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TITLE: Development and Novel Uses of Antibodies in Epithelial Ovarian Cancer

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<b>14. ABSTRACT</b> Immunologic approaches to treat ovarian cancer, a chemosensitive tumor, are in their infancy and have generally represented isolated clinical trial efforts. Further understanding of the host response to epithelial cancers and the potential capability of innovative immunologic technologies to ovarian cancer may play a key role in therapeutic advances. This integrated program proposes to expand the scope of ovarian cancer treatment in general, and immunotherapy in particular by working in three new and interrelated directions. These include: 1) new techniques that may identify relevant new human antibodies and characterize the target antigens, 2) test these in project models to evaluate the potential role in future therapy for ovarian cancer combined with radiation and chemotherapy, and 3) explore their role in allowing for the more accurate targeting of gene therapy. During the first year of the project, we were able to establish a cohesive working group. IRB approval was obtained. Standard opera procedures regarding collection of lymphatic tissue and transfer to PI for Project 1 were established. Initial work was begun on Project 2 and Project 3.								
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## **Introduction**

Ovarian cancer is the leading cause of death due to gynecologic malignancies and is the fourth leading cause of death due to all cancers in women. The current challenges to reducing morbidity and mortality to this disease are well known. The majority of patients are diagnosed with advanced disease and, although the initial response to surgery and combination chemotherapy is high, the majority of patients will suffer a relapse. Second or third line therapies can often induce regression or disease stabilization, providing further evidence that ovarian cancer is a chemosensitive tumor. Therefore, we consider the identification of immune responses and antigens, immunomodulation of current active agents, and the introduction of novel treatment approaches by targeted gene therapy to hold the promise for further advances against ovarian cancer, and worthy of concerted multidisciplinary efforts by our clinical and basic scientists. Accordingly, the principal investigators have sought collaborations within the institution and at collaborating facilities to launch three interrelated preclinical therapeutics projects.

In project 1, investigators will utilize a fusion partner cell line that is capable of producing antibodies when fused with human B-lymphocytes derived from lymph nodes, or peripheral blood from patients with ovarian cancer. The production of human monoclonal antibodies will be characterized for their specificity to epithelial ovarian cancer and the type of antigens they recognize. Cell lines producing promising antibodies can then be further expanded and studied in the other preclinical projects designed to explore therapeutic models of combined modality and gene therapy. However, other potential applications, such as in diagnosis or vaccine development, could also be considered.

Project 2 utilizes monoclonal antibodies to target ovarian cancer cells testing monoclonal the potential therapeutic impact of antibody-linked radioisotopes combined with continuous exposure to topoisomerase 1 inhibitors. . Investigators in project 2 will build on past work with BrE-3 antibody and their long-standing interest in topoisomerase 1 as a target, the role of inhibitors as radiosensitizers, and the pharmacology of the clinically useful inhibitors. The model will also allow for rapid testing of new antibodies discovered in project 1.

The Laboratory shared resource would support these three studies by providing quality control, biodistribution and assessing the pharmacokinetics of the novel antibodies identified. The Administrative & Planning core will assist in the analysis of the results, and help plan experiments that may allow their transfer into clinical testing.

Thus, the studies proposed would integrate the identification of novel monoclonal antibodies with pre-clinical therapeutic potential as either direct anticancer agents, or as adjuncts to better targeting of either radioisotopes or gene therapy virus vectors. We anticipate that these studies would form the basis for exploring their potential in preclinical models of successful radioimmunotherapy and gene therapy transduction for therapeutic purposes, in situations that frequently arise during the course of ovarian cancer (e.g., presence of residual disease after initial chemotherapy, and of drug-resistant recurrences.)

## **Body**

### ***Core Facility B: Administration***

#### **Task Assignments and Status:**

1. Establishment of executive committee to provide oversight of program project and communicate with internal and external participants
  - a. Months 1-3
    - i. Establishment of launch date and identification of any problems or barriers to the successful initiation of individual projects
    - ii. Identification of operational problems and issue resolution
    - iii. Establishment of regular meeting cycles and review, report preparation

#### **Status:**

The initial step was to obtain Institution Review Board approval of the research project. The project was approved. It was also confirmed that any tissue or sera identified for use in this research could be acquired under the existing tumor procurement IRB protocol in place at NYU School of Medicine.

Regular meetings were scheduled and review projects were begun during months 1 - 3. Further meetings were held with the support core to obtain lymphoid tissue for project number 1.

2. Establishment of internal advisory committee to provide feedback regarding program project

#### **Status:**

The internal advisory committee met initially and discussed for the follow up period. This included Dr. Daniel Meruelo who had been awarded a prior Department of Defense Project and was working with a member of the Division of Gynecologic Oncology.

3. Establishment of external scientific advisory committee to provide oversight and scientific evaluation of program.

#### **Status:**

The final task was preparation and review of abstracts with input from Judith Goldberg. After circulation of abstracts, these were submitted and accepted for presentation at Annual Meeting of Society of Gynecologic Oncologists and AACR. (Months 9 - 12).

## ***Core Facility A: Laboratory Core***

### Task Assignments and Status

1. Initial Startup Work, Months 0-13
  - a. Pharmacology Section: Support of Project 2 Studies. Preparation of radiolabeled antibody 90Yhu BrE-3 and quality control radiolabeled preparations. Preparation and calibration of osmotic infusion pumps. Investigation and development of appropriate DOTA chelating constructs for new anticipated humanized antibodies.
  - b. Tissue and Immunopathology: Support projects 1 and 3. Coordinate procurement of fresh lymph nodes from patients undergoing surgical staging for ovarian cancer. Ensure proper selection of appropriate tissue and placement in transport media. Define a workable methodology for minimizing the background effects derived from the use of human and IgM antibodies.

#### Status:

Operational issues were addressed and lymph tissue from patients with known ovarian carcinoma were successfully identified, harvested and transferred to the laboratory of the principal investigator for project 1 during months 3 - 12.

### ***Project 1: Development and Antigen Characterization of Totally Human Monoclonal Antibodies to Ovarian Cancer-Associated Antigens***

#### Task Assignments and Status:

1. To develop a panel of hybridomas using ovarian cancer patients' retroperitoneal or pelvic lymph nodes and peripheral blood lymphocytes (months 1 – 18)
  - a. Collect clinical material (peripheral blood and lymph nodes) to isolate lymphocytes for hybridization with human hybridoma fusion partner cells MFP-2.
  - b. Screen hybridoma panels for the secretion of ovarian cancer cell specific monoclonal antibodies (months 4 – 18)
2. Establish fusion partner cell lines from human B-lymphocytes derived from ovarian cancer patients.
3. Identify candidate antibodies capable of targeting established human ovarian cancer cell lines

#### Status

Task #1 has been successfully accomplished. Working in collaboration with cores #1 and #2 standard operating procedures were established to achieve the aims of the project.

Months 1-4

Issues addressed included:

- Identification of potential candidates who were scheduled for surgery with known or suspected ovarian cancer at NYU Medical Center.
- Establishing process to consent patients for tissue collection at time of surgery.

- Establishment of procedure with pathology regarding the collection of lymph nodes from patients undergoing surgery at NYU Medical Center
- Establish procedure for transfer of tissue from NYU to Dr Trakht laboratory at Columbia University.

Months 4-12

Lymph node samples obtained yielded lymphocytes eligible for hybridization.

Hybrid cells lines were established

Process of screening hybridoma panels for specific ovarian cancer antibodies begun.

***Project 2: Potentiation of radioimmunotherapy by topoisomerase I inhibitor when combined for ovarian cancer treatment.***

Task Assignments and Status:

1. Objective 1: Months 0 – 13: Demonstrate the efficacy of huBrE-3/Topotecan in ovarian cancer in in vivo models and extend this model to a strategy employing: huBrE-3/Topotecan CI or Topotecan IP.
  - a. Test our current combination of hu-BrE-3 and CI TPT in a subcutaneous mouse xenograft model of ovarian carcinoma.
  - b. Determine the MTD of direct IP TPT in combination with 90Y huBrE-3 in this model and compare direct IP administration of TPT to CI TPT in combination with 90Y huBrE-3.

Test the efficacy of adding other chemot

Lymph node samples obtained yielded lymphocytes eligible for hybridization.

Hybrid cells lines were established

Process of screening hybridoma panels for specific ovarian cancer antibodies begun.

- a. KOV-3 cells plated on denatured collagen
- b. Use the best combination in a tumor response experiment

For all tumor response experiments, tumor response and mortality will be endpoints, but potential correlates including pharmacokinetics, resistance mechanisms (e.g. BCRP), tumor apoptosis, topo-I enzyme levels in tumor and marrow cells will be studied (180 nude mice, 24 immunocompetent mice)

2. Identify the most effective antibody in the combinations: huBrE-3/ chemotherapy vs TIP-2 ( or other human antibodies produced by Project 1)/ chemotherapy
3. Month 17- 29 (12 months) Define the optimal isotope for radioimmunotherapy in this system: 90Y vs 188Re

Status

Objective 1

We have demonstrated the efficacy of huBrE-3/Topotecan in ovarian cancer *in-vivo* models and extend this model to a strategy employing : huBrE-3 /Topotecan CI or Topotecan I.P. First, we tested our current combination of huBrE-3 and CI TTN in a subcutaneous mouse xenograft model of ovarian carcinoma versus direct IP TTN in

combination with Y-90 huBrE-3 in this model and compared direct IP administration of TTN to CI TTN in combination with Y-90 huBrE-3. In addition we tested a third schedule which reflected the current clinical protocol being studied in our cancer center (intraperitoneal topotecan given daily for three days for each of two weeks. We have not yet tested the addition of cisplatin to these regimens. As reported at the 2003 AACR-EORTC, both the single weekly IP dose (for two weeks) of topotecan in combination with the Y-90 huBrE-3 and the continuous infusion topotecan given over two weeks in combination with the Y-90 huBrE-3 were more effective than the three daily doses of topotecan in combination with radiolabeled antibody. Also, continuous infusion combined with radioimmunotherapy appeared to be somewhat more effective than the single IP dose given weekly for two weeks combined with radioimmunotherapy. For each of these regimens, the combination therapy was more effective than the single agents alone.

We have also begun to elucidate mechanisms to explain the increased efficacy of the combined regimen. We have established an altered pharmacokinetics for the radiolabeled antibody when combined with topotecan. We have shown in vivo that a marked increase (28%) in the AUC of Y-90 huBrE3 occurs in tumors of mice treated with continuous infusion topotecan ( $310 \pm 6$  %ID/g X hr) compared with mice treated with RIT alone ( $241 \pm 12$  %ID/g X hr). %ID/gm at 24 hr also was increased in tumors treated with TTN and RIT ( $4.01 \pm 0.42$ ,  $p = 0.0255$ ) vs.  $3.11 \pm 0.38$  for mice treated with RIT alone. A 17% decrease in AUC of Y-90-huBrE3 was seen in the blood of mice treated with CI TTN ( $595 \pm 5$  %ID/g X hr) as compared to mice treated with RIT alone ( $719 \pm 2$  %ID/g X hr). %ID/ml in blood at 24 hr was decreased in mice treated with TTN and RIT ( $8.59 \pm 0.72$ ,  $p=0.0068$ ) vs.  $10.5 \pm 0.3$  for mice treated with RIT alone. No comparable differences were seen in other normal organs.

Based on these results we have concluded that adding continuous infusion topotecan to RIT increases the %ID/g and the AUC for tumor and significantly decreases %ID/ml and AUC in blood. We believe that this helps to explain the observed enhancement of therapeutic index for this combined modality.

#### Objective 2

We have not yet conjugated the TIP-2 antibody to DOTA as planned. However, production of sufficient amounts of TIP-2 antibody to permit conjugation with DOTA is now underway.

#### Objective 3: Month 17- 29 (12 months)

Define the optimal isotope for radioimmunotherapy in this system: 90Y vs188Re  
The first step will be to label the chosen antibody with Re-188. This will be done either by direct labeling or by a bifunctional chelating agent. This method will be optimized to provide the best overall labeling yield, immunoreactivity, in-vitro and in-vivo stability.

Over the past months we have determined that direct reduction and labeling of BrE-3 antibody with a lanthanide is not feasible. Attempts to achieve this cause fragmentation of the antibody. However, we have successfully conjugated MAG-3 to



BrE-3 and labeled with Tc-99m with maintenance of immunoreactivity and adequate radiolabeling efficiency. We are now in the process of translating this to Rhenium using non radioactive Rhenium perrhenate.

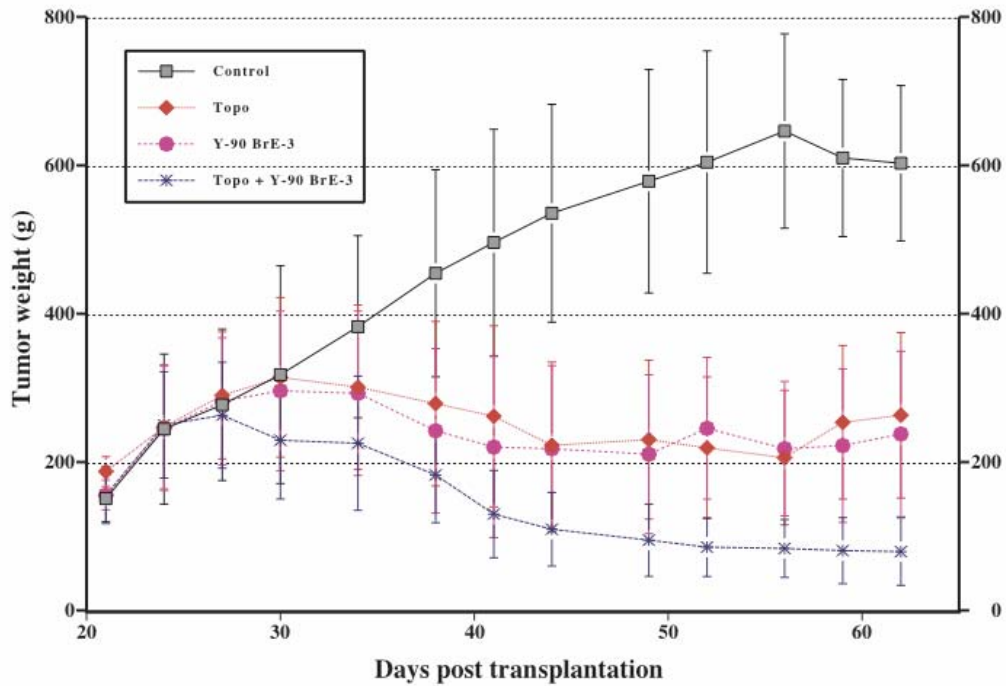


Figure 1. For the administration of topotecan 7 mg I.P. weekly for 2 weeks, each treatment when adjusted for the multiplicity of the treatments showed reduced average tumor weights compared to the control group. In addition, the combination of topotecan, and 90Y-hu-BrE-3 had a greater decrease than either single agent treatment alone.

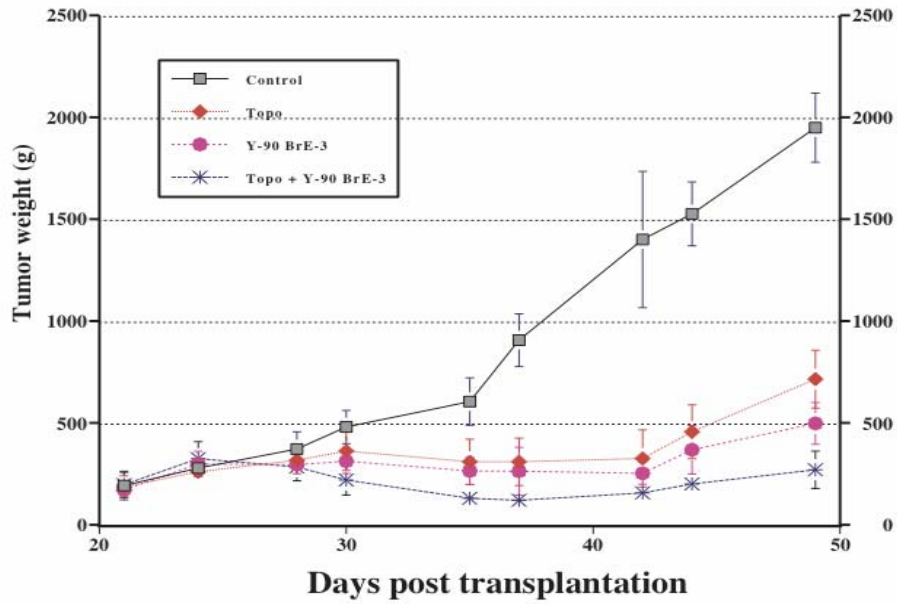


Figure 2. For the administration of the 2.5 mg/d daily x 3 for 2 weeks only the combined treatment of topotecan +  $^{90}\text{Y}$  huBrE-3 showed a greater decrease in tumor weight compared with the single agent treatments ( $p = 0.01$ ).m

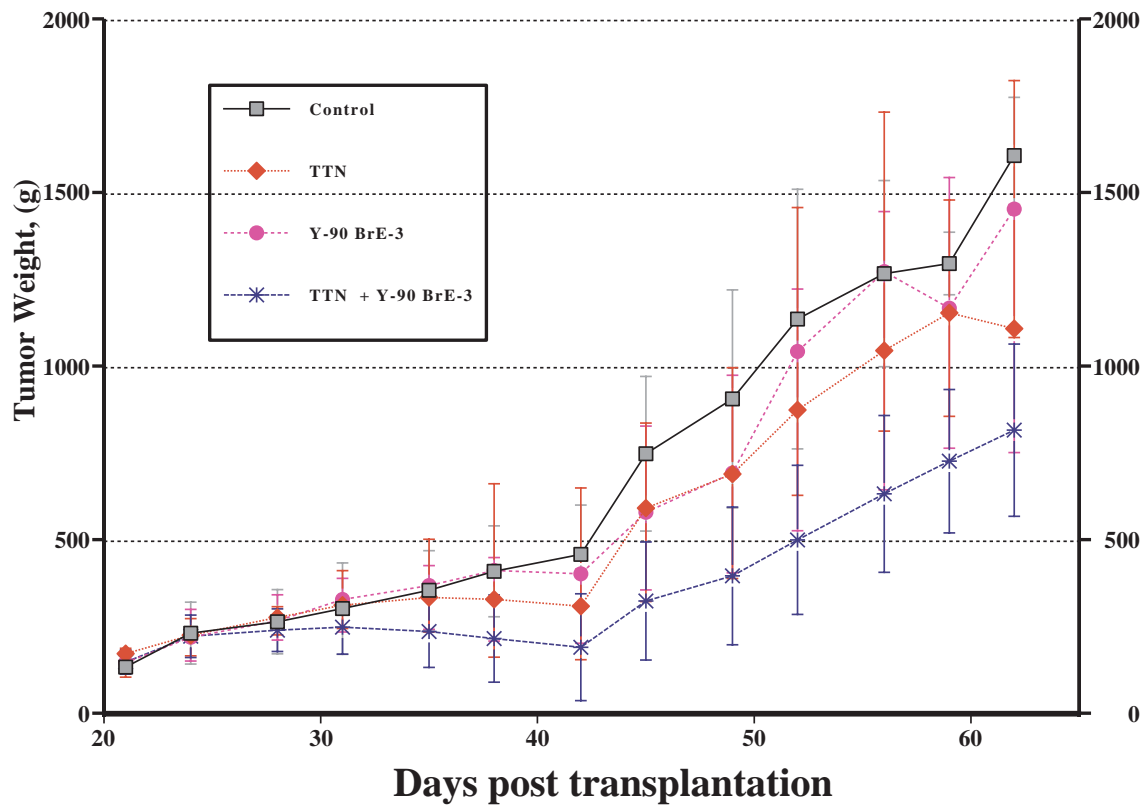


Figure 3. The administration of topotecan by continuous infusion showed significant inhibition by Topo alone compared to untreated animals, and also by Topo + RIT compared to either single agent or untreated control for mean tumor wt (Day 49 – Day21) among the 4 groups (KW;  $p < 0.0001$ ). After adjusting for multiplicity ( $p < 0.01$ ) significant differences were seen between all treatments and control (Topo+ RIT ( $p = 0.0034$ ), Topo ( $p = 0.0058$ )).

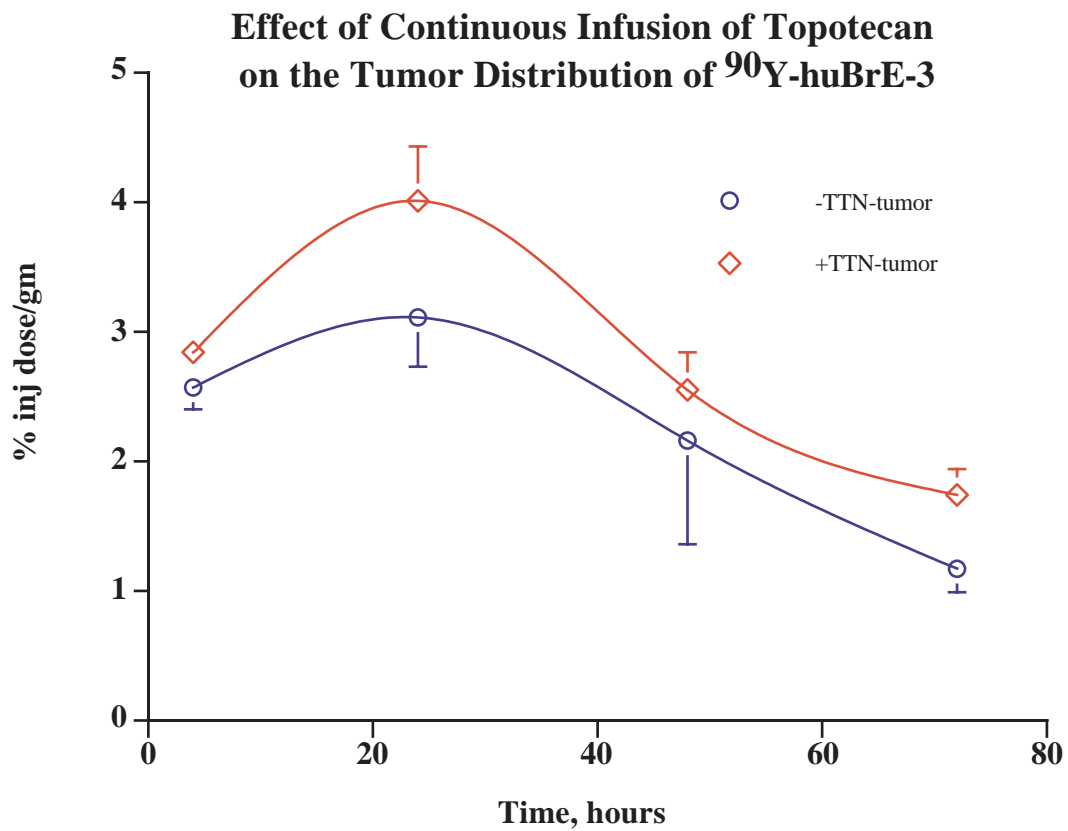


Figure 4. An increase in the tumor uptake of  $^{90}\text{Y}$ -huBrE-3 (AUC = 310.5 % injected dose/gm x hour) as a result of the presence of continuous infusion topotecan was observed compared to animals that were only treated with  $^{90}\text{Y}$ -huBrE-3 alone (AUC = 241.5 % injected dose/gm x hour).

### Effect of Continuous Infusion of Topotecan on the Blood Distribution of $^{90}\text{Y}$ -huBrE-3

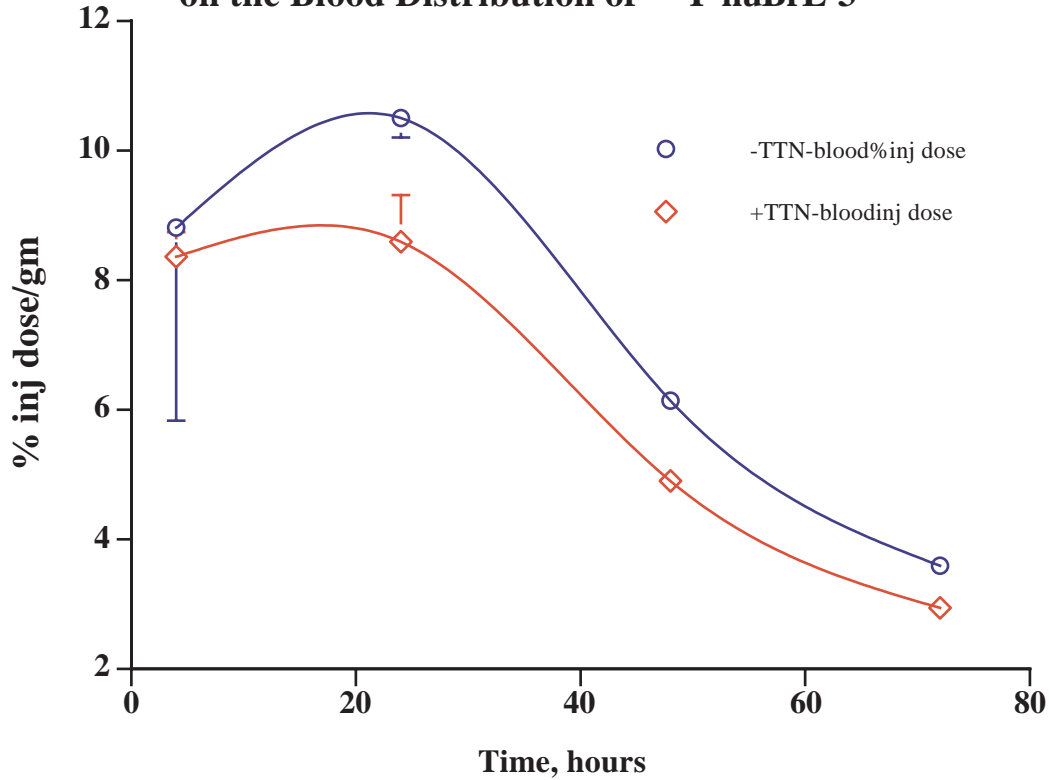


Figure 5. An decrease in the blood distribution of  $^{90}\text{Y}$ -huBrE-3 (AUC = 595.0 % injected dose/gm x hour) as a result of the presence of continuous infusion topotecan was observed compared to animals that were only treated with  $^{90}\text{Y}$ -huBrE-3 alone (AUC = 719.6 % injected dose/gm x hour).

### ***Project 3 Targeting Cryptic Epitopes as a Novel Therapeutic Strategy for Treatment of Ovarian Carcinoma***

#### Task Assignments and Status:

1. Months 0-12. Determine if cryptic epitopes of collagen defined by Mabs HU177 and XL313 are exposed within normal ovaries and ovarian carcinomas and to determine if there is a correlation between expression of these epitopes and the tumor stage. To accomplish this objective, we will begin by determining the optimal immunofluorescence staining parameters. These experiments will include evaluation of the best fixation procedure to reduce any alteration on the structure of collagen within the tissues, and determine the best blocking condition needed to reduce any non-specific binding of both primary and fluorochrome conjugated secondary antibodies. We will then evaluate at least 25 specimens from each tumor stage and 25 normal ovarian specimens for the relative exposure of the cryptic collagen epitopes defined by Mab HU177 and XL313. In particular, we will determine whether a correlation exists between the relative expression levels of the cryptic epitopes defined by Mab HU177 and XL313 and tumor stage. In addition, we will begin to optimize experimental parameters for establishing the two independent models (SKOV-3 and OVCAR-3) of human ovarian carcinoma tumor growth in nude mice. The specific parameters to be established will include proper cell density, growth conditions, and the optimal numbers of cells to be injected per mouse to maintain subcutaneous tumor growth. Finally, we will optimize the time course for *in vivo* tumor growth.

#### Status

1. Expression of HU177 in Normal Ovary and Ovarian Tumors. We have obtained a limited number (n=5) of human ovarian carcinoma tumor specimens and normal ovary specimens. We have established experimental conditions for immunofluorescence staining as well as immunohistochemical methods of analysis of HU177 cryptic isotope. Importantly our preliminary results indicate the HU177 cryptic isotope is specifically expressed within the ECM of malignant human ovarian carcinoma while little if any of the HU177 epitope was detected in normal ovarian tissues (Fig. 1). These preliminary studies indicate a highly selective bio-distribution of the cryptic epitope within human ovarian tumors. Currently, we are establishing the working conditions for the analysis of the XL313 cryptic epitope as well as establishing the optimal experimental conditions for the quantification of both soluble HU177 and XL313 epitopes within human serum samples.
2. Effects of Mab HU177 and XL313 on Human Ovarian Carcinoma Cell Adhesion. Our previous studies have established the experimental conditions to assess the effects of Mab XL313 and HU177 on ovarian carcinoma cell adhesion *in vitro*. Briefly 48-well non-tissue culture plates are coated with denatured collagen type-I and -IV. Human SKOV-3 ovarian tumor cells were

allowed to attach in the presence or absence of Mab HU177 and control antibody. Our results indicate the Mab HU177 potently inhibited (approximately 80%) SKOV-3 cell adhesion to denatured collagen type-I and IV (Figures 2 and 3). Interestingly, similar experiments carried out with Mab XL313 suggested that Mab XL313 does not inhibit SKOV-3 cell adhesion. Additional experiments are underway to assess the effects of these Mab on adhesion of other ovarian carcinoma cell lines.

3. Effects of Mab HU177 on Human SKOV-3 Ovarian Carcinoma Cell Proliferation. Our previous studies have established the experimental conditions to assess the effects of Mab XL313 and HU177 on ovarian carcinoma cell proliferation in vitro. Briefly 96-well non-tissue culture plates are coated with denatured collagen types-I. Human SKOV-3 ovarian tumor cells were resuspended in the presence or absence of Mab HU177 and control antibody. Proliferation was monitored by measuring the tumor cell associated mitochondrial dehydrogenase with a commercially available assay kit (WST-Cleavage). Our results indicate the Mab HU177 (200ug/ml) potently inhibited (approximately 60%) SKOV-3 cell proliferation on denatured collagen type-I (Fig. 4). Additional experiments are underway to assess the effects of Mab HU177 on proliferation of SKOV-3 cell on denature collagen type-IV.
4. Effects of Mab XL313 on SKOV-3 Tumor Growth in Nude Mice. To begin to establish the optimal experimental effects of Mab XL313 on ovarian tumor growth we used a nude mouse xenograft model. Briefly, subconfluent cultures of human ovarian carcinoma cell line SKOVV-3 were harvested, washed, and resuspended in sterile PBC. Female nude mice were injected subcutaneously with SKOV-3 cells ( $1 \times 10^6$ /per mouse). Subcutaneous ovarian tumors were allowed to grow for two weeks (approximately  $80 \text{ mm}^3$ ) at which time mice were treated daily by intraperitoneal injections with Mab XL313 (250ug/day). As shown in Figure 5, Mab XL313 resulted in an approximately 50 – 60% inhibition of tumor growth as compared to either isotope matched control antibody or no treatment.

Figure 1.

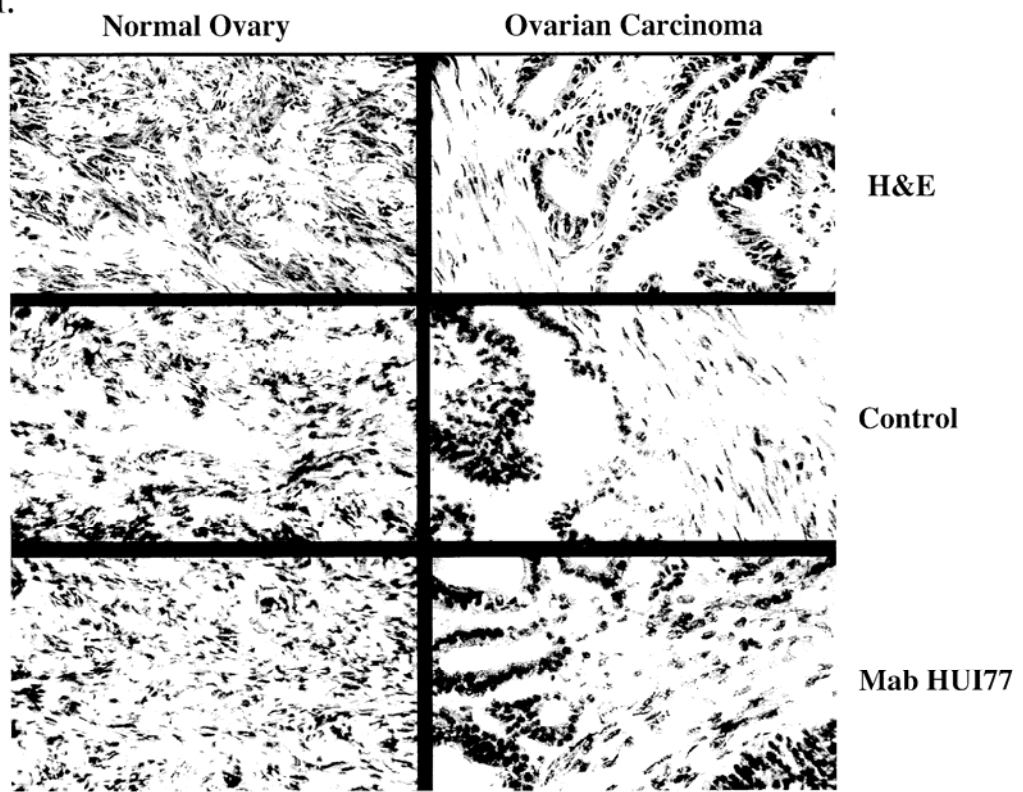




Figure 2.

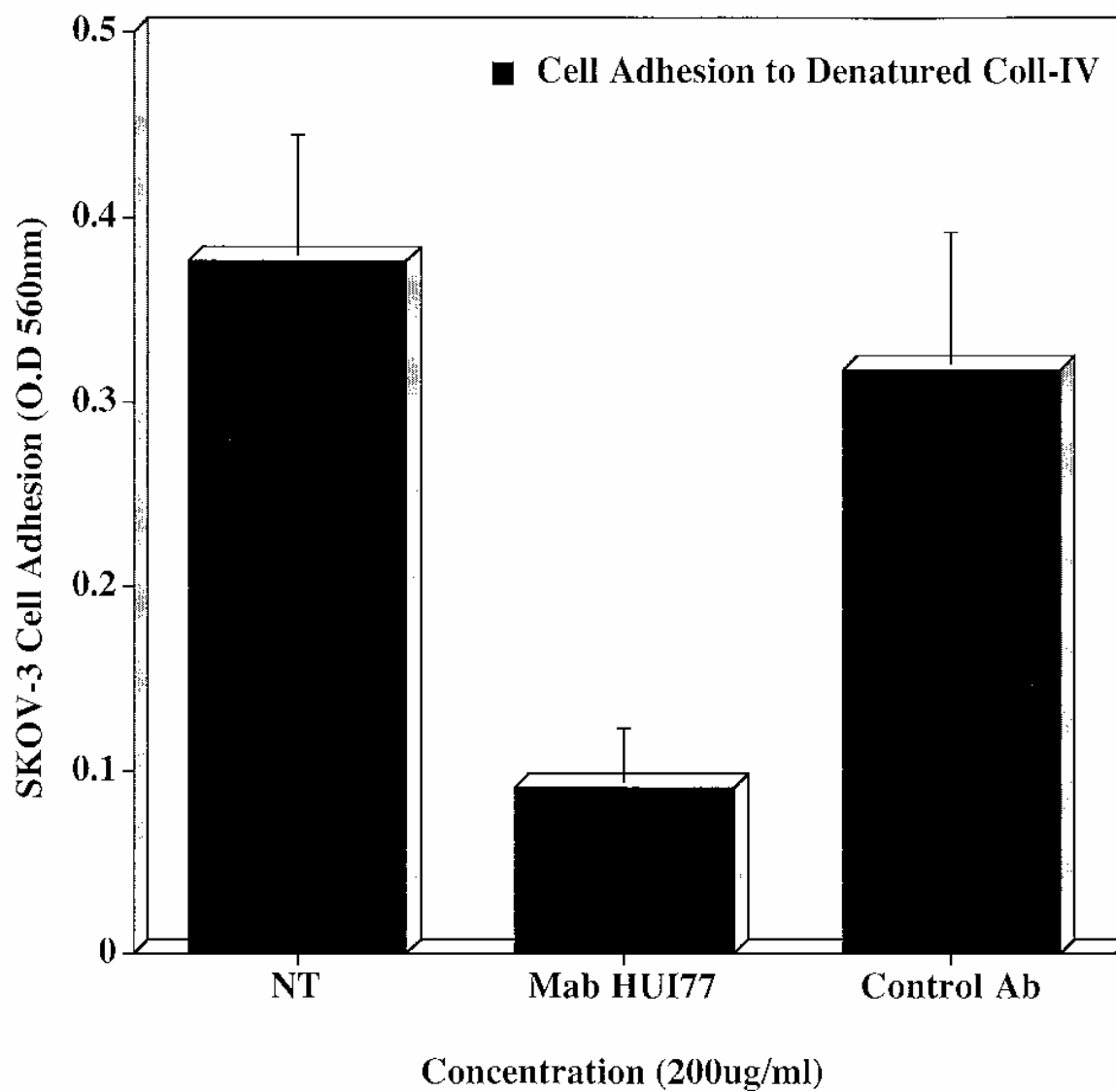


Figure 3.

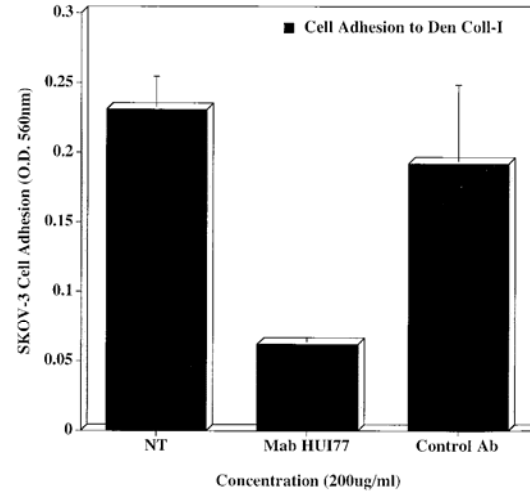


Figure-4.

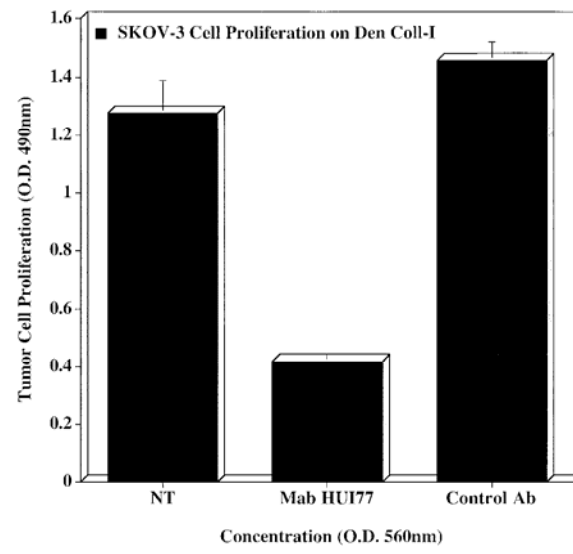
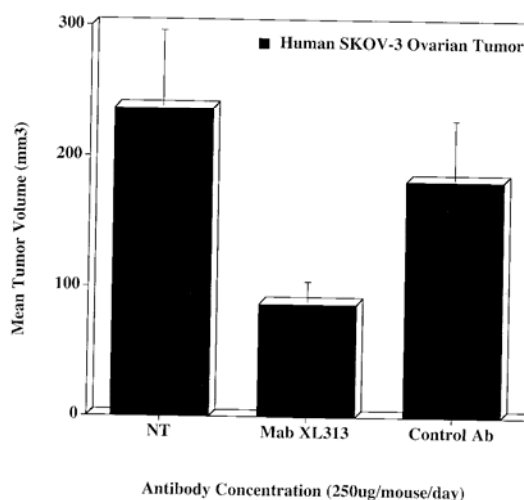


Figure 5.



### Key Research Accomplishments

- Establish internal working group
- Tissue sample collection and processing procedures
- Identification and processing of ovarian cancer tissue from historical tumor bank
- Harvesting of lymphocytes from lymph node tissue from patients with ovarian cancer
- Establishment of fusion partner cell lines from human B-lymphocytes
- Demonstrated efficacy of huBrE-3/topotecan in a mouse model of ovarian cancer.
- Demonstrated expression of HU177 within the ECM of human ovarian carcinoma while demonstrating little expression of HU177 in normal ovarian tissue.

### Reportable Outcomes

Abstract #217. The 34<sup>th</sup> Annual Meeting of the Society of Gynecologic Oncologists. 2003.

### **Treatment of an Ovarian Tumor Xenograft with Combined Chemo-Radimmunotherapy (RI) Using 90Y-BrE-3 Antibody and Intraperitoneal (IP) Administration of Topotecan (TPT)**

Leonard Liebes, Bruce Ng, Elissa Kramer, Phil Furmanski, Franco Muggia, Vandana Mukhi, Judith Goldberg, Roberto Ceriani, John P. Curtin. New York Univ Medical Ctr, New York, NY, Cancer Research Fund of Contra Costa, Walnut Creek, CA.

Objective: Clinical RI of solid tumors holds great promise, but has been hindered by a limited ability to deliver tumoricidal radiation doses with acceptable toxicity. We sought to potentiate the therapeutic action of radioimmunoconjugates at the tumor site and thus improve the efficacy of RI through combination with a treatment modality using the Topo I inhibitor TPT. Many studies have demonstrated that Topo I inhibitors can potentiate the lethal effects of ionizing radiation on tumor cells. TPT itself has been clinically effective for ovarian cancer. Since the primary site of ovarian cancer recurrence and progression is in the peritoneal compartment, intraperitoneal therapy is an effective therapeutic approach.

Methods: Using a human breast cancer xenograft model we had previously demonstrated a dramatic anti-tumor effect of combined administration of 200Ci 90Y-BrE3 monoclonal antibody (Mab) in combination with continuous infusion TPT (1mg/kg/day) for 14 days {Ng et al, Cancer Res, 2001}. To extend these findings to ovarian cancers, athymic female nude Swiss NIH mice were inoculated subcutaneously in the left flank with 106 SKOV3 human ovarian tumor cells on day 0. On day 21 post-tumor inoculation, mice were randomized into four treatment groups: Control (no treatment (tx), n=6), and three tx groups of 8 animals each: TPT alone (1mg/kg i.p. weekly x 2), 90Y-BrE-3 alone (i.p. 200 Ci of 90Y-BrE3) and TPT + 90Y-BrE-3. Tumor dimensions were measured in 3 planes with a Vernier caliper every 3-4 days.

Results: Topo I inhibitor or 90Y-BrE-3 MAb treatment alone delayed overall tumor growth rate with regrowth at day 59. The combination of RI with the topo I inhibitor dramatically reduced growth of human ovarian tumor over the 62 day follow up period compared with either TPT alone (p=0.02) or 90Y-BrE-3 alone (p=0.0014) or control (p<0.0001) using a fixed effects linear model for repeated observations.

Conclusions: The combination of TTN and 90Y BrE-3 shows significantly improved efficacy over the use of either agent alone in this animal model of ovarian cancer. Supported by DOD OC010016, the Chemotherapy Foundation and NIH CA-56129-24.

Abstract presented at AACR/NCI/EORTC Molecular Targets & Cancer Therapeutics, Nov. 17-21, 2003, Boston Ma.

**B217 Optimized schedules of topotecan combined with 90Y huBrE3 radioimmunotherapy show enhanced tumor response in an in vivo ovarian carcinoma model.**

Elissa Kramer, Bruce Ng, Leonard Liebes, Philip Furmanski, Judith Goldberg, Vandana Mukhi, Roberto Ceriani, and John Curtin. NYU School of Medicine, New York, NY, New York University, New York, NY, and Cancer Research Institute of Contra Costa, San Francisco, Cancer Research Institute of Contra Costa, San Francisco, CA.

Radioimmunotherapy(RIT) alone of solid tumors has failed to achieve an adequate therapeutic. we combined RIT with potentially synergistic chemotherapy to enhance the therapeutic index of both. we previously shm synergy in breast cancer between topoisomerase inhibitor, topotecan (topo), and 90Y huBrE3, a humanized monoclonal igg specific for breast epithelial mucin. 90Y huBrE3 reacts with >95% of human ovarian

tumors tested. to optimize the schedule of topo administration when combined with 90Y huBrE3, we tested 3 different schedules of topo in an animal model of ovarian cancer. Cohorts of athymic nude swiss nih mice were inoculated subcutaneously in the flank with 106 SKOV3 ovarian tumor cells on day 0. On day 21, mice were randomized into four treatment groups: control (no treatment), and 3 treatment groups: topo alone, RIT alone (ip 200 uCi of 90YhuBrE3) and topo+ RIT. we tested 3 regimens of topo administration alone and in combination: topo i.p. (7mg/kg) weekly x 2, continuous administration of topo (ci) (1mg/kg/day x 14days) via implanted Alzet pumps, or topo ip 2.5mg/kg for 3 x per week for 2 weeks. We measured tumors in 3 planes with Vernier calipers 2-3 x per week to estimate tumor weight (wt).

Days/Topo Schedule	Control mg tumor	Topo mg tumor	RIT mg tumor	Topo + RIT mg tumor
2.5 mg/kg/d IP x 3 x 2wk				
21	137.6 +/-33.0 (n=8)	176.3 +/- 18.2 (n=10)	149.9 +/- 41.5 (n=5)	152.1 +/-35.1 (n=10)
62	1611.1 +/- 167.6 (n=3)	1112.6 +/- 357.3 (n=6)	1456.8 +/- 370.1 (n=5)	820.0 +/- 248.1 (n=8)
CI 1mg/kg/d x14	n=5	n=7	n=9	n=9
21	198.8 +/- 70.3	193.0 +/- 53.9	178.6 +/- 32.6	203.3 +/- 60.5
49	1955.5 +/- 169.4	721.2 +/- 141.9	504.0 +/- 102.3	276.3 +/- 91.6
7 mg IP/kg/wk x 2	n=6	n=8	n=8	n=8
21	152.8 ± 31.4	189.2 ± 20.0	157.5 ± 46.6	157.5 ± 38.5
62	604.8 ± 104.6	265.0 ± 111.5	239.8 ± 137.1	81.2 ± 45.6

On topo ip x 3 x 2wk, only combined treatment significantly reduced tumor wt relative to controls (4 treatment groups compared in terms of change in mean tumor wt (day 62 - day 21) (Kruskal Wallis test (KW); p = 0.0101) adjusting for multiplicity (5 comparisons, p<0.01), topo+ rit showed a greater decrease in tumor wt over the period (p = 0.0104). CI topo showed significant inhibition by topo alone compared to untreated animals, and also by topo + RIT compared to either single agent or untreated control for mean tumor wt (day 49 - day21) among the 4 groups (kw; p<0.0001). After adjusting for multiplicity (p<0.01) significant differences were seen between all treatments and control ( topo+ RIT(p=0.0034), topo (p=0.0058), RIT(p=0.0034)) and the combination compared to RIT(p=0.0036) or topo alone (p=0.001). For 7 mg ip/kg/wk x 2 single agent and combined treatment showed significant tumor response relative to controls (4 group comparison of mean tumor wt change (day 62- day 21); KW,p = 0.0004). adjusting for 5 multiple comparisons, each treatment group showed reduced average tumor wt compared to control (topo+RIT(p=0.0024), topo (p=0.0024), RIT(p=0.0037)). The combination had a greater decrease than topo alone (p=0.0027). In conclusion, our *in vivo* results confirm the enhanced therapeutic

index of combined topo and 90Y huBrE3 and suggest that either ci topo for two weeks or once weekly ip topo combined with 90Y huBrE3 is more effective than ip topo 3 days/week. Supported by DOD program project OC10016

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**Adding Continuous Infusion of Topotecan to Y-90 huBrE-3 Radioimmunotherapy Improves Biodistribution and Pharmacokinetics of MAb in Ovarian Tumor Xenografts**

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**Objectives:**

Radioimmunotherapy (RIT) is a promising therapeutic modality for the treatment of a wide variety of malignancies. We have shown before that the combination of continuous infusion (CI) of topotecan (TTN) and RIT with Y-90 huBrE-3 reduces ovarian tumor xenografts growth more effectively than either agent given alone. To elucidate underlying reasons for this we compared biodistribution of MAb in tumor and normal organs with and without co-administration of CI TTN.

**Methods:**

Nude mice were inoculated with SKOV3 ovarian cancer cells. 21 days after tumor inoculation 15 animals were treated with RIT alone (200uCi Y-90 huBrE-3, 50 ug, ip injection) and 15 animals received both RIT and CI TTN (1 mg/kg/day for 14 days via implanted Alzet pumps). Cohorts of 3 mice were sacrificed 4, 24, 48, and 72 hours post treatment. Normal organs (liver, lung, spleen, bone, and blood) and tumors were weighed and counted for radioactivity. Activity was expressed as %ID/g +/- S.D. Pharmacokinetic parameters (AUC ± SE) were calculated for normal organs and tumor using WINONLIN (Mountain View,CA).

**Results:**

A marked increase (28%) was seen in the AUC of Y-90 huBrE3 in tumor of mice treated with CI TTN ( $310 \pm 6$  %ID/g X hr) compared with mice treated with RIT alone ( $241 \pm 12$  %ID/g X hr). %ID/gm at 24 hr was increased in tumors treated with TTN and RIT ( $4.01 \pm 0.42$ ,  $p = 0.0255$ ) vs.  $3.11 \pm 0.38$  for mice treated with RIT alone.

A 17% decrease in AUC of Y-90-huBrE3 was seen in the blood of mice treated with CI TTN ( $595 \pm 5$  %ID/g X hr) as compared to mice treated with RIT alone ( $719 \pm 2$  %ID/g X hr). %ID/ml in blood at 24 hr was decreased in mice treated with TTN and RIT ( $8.59 \pm 0.72$ ,  $p=0.0068$ ) vs.  $10.5 \pm 0.3$  for mice treated with RIT alone.

No comparable differences were seen in other normal organs.

**Conclusions:**

Adding CI TTN to RIT increases the %ID/g and the AUC for tumor and significantly decreases %ID/ml and AUC in blood. This suggests that adding CI TTN to RIT of ovarian cancer may both increase the efficacy of RIT and decrease toxicity. This helps to explain the observed enhancement of therapeutic index for this combined modality. Supported by DOD#OC010016 and Chemotherapy Foundation.