detrimental scenario is not realized in the case of P10s at the doses used. Since the pathology would not depend on newly differentiating CTLs but rather on the extent of the distribution of the peptide, it would be monotonously dependent on the dose and therefore the supra-optimal doses used in the safety studies provide an additional level of certainty.

4.2.1 Pharmacokinetic Studies in Mice

Injection site reactions were noted in 18 mice. Reactions were most common during weeks 3-9 of the trial. This represents the time between the 3rd and 4th immunizations. Reactions were noted in 3 animals at 20 weeks, the week following immunization number 5. The most common reaction was hair loss, followed by ulceration, redness, and swelling at the injection site. Reactions were mild and transient in all mice, and were present in mice from both control and high-dose groups. Within ½ hour of immunization 5, 1 animal from each of 3 cages, exhibited decreased

motility, hunched posture, and rapid breathing. This reaction was transient and mice recovered within 1 day without incident.

- **Body weights:** Body weights showed no statistically significant group differences, and no net trends with dose. This was true for all five procedure times.
- **Heart:** Organ Weights were analyzed by descriptive statistics, using K-W test results and Spearman correlations, and scatterplots. No statistically significant differences or trends were seen at any of the three necropsy times.

- **Kidney:** Organ Weights were analyzed by descriptive statistics, using K-W test results and Spearman correlations, and scatterplots. No statistically significant differences or trends were seen at any of the three necropsy times.

- **Liver:** Organ Weights were analyzed by descriptive statistics, using K-W test results and Spearman correlations, and scatterplots. At second and third necropsies, liver percent weights showed significant K-W test results, and second necropsy also showed significant negative trends with dose in both gram weights and percent weights. The scatterplots show that similar but non-significant trends may be present at first necropsy, but do not show trend-like behavior at the third necropsy.

- **Spleen:** Organ Weights were analyzed by descriptive statistics, using K-W test results and Spearman correlations, and scatterplots. First necropsy yielded a statistically significant K-W test results for both gram weight and percent weight that were not accompanied by significant evidence for trend.

4.2.2 Excretion and Metabolism

Urine was collected for three days in individual metabolic cages according to Standard Operating Procedure EQU007 and prior to scheduled necropsy for complete urinalysis. Five out of eight mice per group were chosen randomly using a Microsoft® Office Excel 2003 Randomization spreadsheet for urinalysis testing . Appearance, volume, specific gravity, pH, Ketones, Bilirubin, Glucose, occult blood, and Urobilinogen of urine samples were evaluated under GLP conditions at: Rodent Clinical Pathology Core Laboratory Central Arkansas Veterans Healthcare System Research Services, (Little Rock, AR). Blood was collected via cardiac puncture immediately postmortem, according to Standard Operating Procedure ANCA014 and via tail vein one week before 4^{th} and 5^{th} immunization according to Standard Operating Procedure ANCA016.

The following Blood and Chemistry parameters (Tables 2 and 3) were evaluated under GLP conditions at the Rodent Clinical Pathology Core Laboratory: Central Arkansas Veterans Healthcare System, Little Rock, AR.

Table 2 Blood Parameters Evaluated

Leukocyte count, total and differential
Erythrocyte count
Hematocrit
Hemoglobin
Mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration (calculated)
Platelet count

Table 3 Chemistry Parameters Evaluated

Alkaline phosphatase
Aspartate Aminotransferase
Bilirubin, total
Calcium
Chloride
Creatinine
Gamma glutamyl transferase
Glucose
Lactate Dehydrogenase
Magnesium
Phosphorus
Potassium
Sodium
Total Protein
Urea Nitrogen

- **Hematology (Hemavet):** At the first tail-bleed, 15 of the 20 Hemavet measures produced statistically significant K-W test results, but only one of the 15 (Monocyte %(MO %)) also yielded a statistically significant net trend (negative) with dose. This measure also yielded a significant K-W test and trend at the third necropsy, but the trend with dose changed from negative to positive. Monocyte (MO) concentrations also yielded numerous statistically significant results that did not, however, coincide in time with MO %. Neutrophils (NE) showed a number of significant test results in the concentration measure but none in the percentage measure. Apart from the first tail-bleed, the other Hemavet measures showed only sporadic statistically significant results that were not sustained over time. The overall conclusion was that the results for first tail-bleed did not reflect a vaccine-related biological signal, and that results at other times were consistent with study variability.
- **Serum chemistry (Vetscan):** At 2nd necropsy, two Vetscan measures produced a statistically significant K-W test accompanied by a statistically significant net trend with dose. The net negative trend in serum glucose arises from a discontinuous drop between the lower two and upper two doses, while it was observed that the net positive trend in serum phosphate similarly arises from a discontinuous rise

between the lower two and upper two doses. For both these parameters, the dose discontinuities disappeared by 3rd necropsy. At 1st necropsy, two significant K-W tests unaccompanied by significant trends were seen, and three significant trends were seen that were unaccompanied by significant K-W tests. The overall conclusion was that serum levels of glucose and phosphate showed transient changes that possibly could have been related to the vaccination dose, but that other Vetscan measures showed no evidence of a biological signal.

- **Urinalysis:** Leukocytes produced the only significant test result, which was at 3rd necropsy. Total bilirubin was noted to be at abnormal levels in all animals at first necropsy, and at abnormal levels in many animals at subsequent times. All measurements of urine ketone and urine protein were high enough to be numeric, which meant that the urine of all mice tested had abnormal levels of ketone and protein. No dose-group differences in prevalence of urinary abnormalities were detected. The overall conclusion was that the leukocyte result was not a biological signal, because urinary tract infection was not confirmed by histology. Likewise, renal lesions consistent with development of proteinuria were also lacking. Urinary bilirubin and protein results likely reflected fecal contamination of the urine. Biological explanation for elevated ketones in these mice are lacking as there is no evidence of renal disease or diabetes.

4.3 Mechanisms of Action and Toxicology

4.3.1 Vaccine - P10s-PADRE -

a. Antibody Quantity and Isotype

P10s is shown to bind to monoclonal antibodies that are reactive with several gangliosides and with the neolactoseries antigen LeY. P10s-PADRE immunization induces both IgG and IgM antibodies to P10s as determined by ELISA assays. These antibodies are cross-reactive with human and murine breast cancer cell lines. P10s is also reactive with human antibodies of the IgG2 and IgG1 isotype that display broad spectrum TACA reactivities.

b. Functional Antibody Responses

P10s-PADRE and P10s-KLH formulations induce antibodies that mediate CDC killing of tumor cell lines. Data also suggest that P10s-PADRE induces antibodies that have catalytic properties with TACA exposing TACA forms that are purported to interact with NK cells. Immunization with P10s suggest that it enhances NK cell infiltration into tumor cells in vivo in experimental animals.

c. Antigen Dosing

In preclinical studies, mice immunized with P10s-PADRE mount an anti-P10s response that is similar at 300μg and 500μg doses as determined by ELISA. However the cross-reactivity against the ganglioside is observed to be more robust at the 300μg dose. TACA are known to suppress the immune response. As P10s is a TACA mimetic it might be possible that it triggers T independent high tolerance pathways. Another possible mechanism is increased signaling through

C-type lectins on antigen presenting cells that are generally antagonistic to the stimulatory Toll like receptors. With this respect the dose dependence of the immunogenicity, the potential autoimmune effects and the potential toxicity may be considerably different qualitatively and quantitatively with immunogenicity optimal at relatively low doses far below the potential toxicity levels. Autoimmunity seems to be excluded reliably since in toxicity studies at the 300 µg dose the antibody responses are comparable to those observed earlier with lower doses but no autoimmune pathology was found.

d. Persistence of Response/Immunological Memory

P10s was shown to induce CD4+ T cells as P10s stimulates splenocytes cross-reactive with its homologue P10. CD4+ T cells are a necessary component of memory responses. While studies with P10s are on going to assess the longevity of the response other studies with other TACA mimotopes related to P10s have suggested anti-tumor responses up to 4 months post vaccination.

4.3.2 Stimulon - QS-21

QS-21 is an immunological adjuvant that has been shown to stimulate both humoral and cell-mediated immunity.

a. Antibody Quantity and Isotype

QS-21 has been shown to stimulate antibody responses to various vaccine antigens in mice, guinea pigs, rats, rhesus monkeys and baboons. The IgG response to a QS-21adjuvanted antigen is typically increased 10- to 1000-fold compared to that induced by unadjuvanted antigen. Whereas 5-20 µg of QS-21 has adjuvant activity in mice, doses of 50 to 100µg are effective in nonhuman primates.

b. Functional Antibody Responses

QS-21 improves functional antibody responses (viral neutralizing and bactericidal antibody) in animals. QS-21 was shown to stimulate a substantially higher serum neutralizing antibody titer to HIV-1 after immunization of baboons with HIV glycoprotein gp120 in comparison to the viral neutralizing response raised by gp120/aluminum hydroxide.

c. Antigen Dose-Sparing

Two biweekly SC doses of 5 µg of ovalbumin, adjuvanted with 10µg QS-21, induced titers in C57BL/6 mice that were over 100-fold higher than the titers induced by 125 µg of unadjuvanted ovalbumin. A similar dose-sparing effect was noted with HIV-1 gp120 in guinea pigs and baboons. These findings suggest that QS-21 adjuvant could be used to decrease the minimum immunogenic dose of antigen (antigen dose-sparing).

d. Persistence of Response/Immunological Memory

Adjuvants have also been shown to affect the duration of the antibody response. This was evaluated with various HIV-1 gp120 formulations with or without QS-21 in guinea pigs. After a single immunization, a peak serum antibody titer was observed, followed by a pseudo first-order decay of antibody. This was followed by a plateau of low-level antibody titers that were approximately 10-fold lower than the peak. This low-level antibody plateau following the decay phase is called antibody persistence. Various antigen formulations including QS-21, MF-59, aluminum hydroxide and no adjuvant yielded similar antibody decay kinetics. However, considerable differences were observed between adjuvants for peak titers and plateau titers. A strong correlation between peak titers and plateau titers was observed. Of formulations tested, QS-21 induced the highest peak titers and the highest plateau titers.

5.0 EFFECTS IN HUMANS

5.1 Risks/Safety –P10s-PADRE

At present P10s-PADRE has not been tested in Humans. The risks and safety of administering Mimotope P10s-PADRE to humans will be determined in clinical studies required by the FDA. Technical reports documenting proof of manufacturing integrity, concept studies, assay development and toxicology assessments will be written and reviewed prior to submission of the Investigational New Drug (IND) application to the FDA. In preclinical animal studies, Mimotope P10S-PADRE was determined as safe by meeting the following endpoint criteria: (i) mortality < 5% with no vaccine-attributable deaths, (ii) incidence of morbidity < 10% with no early sacrifice, (iii) absence of severe injection-site reactions (e.g. skin ulceration and/or severe myocyte necrosis) and (iv) immunopathology was absent or mild in non-targeted organs. The results of the preclinical safety study indicate that the vaccine does not lead to significant immunotoxicity or immunopathologies and therefore presents an acceptable safety profile in experimental animals. Like other carbohydrate targeting vaccines it is possible that P10s-PADRE might lead to Guillain-Barre Syndrome (GBS). GBS is the most frequent cause of acute flaccid paralysis in humans, occurring with an annual incidence of 1 to 2 cases per 100,000 people. In recent years, studies have shed new light on a number of disease aspects that have enhanced the understanding of the pathogenic mechanisms of GBS. GBS is an acute inflammatory polyradiculoneuropathy that usually develops following a gastrointestinal infection. Clinical symptoms often occur 1 to 3 weeks after a bacterial or viral infection, which have carbohydrate antigens in common with peripheral nerve tissue. Rabbits are an appropriate model to test formulations such as KLH for the induction of GBS. Despite that KLH induces GBS in experimental rabbits there are limited indications that this translates to humans as KLH is a carrier protein for many TACA-based vaccine that have been tested in humans. In a non-GLP study two rabbits were immunized with 500 µg P10S-PADRE and 20ug QS-21, one immunization every week for a total of three immunizations (3 weeks), emulating the scheduling proposed in the Phase I trial for the first three immunizations. While a short duration study (4 weeks),

no evidence of paralysis in these animals, nor any evidence of immunopatholgy was observed after necropsy. GBS is associated with reactivity to the ganglioside GM1. Human antibody fractions reactive with P10s and antibodies induced by P10s are not observed to cross-react with the GM1 ganglioside.

5.2 Risk/Safety – QS-21

Clinical studies containing QS-21 to date have focused primarily on its use as an adjuvant to enhance immune response evoked by preventative as well as therapeutic vaccines against infectious agents and cancers. Studies were and continue to be conducted by many sponsors with a wide variety of antigens and in varying indications.

QS-21 has been evaluated in over 120 different clinical trials of various experimental vaccines in cancer, infectious disease, and neurodegenerative disorders. Mild to moderate pain, erythema and edema at the injection site are common side effects of QS-21-containing vaccines. Low-grade fever and severe pain at the injection site may occur, but these effects are uncommon and short-lived. No significant hematological and biological alterations have been documented.

Vaccines containing QS-21 at doses of 50 and 100 µg induce local and systemic side-effects that tend to be more frequent, and of greater intensity compared to the same vaccines in an aluminum hydroxide formulation. However, aluminum hydroxide-induced tissue reactions such as granuloma, necrosis and nodules have never been reported in volunteers administered QS-21 alone or combined with vaccine antigens. Nearly 90% of volunteers injected with vaccines mixed with 50 or 100 µg of QS-21 experience injection site pain of variable intensity and onset, mild to moderate erythema, induration and some arm soreness. Some volunteers have reported immediate and severe pain on injection following administration of QS-21 preparations. In most studies, this effect occurs in a small percentage of subjects. Data from completed clinical studies indicate these side-effects to be transient, resolving within 7 days without sequelae. Systemic side effects have also been reported following the administration of vaccines combined with 50 µg, 100 µg, and 200 µg of QS-21. The overall incidence and severity of these reactions are generally similar to those of other vaccines, except for a marked increase in reactogenicity among subjects given the 200 µg QS-21 dose. Systemic events reported in recipients of QS-21 formulations include low-grade fever, flu-like symptoms with body aches, malaise, chills, myalgia, headache and dizziness. These symptoms are transient, resolving within 48 hours in most subjects. Severe adverse events (SAEs) (vaso-vagal reactions with hypotension and fainting) have also been reported, but they are extremely rare, occurring in less than 1% of QS-21 recipients.

Severe allergic reactions have been noted among recipients of QS-21-containing malaria synthetic peptide vaccines in two trials. Two out of ninety volunteers administered 2 mg of the SPf66 antigen mixed with 50 µg of QS-21 developed generalized pruritus with a few pruritic hives and minor bronchospasm (one volunteer), facial erythema, palpebral edema and dysphonia (one volunteer). These manifestations occurred 5 to 10 minutes after administration of the third vaccine dose, and were accompanied by hypotension. The reactions resolved completely within 60 minutes following systemic therapy with

epinephrine, hydrocortisone and anti-allergic drugs. Follow up exams performed 24 hours and 48 hours after the incidents were normal.

Data collected from Phase I and Phase II clinical trials of QS-21-containing vaccines indicate that the vast majority of adverse events (AEs) are transient reactions confined to the site of injection. The potential health risks associated with these vaccines will depend mainly upon the type of antigen being used, and are likely to be a greater concern if the vaccine antigen has homology with normal tissue constituents. Tumor vaccines admixed with QS-21 should not be administered to persons who have a documented history of autoimmune diseases, unless skin testing or lab results have unequivocally excluded the antigen as a potential source of tissue reaction.

Pregnancy and Lactation

There are no data available on the reproductive effects of QS-21. Therefore, the potential risks posed by QS-21 alone or combined with vaccine antigens are unknown. For this reason, QS-21 formulations should not be given to pregnant or lactating women.

Treatment of Overdose

No information is available regarding the potential toxicity resulting from an overdose of QS-21 in humans.

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