AWARD NUMBER:	W81XWH-10-1-0699
TITLE:	Randomized Phase II Trial of Adjuvant WT-1 Analog Peptide Vaccine in Patients with Malignant Pleural Mesothelioma after Completion of Multimodality Therapy
PRINCIPAL INVESTIGATOR:	Marjorie G. Zauderer
CONTRACTING ORGANIZATION:	Sloan Kettering Institute for Cancer Research New York, NY 10065
REPORT DATE:	November 2017
TYPE OF REPORT:	Final
PREPARED FOR:	U.S. Army Medical Reserarch and Material Command Fort Detrick, Maryland 21702-5012
DISTRIBUTION STATEMENT:	Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
November 2017	Final	15 AUG 2010 - 14 AUG 2017

4. TITLE AND SUBTITLE

Randomized Phase II Trial of Adjuvant WT-1 Analog Peptide Vaccine in Patients with Malignant Pleural Mesothelioma after Completion of Multimodality Therapy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0699

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Marjorie G. Zauderer, MD, MS, FACP

E-Mail: zauderem@mskcc.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Sloan Kettering Institute for
Cancer Research
1275 York Avenue
New York, NY 10065

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The Wilms' tumor gene, WT1, encodes transcription factors that regulate cell proliferation, differentiation, and apoptosis. WT1 protein is highly expressed in malignant pleural mesothelioma (MPM), and is a rational target for immunotherapy. We have developed a vaccine comprised of four WT1 heteroclitic peptides that are given together with Montanide and GM-CSF as immunologic adjuvants. This WT1 vaccine was previously tested in a pilot trial, and shown to be safe and immunogenic. This study tested the efficacy of this vaccine in MPM patients who have minimal disease burden after completion of multimodality therapy, but remain at exceedingly high risk for recurrence. A multicenter, blinded, randomized trial was conducted comparing treatment with the WT-1 peptide vaccine + Montanide/GM-CSF to treatment with Montanide/GM-CSF alone in patients with MPM who have completed multimodality therapy. The primary endpoint is progression free survival rate at 1 year. The trial has completed and the results are being published in Clinical Cancer Research.

15. SUBJECT TERMS
Mesothelioma, WT1, vaccine

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	60	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

Introduction:

The specific aim of this project was to conduct a randomized phase II trial of a WT1 peptide vaccine in patients with malignant pleural mesothelioma (MPM) as adjuvant therapy after completion of multimodality therapy. The Wilms' tumor gene, WT1, encodes transcription factors that regulate cell proliferation, differentiation, and apoptosis (1-7). WT1 protein is highly expressed in MPM, and is a rational target for immunotherapy. We have developed a vaccine comprised of four WT1 heteroclitic peptides that are given together with Montanide and GM-CSF as immunologic adjuvants (8). This WT1 vaccine was previously tested in a small pilot trial, and shown to be safe and immunogenic (9-11). We chose to test the efficacy of this vaccine in MPM patients who have minimal disease burden after completion of multimodality therapy (12-14), but remain at exceedingly high risk for recurrence. The study design was a multicenter, blinded, randomized trial comparing treatment with the WT-1 peptide vaccine + Montanide/GM-CSF to treatment with Montanide/GM-CSF alone in patients with MPM who have completed multimodality therapy. The primary endpoint was the progression free survival rate at 1 year.

Body:

Work Statement	Outcome
Peptides will be purchased, manufactured, and undergo sterility testing.	The peptides were ordered from AmbioPharm, Inc. They were vialed by the University of Iowa Pharmaceuticals under GMP conditions and underwent sterility and stability testing.
The protocol will be reviewed by the various committees at MSKCC leading to IRB approval.	The protocol was reviewed by the Office of Clinical Research, Biostatistics, Medicine Steering Committee, Research Council, IND Committee, and the IRB which granted approval in 2010 and the study opened to accrual in May 2011.
After the approval at MSKCC, M. D. Anderson Cancer Center will submit the documents for a similar review process at their center.	This protocol was approved at M. D. Anderson through a separate IND and opened to accrual in May 2013.
Additional sites may be recruited for participation in the study. This depends on the ability to secure additional funds and will not be covered under the auscpices of this grant.	Additional sites were not added due to funding constraints.
Patients will be recruited among those already seen at MSKCC and MDACC which have high volumes of mesothelioma patients. Patients treated elsewhere will also be referred to those centers for participation in the trial.	MSKCC and MDACC patients were enrolled. Additionally, patients were referred from other surgical centers to enroll on this adjuvant vaccine trial.
This is a randomized trial in which patients will receive WT1 peptides plus GM-CSF or	Based on the prespecified futility analysis, the control arm closed early to accrual in May

GM-CSF alone. The sample size is 78 patients (39 in each arm). Thus 16-20 patients will need to be enrolled annually.	2015 with 21 patients. Because subsequent patients would not be blinded, the vaccine arm was closed in November 2015 with 20 patients. The futility threshold was met, in part, because the design of this trial used progression-free survival from multimodality series which calculated from the date of surgery (15-21). This study, however, used the beginning of study treatment which was typically several months later. This likely accounted for a substantial component of the "early" progressors relative to historical controls.
We anticipate about equal numbers of patients to be enrolled at each site.	About 25% of patients were enrolled from MDACC. Because the study opened at MDACC two years after opening at MSKCC, MSKCC enrolled more patients.
Each patient will have a baseline sample and one at the completion of the course of vaccinations (156 samples in total).	Samples were collected as specified for the enrolled patients (41). However, there were unanticipated issues with cell viability both in the MSKCC and MDACC samples which limited the correlative analyses.
The assays will be performed in the laboratory of Dr. David Scheinberg at MSKCC by Tao Dao, PhD and by Viktoriya Zakhaleva.	The immunologic testing was performed in the laboratory of Dr. David Scheinberg.
Samples obtained at MDACC will be shipped to MSKCC for analysis.	Samples were collected at MDACC and shipped to MSKCC for analysis. Unfortunately, cellular viability was limited in these samples and there were only 10 samples on each arm of the trial suitable for analyses. This challenge has been very illustrative for the

	handling and processing of any future specimens.
Continued follow up of patients for the primary endpoint of progression-free survival.	Patients were followed for progression-free survival. The study was closed to follow up in August 2016 and final analyses performed.
Analysis of the data by the study biostatistician.	Dr. Panageas performed all statistical analyses for this study. The progression free survival rate at 1 year was 45% in the vaccine arm and 33% in the vaccine arm. Median progression-free survival was 10.1 months in the vaccine arma nd 7.4 months in the control arm. It is important to note that this difference was not statistically significant. It is unclear whether this was due to early termination of the study, styudy sample size, or vaccine efficacy. Even with 78 patients, this trial was not powered for comparison between the two arms. Please see further discussion in the attached manuscript.
Submission of abstracts to international meetings such as the American Society of Clinical Oncology or the International Mesothelioma Interest Group.	The preliminary data were presented as a plenary oral abstract at the 2016 International Mesothelioma Interest Group Meeting and a poster discussion at the 2016 American Society of Clinical Oncology Annual Meeting. These presentations are attached as appendices.
Preparation of manuscript and submission for publication.	The manuscript is currently in press at Clinical Cancer Research. A copy is attached to this report as an appendix.

Key research accomplishments:

- This trial further substantiated the safety profile of the WT1 vaccine.
- This work established robust “control” progression-free and overall survival data for patients after receipt of multimodality therapy. These outcomes will be used to inform all future clinical trials in the maintenance space after multimodality therapy.
- This trial helped identify challenges and opportunities for recruiting patients after multimodality therapy.
- This trial demonstrated a trend toward improved survival with receipt of the vaccine. The study was underpowered for comparison between the treatment and control arms.

Reportable Outcomes:

1) Oral presentation at International Mesothelioma Interest Group Meeting 2016, Birmingham UK

2) Poster discussion at American Society of Clinical Oncology Annual Meeting 2016, Chicago USA

3) Oral presentation at NCI-IASLC-MARF Mesothelioma Clinical Trials Planning Meeting 2017, Bethesda USA

4) Manuscript in press at Clinical Cancer Research

5) Dr. Victoria Lai applied for and received an American Society of Clinical Oncology Young Investigator Award to support a clinical trial of the WT1 vaccine in combination with nivolumab

6) Dr. Marjorie G. Zauderer secured drug and funding from Bristol Meyers Squibb to support the aforementioned clinical trial of the WT1 vaccine in combination with nivolumab

Conclusion:

There is a scientifically interesting trend toward improved survival with receipt of the WT1 vaccine. It was difficult to assess immune responses as samples with viable cells were only available for half of the patients. Future work will focus on ensuring robust correlatives that will be collected for all patients. Additionally, we are focusing efforts on combining the vaccine with immunomodulatory agents to improve the immune response.

References:

1. Mundlos S, Pelletier J, Darveau A, Bachmann M, Winterpacht A, Zabel B. Nuclear localization of the protein encoded by the Wilms' tumor gene WT1 in embryonic and adult tissues. *Development* 1993; **119**(4): 1329-41.

2. Amin KM, Litzky LA, Smythe WR, et al. Wilms' tumor 1 susceptibility (WT1) gene products are selectively expressed in malignant mesothelioma. *The American journal of pathology* 1995; **146**(2): 344-56.

3. Inoue K, Ogawa H, Sonoda Y, et al. Aberrant overexpression of the Wilms tumor gene (WT1) in human leukemia. *Blood* 1997; **89**(4): 1405-12.

4. Keilholz U, Menssen HD, Gaiger A, et al. Wilms' tumour gene 1 (WT1) in human neoplasia. *Leukemia* 2005; **19**(8): 1318-23.

5. Oji Y, Ogawa H, Tamaki H, et al. Expression of the Wilms' tumor gene WT1 in solid tumors and its involvement in tumor cell growth. *Japanese journal of cancer research : Gann* 1999; **90**(2): 194-204.

6. Rosenfeld C, Cheever MA, Gaiger A. WT1 in acute leukemia, chronic myelogenous leukemia and myelodysplastic syndrome: therapeutic potential of WT1 targeted therapies. *Leukemia* 2003; **17**(7): 1301-12.

7. Cheever MA, Allison JP, Ferris AS, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2009; **15**(17): 5323-37.

8. Pinilla-Ibarz J, May RJ, Korontsvit T, et al. Improved human T-cell responses against synthetic HLA-0201 analog peptides derived from the WT1 oncoprotein. *Leukemia* 2006; **20**(11): 2025-33.

9. Krug LM, Dao T, Brown AB, et al. WT1 peptide vaccinations induce CD4 and CD8 T cell immune responses in patients with mesothelioma and non-small cell lung cancer. *Cancer immunology, immunotherapy : CII* 2010; **59**(10): 1467-79.

10. May RJ, Dao T, Pinilla-Ibarz J, et al. Peptide epitopes from the Wilms' tumor 1 oncoprotein stimulate CD4+ and CD8+ T cells that recognize and kill human malignant mesothelioma tumor cells. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2007; **13**(15 Pt 1): 4547-55.

11. Maslak PG, Dao T, Krug LM, et al. Vaccination with synthetic analog peptides derived from WT1 oncoprotein induces T-cell responses in patients with complete remission from acute myeloid leukemia. *Blood* 2010; **116**(2): 171-9.

12. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural

mesothelioma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2003; **21**(14): 2636-44.

13. Rimner A, Zauderer MG, Gomez DR, et al. Phase II Study of Hemithoracic Intensity-Modulated Pleural Radiation Therapy (IMPRINT) As Part of Lung-Sparing Multimodality Therapy in Patients With Malignant Pleural Mesothelioma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2016; **34**(23): 2761-8.

14. Rosenzweig KE, Zauderer MG, Laser B, et al. Pleural intensity-modulated radiotherapy for malignant pleural mesothelioma. *International journal of radiation oncology, biology, physics* 2012; **83**(4): 1278-83.

15. Weder W, Kestenholz P, Taverna C, et al. Neoadjuvant chemotherapy followed by extrapleural pneumonectomy in malignant pleural mesothelioma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2004; **22**(17): 3451-7.

16. Rea F, Marulli G, Bortolotti L, et al. Induction chemotherapy, extrapleural pneumonectomy (EPP) and adjuvant hemi-thoracic radiation in malignant pleural mesothelioma (MPM): Feasibility and results. *Lung cancer* 2007; **57**(1): 89-95.

17. Weder W, Stahel RA, Bernhard J, et al. Multicenter trial of neo-adjuvant chemotherapy followed by extrapleural pneumonectomy in malignant pleural mesothelioma. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2007; **18**(7): 1196-202.

18. Batirel HF, Metintas M, Caglar HB, et al. Trimodality treatment of malignant pleural mesothelioma. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2008; **3**(5): 499-504.

19. Bolukbas S, Manegold C, Eberlein M, Bergmann T, Fisseler-Eckhoff A, Schirren J. Survival after trimodality therapy for malignant pleural mesothelioma: Radical Pleurectomy, chemotherapy with Cisplatin/Pemetrexed and radiotherapy. *Lung cancer* 2011; **71**(1): 75-81.

20. Krug LM, Pass HI, Rusch VW, et al. Multicenter phase II trial of neoadjuvant pemetrexed plus cisplatin followed by extrapleural pneumonectomy and radiation for malignant pleural mesothelioma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2009; **27**(18): 3007-13.

21. Van Schil PE, Baas P, Gaafar R, et al. Trimodality therapy for malignant pleural mesothelioma: results from an EORTC phase II multicentre trial. *The European respiratory journal* 2010; **36**(6): 1362-9.

21. Hasani A, Alvarez JM, Wyatt JM, et al. Outcome for patients with malignant pleural mesothelioma referred for Trimodality therapy in Western Australia. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2009; **4**(8): 1010-6.

Appendices:

Appendix 1 – poster from American Society of Clinical Oncology Annual Meeting 2016

Appendix 2 – plenary oral session at International Mesothelioma Interest Group Meeting 2016

Appendix 2 – manuscript in press at Clinical Cancer Research

Randomized phase II study of adjuvant WT1 vaccine for malignant pleural mesothelioma (MPM) after multimodality therapy

Zauderer MG[1], Dao T[1], Rusch V[1], Ginsberg M[1], Tsao A[2], Mehran R[2], Panageas K[1], Stergiou A[3], Scheinberg DA[1], Krug LM[1*]

[1] Memorial Sloan Kettering Cancer Center, New York, NY; [2] MD Anderson Cancer Center, Houston, Texas;
[3] SELLAS Life Sciences Group, Zug, Switzerland
*current address Bristol Myers Squibb Scotch Plains, NJ

Disclosures

Institutional research funding:	SELLAS Life Sciences Group, MedImmune, Verastem, Lilly, Genentech, GSK, Genelux
Advisory Board:	AstraZeneca
Grant support:	Department of Defense, National Cancer Institute, Meso Foundation

MSK has licensed patents on this vaccine.

DAS is an inventor on the vaccine patents and receives research funding and consulting fees from SELLAS Life Sciences Group.

WT1 is ranked as #1 cancer antigen by the NCI

Human Cancer Biology

The Prioritization of Cancer Antigens: A National Cancer Institute Pilot Project for the Acceleration of Translational Research

Martin A. Cheever,[1] James P. Allison,[2] Andrea S. Ferris,[3] Olivera J. Finn,[4] Benjamin M. Hastings,[3] Toby T. Hecht,[5] Ira Mellman,[7] Sheila A. Prindiville,[6] Jaye L. Viner,[6] Louis M. Weiner,[8] and Lynn M. Matrisian[6]

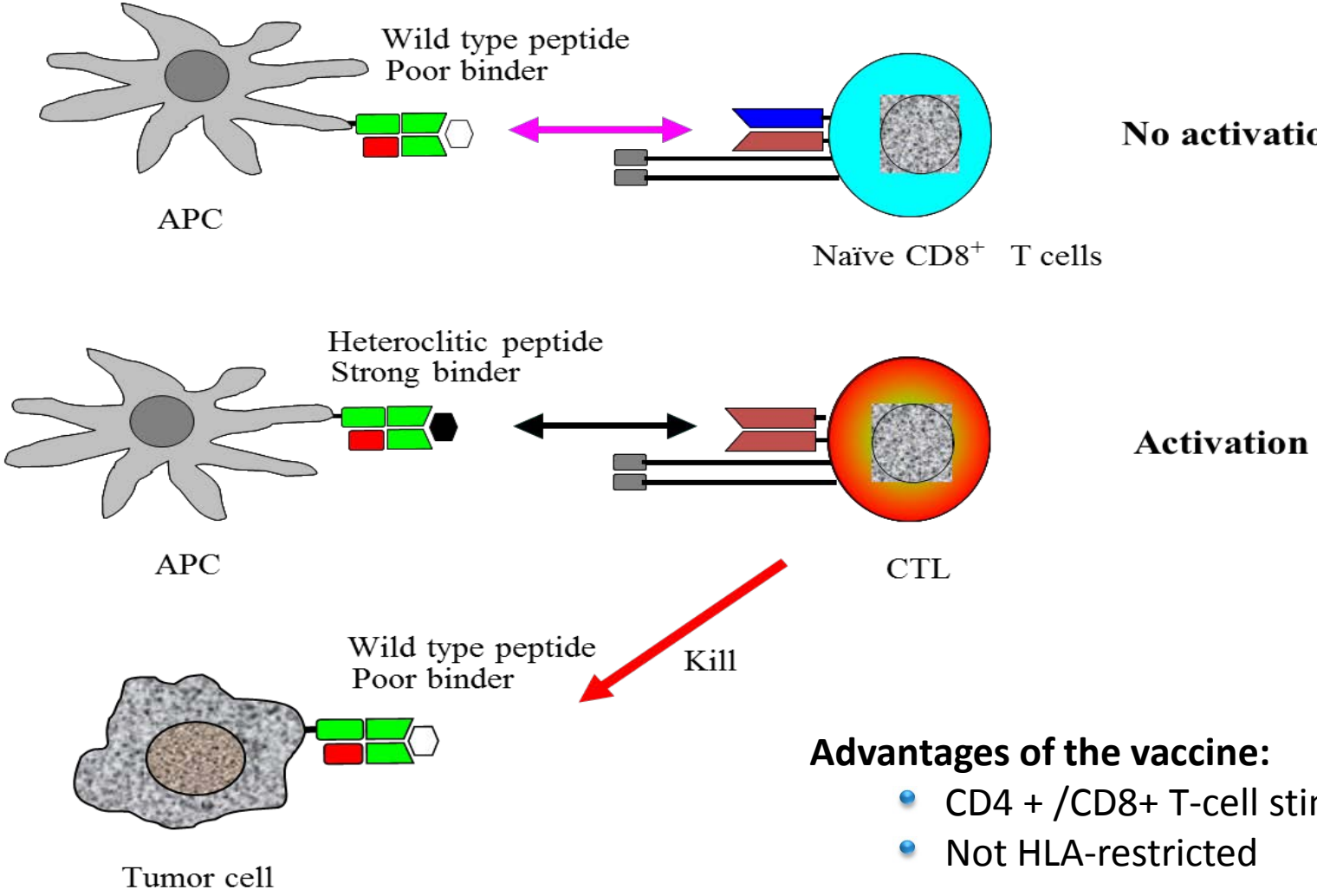
Antigens (rank/reference number and name)	Cumulative score
1. WT1	0.81
2. MUC1	0.79
3. LMP2	0.78
4. HPV E6 E7	0.77

WT1 in MPM

- Limited WT1 expression in normal adult tissues
- WT1 expression up-regulated in MPM
  - Acts as an oncogene
  - Used as prognostic /diagnostic marker in MPM
- WT1 target cells recognized and killed by T cells

Cheever CCR 2009; Pinilla-Ibarz Leukemia 2006; May CCR 2007

WT1 immunogenicity was improved by creating heteroclitic peptides, Galinpepimut-S



Advantages of the vaccine:

- CD4 + /CD8+ T-cell stimulus
- Not HLA-restricted

In a pilot study, Galinpepimut-S was safe and induced immune responses in MPM

Pinilla-Ibarz Leukemia 2006; May CCR 2007;
Krug Cancer Immunol Immunother 2010

Galinpepimut-S' peptide sequences elicit CD4+/CD8+ activation

Memorial Sloan Kettering Cancer Center

Sequences (position)	Immune Response
WT1-A1: *YMFPNAPYL (126-134) **mutated vaccine peptide* *(native has R not Y)*	CD8+ CTL
427 long: RSDELVRHHNMHQRNMTKL (427- 445) 331 long: PGCNKRYFKLSHLQMHSRKHTG (331-352) 122A1 long *SGQAYMFPNAPYLPSCLES (122-140) **Vaccine peptide (native has R not Y)*	CD4+ T cells CD4+ T cells CD4+ and CD8+ T-cells

Pinilla-Ibarz Leukemia 2006; May CCR 2007

Galinpepimut-S was studied in a randomized trial after multimodality treatment for MPM

- Malignant pleural mesothelioma
- WT-1 positive
- 4-12 weeks since completion of multi-modality treatment including surgery
- KPS ≥ 70%

N= 78 patients (39 per arm)

RANDOMIZE

1:1

Specific Immunotherapy x 6 (q2w):

- Galinpepimut-S (800 µg/dose) +
- Montanide (500 µl/dose)
- GM-CSF (70 µg/dose; d-2 d-0)

Non-specific Immunotherapy x 6 q2w):

- Montanide + GM-CSF at doses mentioned above

Primary endpoint: # progression-free @ 1 year
Secondary endpoints: OS, immune response

Closed by DSMB after N=40 due to control arm futility
Study un-blinded and analyzed in November 2015

NCT01265433; DoD W81XWH-10-1-0699

Patient characteristics were well balanced in the two arms of the study

	WT1 (n=19)	Control (n=21)	Total (n=40)
Age (Mean ± SD)	67±11.17	65.71±8.39	66.35±9.7
Male (%)	84.2	85.7	85
Histology			
Epithelioid	17 (89.5%)	20 (95.2%)	37 (92.5%)
Non-Epithelioid	2 (10.5%)	0 (0%)	2 (5%)
KPS	0.87 ± 0.06	0.81 ± 0.07	0.84 ± 0.07
Surgery			
Macroscopic complete resection	4 (21%)	4 (19%)	8 (20%)
Other	9 (47.4%)	12 (57.1%)	21 (52.5%)
Unknown	6 (31.6%)	5 (23.8%)	11 (27.5)
Laboratory Tests (Medians)			
Leukocytes (10^9/L)	5.6	5.55	5.45
Hemoglobin (g/dL)	12.3	11.7	11.85
Platelets (10^9/L)	188	205	195

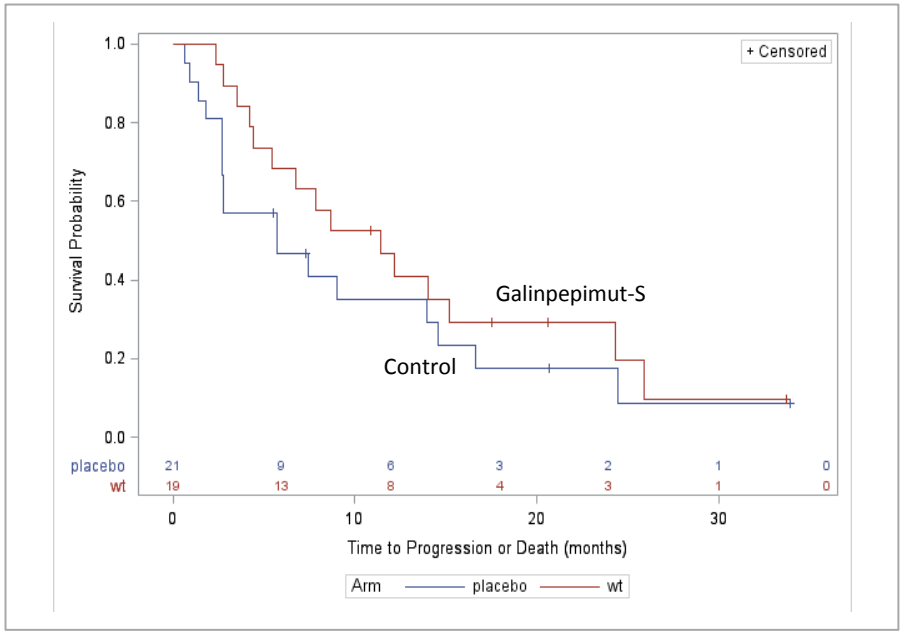
DBL as of 19Nov15

Incidence of Related Adverse Events ≥ 5%: Grade 1-2 and Grade 3-4

	Grade 1/2 n (%)	Grade 3 n (%)
Injection site reaction	23 (57.9)	--
Fatigue	15 (37.5)	--
Fever	4 (10)	--
Arthralgias	2 (5)	--
Rash maculo-papular	2 (5)	--
Lymphocyte count decreased	--	2 (5)

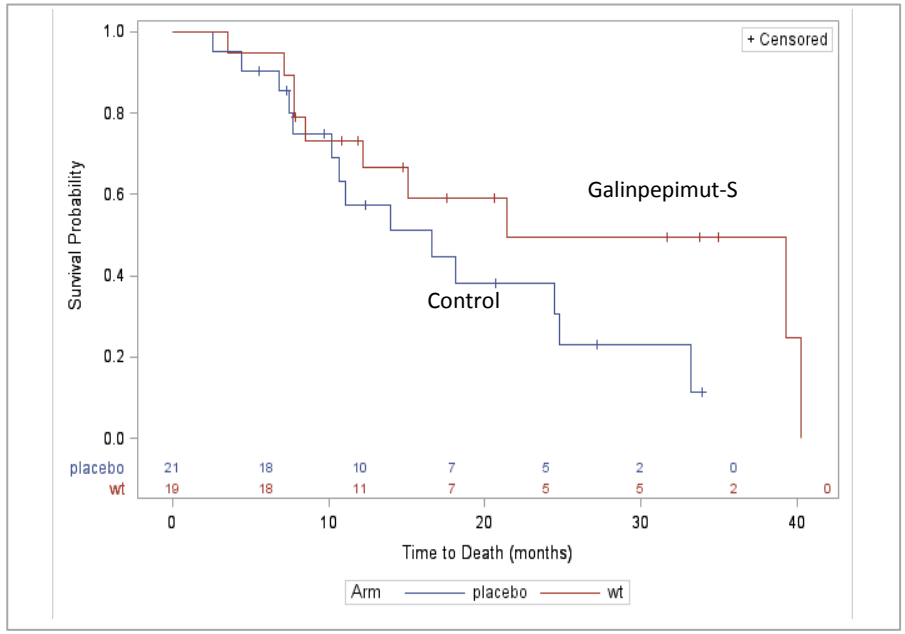
Galinpepimut-S was associated with improved progression-free and overall survival

Progression Free Survival (from 1st vaccine treatment)



- **Improved on vaccine:**
  - HR=0.69
  - Median PFS 5.7 mos. on placebo vs 11.4 mos. on Galinpepimut-S

Overall Survival (from 1st vaccine treatment)

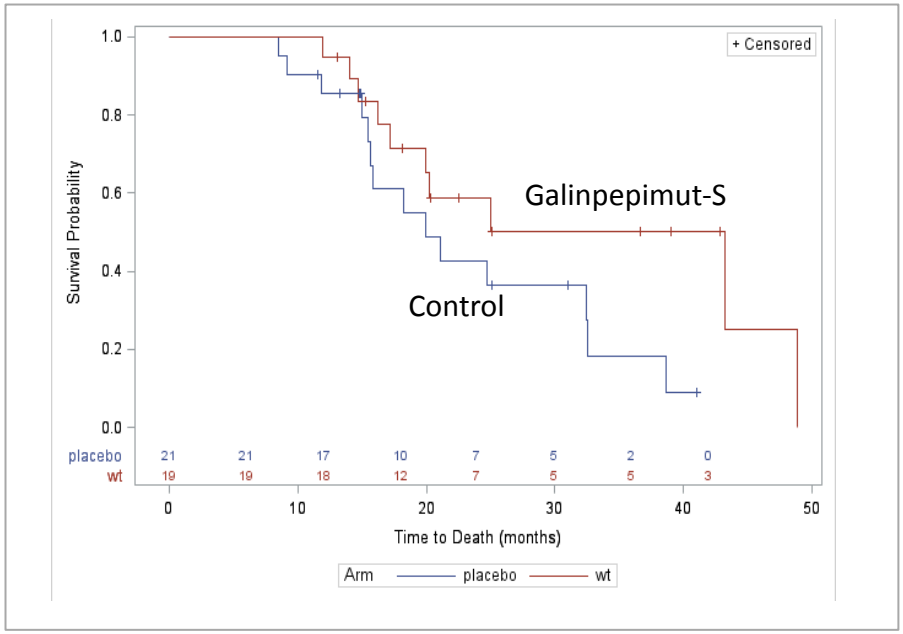


- **Improved on vaccine:**
  - HR=0.52
  - Median OS 16.6 mos. on placebo vs 21.4 mos. on Galinpepimut-S

DBL as of 19Nov15

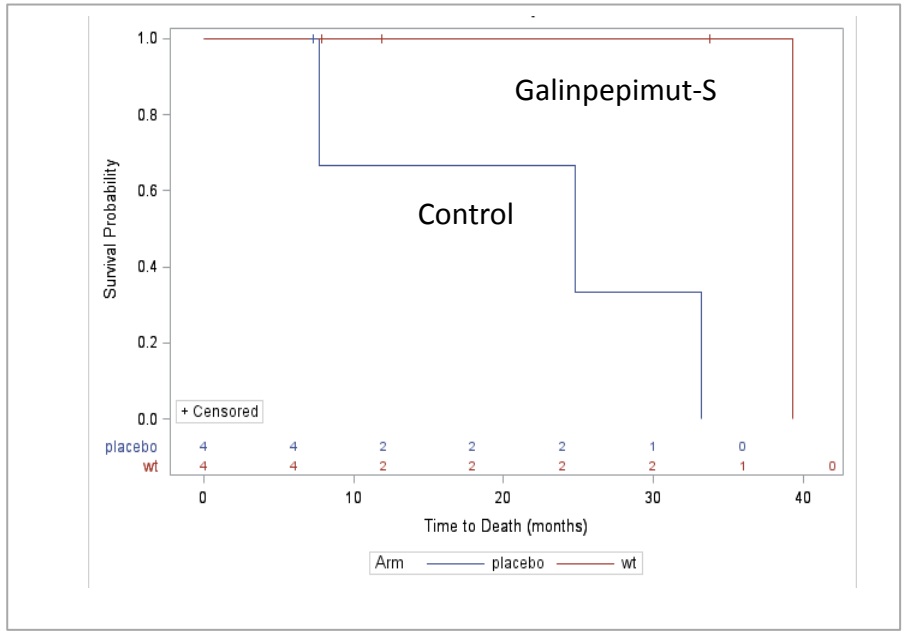
Galinpepimut-S was particularly effective in those who had a complete resection

Overall Survival (from date of resection)



- **Improved on vaccine:**
  - Median OS 19.9 mos. on control vs. 43.2 mos. on Galinpepimut-S

Overall Survival for Galipepimut vs Control in MCR (from date of resection)



- **Improved on vaccine:**
  - Median OS 24.8 mos for control vs 39.3 mos for Galinpepimut-S

DBL as of 19Nov15

*MCR = macroscopic complete resection

Galinpepimut-S induced immune responses in MPM

Immuno-response data available from 22 (11 in each arm) of 31 patients treated at MSKCC

- Vaccine arm: 2 of 3 HLA-A02 patients showed positive CD8 responses

  4 of 8 patients tested positive in a CD4 proliferation assay

- Control arm: 0 of 4 HLA-A02 patients showed a positive CD8 response

  1 of 8 patients tested positive in a CD4 proliferation assay

Galinpepimut-S is a promising new therapy for mesothelioma in combination with multimodality treatment

- Galinpepimut-S was well tolerated
  - AEs were mainly Grade 1 /2 at the site of injection
- Galinpepimut-S increased OS and PFS vs. control group
  - OS 21.4 vs 16.6 months & PFS 11.4 vs 5.7 months
- Minimal residual disease may be the optimal clinical setting
  - OS for MCR with Galinpepitum-S 39.3 vs 24.8 months in the control group
- Galinpepimut-S induced CD8+ and CD4+ T cell activation
- Recently granted Orphan Drug Designation by FDA and EMA
- Phase 3 study is planned to start in 3Q2016

Acknowledgments

- Patients and their families
- United States Department of Defense (Funding)
- Meso Foundation (Funding and patient referrals)
- SELLAS Life Sciences Group, Switzerland (Funding and Data Analysis)

Randomized Phase II Study of Adjuvant WT1 Vaccine (SLS-001) for Malignant Pleural Mesothelioma (MPM) after Multimodality Therapy – Updated Data

Abstract 8519

M. G. Zauderer[1,2], T. Dao[1], V. W. Rusch[1], M. S. Ginsberg[1], A. S. Tsao[3], R. Mehran[3], K. Panageas[1], D. A. Scheinberg[1], L. M. Krug*

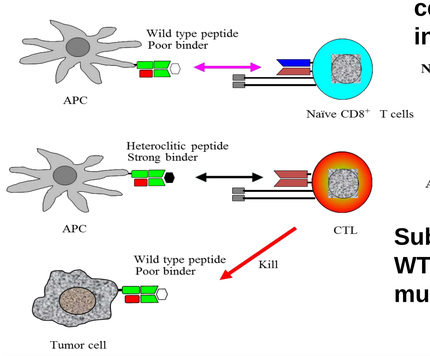
[1]Memorial Sloan Kettering Cancer Center; [2]Weill Cornell Medical College; [3]MD Anderson Cancer Center, *affiliation with MSK and WCMC during this project

Contact zauderem@mskcc.org for additional info

Supported by Department of Defense Grant W81XWH-10-1-0699 and the Meso Foundation

Background

WT1 is an ideal candidate for a tumor selective cancer vaccine in malignancies that express WT1, such as MPM. Using native and synthetic WT1 peptide sequences, a multivalent peptide vaccine, SLS-001 (galinpepimut-S), was created to stimulate both CD4 and CD8 T cell responses. In a pilot trial including those with previously treated MPM, SLS-001 was well-tolerated and CD4/8 immune responses were generated.



Subsequently, we began this trial in WT1 expressing MPM after multimodality therapy.

Study Schema

- MPM
- WT-1 positive by IHC
- 4-12 weeks end of treatment
- KPS ≥ 70%

N= 78 patients (39 per arm)

RANDOMIZE 1:1

Specific Immunotherapy x 6 (q2w):
- SLS-001 (800 µg/dose)
- Montanide (500 µl/dose)
- GM-CSF (70 µg/dose; d-2 d-0)

Control immunotherapy x 6 q2w):
- Montanide (500 µl/dose)
- GM-CSF (70 µg/dose; d-2 d-0)

NCT01265433

Patients followed with imaging every 3 months.
Primary endpoint = 1-year progression-free survival.
Closed early for futility analysis in each arm independently (>10 pts with progression <1 year).

Patient Characteristics

	SLS-001 (n=19)	Control (n=21)	Total (n=40)
Age (Mean ± SD)	67±11.17	65.71±8.39	66.35±9.7
Male (%)	84.2	85.7	85
Histology: Epithelioid	17 (89.5%)	20 (95.2%)	37 (92.5%)
Non-epithelioid	2 (10.5%)	0 (0%)	2 (5%)
KPS	0.87 ± 0.06	0.81 ± 0.07	0.84 ± 0.07
Known R0 resection	4 (21%)	4 (19%)	8 (20%)

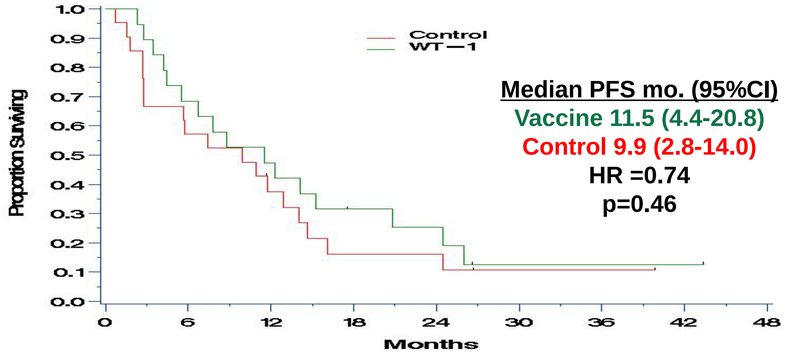
Common Adverse Events

	Grade 1/2 n (%)	Grade 3 n (%)
Injection site reaction	23 (57.9)	--
Fatigue	15 (37.5)	--
Fever	4 (10)	--
Arthralgias	2 (5)	--
Rash maculo-papular	2 (5)	--
Lymphocyte count decreased	--	2 (5)

Injection site reactions were common, mild, and self-limited.

Clinically significant severe events did not occur.

Progression-Free Survival



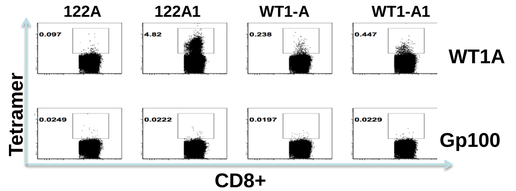
Median PFS mo. (95%CI)
Vaccine 11.5 (4.4-20.8)
Control 9.9 (2.8-14.0)
HR =0.74
p=0.46

Immunoresponse Data

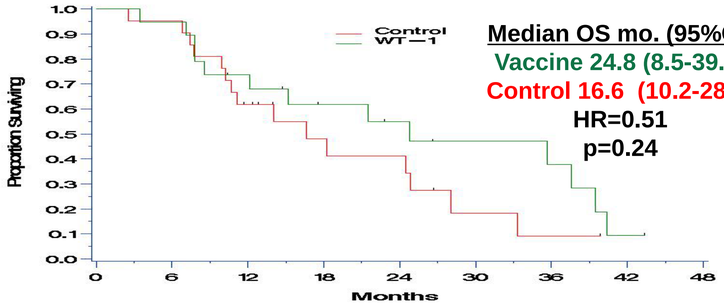
Data available for 22 patients (11 from each arm). An example of CD8 tetramer assay on right (highly specific for MHC allele and peptide).

Vaccine:
- 2/3 CD8+
- 4/8 CD4+

Control:
- 0/4 CD8+
- 1/8 CD4+



Overall Survival



Median OS mo. (95%CI)
Vaccine 24.8 (8.5-39.4)
Control 16.6 (10.2-28.1)
HR=0.51
p=0.24

Conclusions and Future Directions

Despite early stopping of the study, PFS and OS were promising.
SLS-001 is well tolerated and induced CD4/8 T cell activation.
SLS-001 (licensed by SELLAS Life Sciences Group) has obtained Orphan Drug Designation for MPM in EU/US. A pivotal study is to be started in 4Q2016.

References

Pinilla-Ibarz J. Leukemia 2006 Nov;20(11):2025-33.
Krug LM. Cancer Immunol Immunother 2010 Oct;59(10):1467-79.
Maslak PG. Blood 2010 Jul 15;116(2):171-9.
May RJ. Clin Cancer Res 2007 Aug 1;13(15 Pt1):4547-55.

A Randomized Phase II Trial of Adjuvant Galinpepimut-S, WT-1 Analog Peptide Vaccine, after Multimodality Therapy for Patients with Malignant Pleural Mesothelioma

Marjorie G. Zauderer[1], Anne S. Tsao[2], Tao Dao[3], Katherine Panageas[4], Victoria Lai[1], Andreas Rimner[5], Valerie W. Rusch[6], Prasad S. Adusumilli[6], Michelle S. Ginsberg[7], Daniel Gomez[8], David Rice[9], Reza Mehran[9], David A. Scheinberg[3,10], Lee M. Krug[1*]

[1]Department of Medicine, Division of Solid Tumor Oncology, Thoracic Oncology Service Memorial Sloan Kettering Cancer Center and Weill Cornell Medical College

[2]Depart of Thoracic/Head and Neck Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center

[3]Molecular Pharmacology Program, Sloan Kettering Institute

[4]Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center

[5]Department of Radiation Oncology, Memorial Sloan Kettering Cancer Center

[6]Department of Surgery, Memorial Sloan Kettering Cancer Center

[7]Department of Radiology, Memorial Sloan Kettering Cancer Center

[8]Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center

[9]Department of Surgery, The University of Texas MD Anderson Cancer Center

[10]Deparment of Medicine, Memorial Sloan Kettering Cancer Center

[*]Affiliation as above during this work. Presently at Bristol-Myers Squibb.

Supported by the Department of Defense Grant W81XWH-10-1-0699 (LMK & MGZ), the Mesothelioma Applied Research Foundation (DAS & LMK), NCI R01 CA55349 (DAS), NCI P01 CA23766 (DAS), The Lymphoma Foundation (DAS), Tudor Foundation (DAS), Sellas Life Sciences (MGZ & DAS), and the National Cancer Institute Core Grant P30 CA 008748 (all).

Corresponding Author:

Marjorie G. Zauderer

Co-Director MSK Mesothelioma Program

Assistant Attending

Memorial Sloan Kettering Cancer Center

300 East 66th Street, 1219

New York, NY 10065

T: 646-888-4656; F: 646-227-7276

Email: zauderem@mskcc.org

Running title: Adjuvant galinpepimut-s in malignant pleural mesothelioma

Word Count: 2972 (Tables: 3; Figures: 4 ; Supplementary Tables: 2; Supplementary Figures: 2)

Prior presentations: A preliminary analysis of this trial was presented at the International Mesothelioma Interest Group meeting in Birmingham, UK. Updated data were presented at the ASCO Annual Meeting 2016.

Abstract

Purpose: Determine the 1-year progression-free survival (PFS) among patients with malignant pleural mesothelioma (MPM) receiving the WT1 peptide vaccine galinpepimut-S after multimodality therapy vs those receiving control adjuvants.

Patients and Methods: This double-blind, controlled, two center phase II trial randomized MPM patients after surgery and another treatment modality to galinpepimut-S with GM-CSF and Montanide or GM-CSF and Montanide alone. An improvement in 1-year PFS from 50% to 70% was the predefined efficacy threshold, and 78 patients total were planned. The study was not powered for comparison between the two arms.

Results: 41 patients were randomized. Treatment related adverse events were mild, self-limited, and not clinically significant. Based on a stringent prespecified futility analysis (futility = $\geq$ 10 of 20 patients on one arm experiencing progression < 1 year), the control arm closed early. The treatment arm was subsequently closed because of the resultant unblinding. The PFS rate at 1 year from beginning study treatment was 33% and 45% in the control and vaccine arms, respectively. Median PFS was 7.4 months vs 10.1 months and median OS was 18.3 months vs 22.8 months in the control and vaccine arms, respectively.

Conclusion: The favorable safety profile was confirmed. PFS and OS were greater in those who received vaccine but the trial was neither designed nor powered for comparison between the arms. Based on these promising results, the investigators are planning a larger randomized trial with greater statistical power to define the optimal use and benefit of galinpepimut-S in the treatment of MPM.

Translational Relevance

The development of novel therapeutic strategies in malignant pleural mesothelioma (MPM) is dependent on exploiting its molecular aberrations. The high expression of WT-1 in most MPM and its absence in normal adult tissues make it a promising target for new treatments and, in particular, for a tumor selective vaccine. Here, we report the results of a randomized phase II evaluating a multivalent WT-1 peptide vaccine, galinpepimut-S, in the treatment of MPM after multimodality therapy. In addition to demonstrating a signal for efficacy, we show that the vaccine stimulates immune responses in certain individuals and an immune response was associated with improved survival, although this did not reach statistical significance. Based on these promising results, a large randomized phase III trial is planned for patients with WT-1 expressing mesothelioma.

Introduction

Malignant pleural mesothelioma (MPM) remains difficult to treat with only one FDA approved chemotherapy regimen (cisplatin and pemetrexed)[1] for patients with advanced disease. For patients with early stage disease, multimodality therapy is a preferred approach which includes cytoreductive surgery (such as extended pleurectomy/decortication), pemetrexed-based chemotherapy, and, in some cases, thoracic radiation.[2] However, even with this aggressive approach to early stage disease, the majority of patients experience recurrence due to persistent microscopic disease. Therefore, it is imperative that efforts continue to further improve outcomes.

One promising avenue involves exploiting the Wilms tumor-1 protein in MPM. In normal adult tissues, WT1 expression is limited, but WT1 is highly overexpressed in MPM as well as several other hematologic and solid tumors,[3] making it an ideal candidate for a tumor selective cancer vaccine in WT1 expressing malignancies. Although WT1 is a nuclear and cytoplasmic protein that functions as a transcription factor regulating genes involved in cellular proliferation, differentiation, apoptosis, organ development, and sex determination, the protein is processed by the proteasome and the derived peptides are presented on the cell surface making it an attractive target for immunotherapy.[4-8] WT1 was ranked as the top cancer antigen by a working group organized by the National Cancer Institute in 2009.[9]

Because WT1 is a self-antigen, overcoming immune tolerance is challenging and a potential obstacle in vaccine development. To address this, we enhanced the immunogenicity of WT1 by designing synthetic immunogenic peptide analogs that generate cross-reactivity to native peptides, known as a heteroclitic response. Single amino acid substitutions were introduced to

improve HLA-A*02:01 major histocompatibility complex binding affinity of two of the vaccine peptides. These new peptides had improved stability, elicited WT1 specific T cell recognition and cytotoxic T cell lymphocytes, and stimulated T cells to react with native WT1.[10] To provide immunogenicity over a broader range of HLA subtypes, and to elicit CD4 as well as CD8 responses, four WT1 peptides ranging in length from 9 to 22 amino acids (Supplementary Table 1) were combined into a vaccine, galinpepimut-S. All four peptides were shown to be immunogenic in preclinical studies and in pilot human trials.[11,12] Immunologic adjuvants (Montanide and GM-CSF) were co-administered as part of the vaccination regimen to retain the peptides at the injection site and to induce local inflammation near the peptide.

A prior pilot study to assess the safety, activity, and immunogenicity of galinpepimut-S included nine patients with MPM and 3 with NSCLC.[11] No severe toxicity was associated with treatment and immune responses occurred in a high proportion of patients. These results were the rationale for the subsequent randomized phase II trial of galinpepimut-S in MPM described herein. Of note, a similar pilot study in 9 patients with acute myeloid leukemia yielded similar safety and immunologic data.[13] Based on the data from these first two trials, we chose to evaluate galinpepimut-S in patients who have minimal disease burden after completion of multimodality therapy but remain at exceedingly high risk for recurrence.

Materials and Methods

This randomized, double-blinded, controlled phase II study of galinpepimut-S in patients with MPM after multimodality treatment (NCT 01265433) was reviewed and approved by the Institutional Review Boards at Memorial Sloan Kettering Cancer Center (MSK) and MD

Anderson Cancer Center (MDACC) as well as the Human Research Protection Office of the U.S. Army Medical Research and Material Command. The study was conducted in accordance with good clinical practice and followed the guiding principles of the Declaration of Helsinki, as well as local laws and regulations. Eligibility criteria were as follows: pathologically confirmed MPM, IHC positive for WT1 (clone WT49) in greater than 10% of cells, completion of multimodality therapy (including surgical resection by either pleurectomy/decortication or extrapleural pneumonectomy and chemotherapy or radiation therapy or both), 4 to 12 weeks elapsed since completion of multimodality therapy, age $\geq$18 years, Karnofsky Performance Status $\geq$ 70%, and adequate hematologic, renal, and hepatic function (ANC $\geq$ 1000/μL, platelets > 50 K/μL, total bilirubin $\leq$ 2.0 mg/dl, creatinine $\leq$ 2.0 mg/dl, and AST and ALT $\leq$2.5 x upper limits of normal). Exclusion criteria were pregnancy, active infection requiring systemic treatment, use of systemic corticosteroids, known immunodeficiency syndrome, other serious unstable medical illness, or another active cancer.

Treatment Plan

After obtaining written informed consent and confirmation of eligibility, patients were stratified by surgery type (extrapleural pneumonectomy vs pleurectomy/decortication) and clinical stage (I/II vs III/IV) and randomized to receive granulocyte-macrophage colony-stimulating factor (GM-CSF) 70 μg, Montanide 500 μg, and galinpepimut-S 800 μg (total weight; 200 μg of each of the 4 peptides within the mixture) versus the adjuvants only (GM-CSF 70 μg and Montanide 500 μg). Patients, caregivers, and investigators were blinded as to treatment arm. After injection teaching, GM-CSF 70 μg was self-administered 2 days prior and the day of each vaccine

treatment in the site of prospective vaccination on a limb. A series of 6 vaccines were given every 14 days (weeks 0, 2, 4, 6, 8, and 10, +/- 3 days). On treatment days, depending on randomization, nurses administered Montanide, GM-CSF and galinpepimut-S or Montanide and GM-CSF alone to the same anatomical site where the GM-CSF was administered 2 days prior. Patients were assessed at baseline, weeks 2, 6, and 12 and every 3 months for up to 2 years or until disease progression with history and physical examination. CT scans of the chest were performed at baseline, week 12, and every 3 months for 2 years or until disease progression and assessed using the modified RECIST for mesothelioma with reference study radiologists.[14] Toxicities were graded using the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) version 4.0.

Galinpepimut-S Formulation

Galinpepimut-S contains 4 peptides (Supplementary Table 1) that stimulate both CD4 and CD8.[10,12] For this study, galinpepimut-S was manufactured at AmbioPharm, Inc. and provided in a sterile solution with phosphate buffered saline. Each vial contained a final injectable dose of 200 μg of each peptide in a volume of 0.5 ml vial, overfill was 40%. Vialing under Good Manufacturing Practice conditions and sterility testing was performed by University of Iowa Pharmaceuticals. For administration, the 0.5 ml of vaccine was mixed with Montanide ISA 51 VG (Seppic Pharmaceuticals, Fairfield, NJ) in a 1:1 ratio and then vortexed in a Fisher Scientific vortex machine >3000 rpm for 12 minutes with the use of an attachment.

T-cell Immune Response Assays

Peripheral blood was collected for T cell immune proliferation assessment as well as gamma-interferon release as measured by ELISPOT at baseline and at week 12. All measurements were done in quadruplicate at each time point. A response was considered positive for reactivity with the test peptides if the result was at least 2-fold higher for the test peptides as compared to the control peptides, statistically significant with $p<0.05$, and a minimum number of spots were measured (>200 for CD4 and >30 for CD8).

Statistical Analysis

The primary endpoint of this trial was 1-year progression free survival (PFS) rate. PFS was calculated from the date of randomization to the date of progression, death, or last follow-up. Extrapolating from prior multicenter trials of neoadjuvant chemotherapy followed by extrapleural pneumonectomy and hemithoracic radiation (Supplementary Table 2), the 1-year PFS after multimodality therapy was expected to be 50%. An improvement in 1-year PFS to 70% was considered to be of interest in the vaccine arm. Thus, two parallel arms of single-stage design were employed to assess PFS at 1-year in each arm separately. For each arm, a 50% PFS rate at 12 months was defined as not promising and a 70% PFS rate at 12 months was considered promising. The probabilities of a type I and type II error were set at 0.10 and 0.10, respectively. Based on this, thirty-nine patients were planned for accrual to each arm. All patients who received at least one vaccination were considered evaluable in an intent-to-treat analysis. All patients were followed for a minimum of 12 months.

A stopping rule for futility was implemented such that accrual to an arm was to be stopped for futility if: $\underline{\geq}$ 7 of the first 10, $\underline{\geq}$10 of the first 20, or $\underline{\geq}$ 14 of the first 30 patients accrued

experience progression within 1 year. Overall survival was calculated from the date of randomization to the date of death or last follow-up. Survival distributions for each arm were estimated using Kaplan-Meier methodology. Exploratory comparisons between treatment arms were assessed using the logrank test.

Results

Patient Characteristics

Forty-six patients were consented to this protocol between May 2011 and August 2015. MD Anderson Cancer Center (MDACC) joined in May 2013. Three patients were unable to proceed with vaccine therapy due the development of radiation pneumonitis and the need for treatment with systemic corticosteroids. Two patients elected to withdraw consent prior to receipt of any study interventions. Forty-one patients were randomized to receive at least one dose of galinpepimut-S or control and were considered evaluable (Figure 1).

The characteristics of the 41 evaluable patients are listed in Table 1. Baseline characteristics were similar in the two arms, and typical for this patient population. The median age at enrollment was 68 (range 34 to 84) and the median KPS at enrollment was 80% (range 70 to 100%). As expected based on the eligibility requirement for WT1 expression, no sarcomatoid patients were included. Ninety-five percent of patients had purely epithelioid tumors, while 5% had tumors with mixed histology. All patients underwent some type of surgery: 7% extrapleural pneumonectomy; 15% extended pleurectomy decortication; 34% pleurectomy-decortication (removal of all gross tumor with a parietal and visceral pleurectomy but without diaphragmatic or pericardial resection); and 44% partial pleurectomy decortication (partial removal of parietal

and/or visceral and/or cases with residual gross tumor).[15] Forty-nine percent achieved a macroscopic complete resection (MCR defined as R0 or R1 resection). All but one patient received chemotherapy and all regimens contained pemetrexed and platinum. The vast majority of patients, 76%, received intensity modulated pleural radiation therapy (IMPRINT)[2,16] with 7% receiving a different type of radiation and 17% receiving no radiation prior to enrollment. Twenty patients were randomized to galinpepimut-S and 21 to the control arm. There were, on average, 61 days (range 29-181) between last treatment and beginning injections on this study. This time interval was not statistically different between vaccine and control groups or between types of surgery.

Progression-free and Overall Survival

Based on the protocol specified futility analysis and the recommendation of the Data Safety Monitoring Board, the control arm closed to accrual in May 2015. Subsequently, the vaccine arm was closed in November 2015 because there was no way to maintain blinding as both investigators and patients would know that the new enrolled participants were getting the active vaccine treatment. After all patients were on study for 1 year, the database was locked, patients were unblinded, and progression-free and overall survival were calculated. The progression-free survival (PFS) rate at 1 year from start of galinpepimut-S was 33% and 45% in the control arm and vaccine arm, respectively. Among the control patients, median PFS was 7.4 months (95% CI 2.8-14.6 months) and median overall survival (OS) was 18.3 months (95% CI 10.2-28 months). For the patients randomized to galinpepimut-S, median PFS was 10.1 months (95% CI 5.5—20.8 months) and median OS was 22.8 months (95% CI 9.1-37.6 months).

Although the study was not powered for comparison between the treatment arms, these exploratory analyses were performed (Figures 2 and 3) and revealed a hazard ratio for PFS of 0.78 (95% CI 0.4-1.5, p=0.46) and a hazard ratio for OS of 0.79 (95% CI 0.4-1.7, p=0.54). A subset analysis was performed for PFS and OS among the 20 patients who had a macroscopic complete resection (MCR), R0/1, (Figures 4A and B). Among the control patients with MCR, median PFS was 5.7 months (95% CI 0.69-14 months) and median OS was 16.6 months (95% CI 2.3-24.5 months). For patients randomized to galinpepimut-S with MCR, median PFS was 8.3 months (95% CI 2.3-24.5 months) and median OS was 22.8 months (95% CI 7.1-37.6 months).

Toxicities

Treatment related adverse events were mild and self-limited (Table 2). Injection site reactions were more common among those receiving vaccine compared to those receiving control injections with GM-CSF and Montanide alone, 85% versus 43%, all grade 1. Fatigue was comparable in both arms at 50% with galinpepimut-S and 48% with control injections. Interestingly, fever and arthralgias occurred only among those treated with control injections, while nausea occurred in 10% of those receiving galinpepimut-S. The two cases of lymphopenia were considered possibly related and, while grade 3, resolved without any intervention and there were no negative sequelae of this laboratory abnormality.

Immune Response

Data were available from 22 (11 in each arm) of the 41 patients for immunologic assessment (Table 3, Supplementary Figures 1A, 1B, and 2 include illustrative response data from patient 10). There were technical issues in maintaining fully viable cells arriving from MDACC at MSK

which precluded reliable analysis and therefore these samples are not included in the analyses. In the vaccine arm, 2 of 3 HLA-A*02:01 patients showed positive responses in an ELISPOT assay and in an MHC tetramer assay to the RMF (WT1A) peptide or the longer 122A peptide with the imbedded HLA-A*02:01 epitope (the latter being able to evoke both CD4 and CD8 immune responses by design). Four of 8 patients tested positive in a CD4 proliferation assay in response to 1 or more of the longer peptides. In the control arm, 0 of 4 HLA-A02 patients showed a response in the ELISPOT assay or the tetramer assay. One of 8 tested patients showed increased CD4 responses after vaccination. One patient was positive before vaccination and after vaccination had a reduced response. One other patient was positive before vaccination and after vaccination had no response. A fraction of patients have been reported to mount IgG and T cell responses to WT1 epitopes without vaccination.[17] In addition, the CD8 test involves repeated stimulation ex vivo and the WT1 peptides are self-peptides to which T cells may have been exposed and repeated stimulations can generate responses even in unvaccinated donors.[10,12]

As an exploratory analysis, the PFS and OS were examined in various subgroups related to their immune response (IR) to interrogate possible prognostic trends and to see if the patients in whom IR data were available differed from the group as a whole, thereby introducing bias. Patients who had enough cells to perform the IR tests tended to have longer median PFS, but not OS. Patients who were vaccinated and made a positive IR or patients who got vaccine and mounted no IR did not differ in their outcomes appreciably from the larger cohorts.

Discussion

Treatment options for patients with MPM remain limited and, despite aggressive multimodality therapy for early stage disease, MPM remains highly lethal. This randomized, double-blinded, controlled phase II trial evaluated the use of the analog WT1 peptide vaccine, galinpepimut-S, in patients who completed multimodality therapy to improve outcomes for MPM. The results confirmed earlier pilot trials in MPM and leukemia that administration of galinpepimut-S is safe, well-tolerated and feasible in the outpatient setting. Importantly, the data demonstrated that vaccine administration was associated with a non-statistically significant increase in PFS and OS (PFS HR 0.78 95% CI 0.4-1.5 p=0.46 and OS HR 0.79 95% CI 0.4-1.7 p=0.54). Median PFS and OS were 36% longer and 25% longer, respectively, in the galinpepimut-S arm as compared to the placebo arm. This pattern was also noted among patients who had a MCR prior to study enrollment. Importantly, the control group was well-matched, and received the same Montanide and GM-CSF doses, and had the same adverse effects from them, contributing to complete blinding of the patients and investigators, thereby minimizing possible alternative effects from investigator bias contributing to the PFS outcomes.

However, this pilot trial was not powered for comparison between the two treatment arms with the planned accrual of 78 patients and, due to the early closure, accrued only 53% of the planned sample. Additionally, because this is an understudied population with complex and variable initial therapies, the selection of 1 year PFS-rate was challenging and, in retrospect, the initially prespecified 50% threshold at 1 year from randomization was too high. Notably, survival in the historical controls (Supplementary Table 2) was calculated from the date of surgery. In contrast, the futility threshold in this study was based on the date of randomization which occurred, on average, 5 months after surgery. Thus, when the expectations for 12-month

PFS from the historical controls were applied to the initial design of this trial, the median lapse of 5 months between surgery and randomization on the clinical trial was not taken into account.

The interpretation of IR in this cohort was limited as half of patients did not have adequate samples suitable for IR analysis. Additionally, any association between IR and outcomes may be confounded by patient selection, in that those who had enough cells to perform the IR testings remained alive and healthy and without recurrence or subsequent therapy that could adversely impact IR analysis. Because the vaccinated patients did well regardless of whether an IR was capable of being measured, this suggests that the assays used were not sensitive enough to predict clinical response in this small sample or that technical issues precluded a significant analysis. It is also possible that patients mounted an immune response and lost it over the 12 weeks before they were retested.

In summary, PFS and OS were greater among MPM patients who received galinpepimut-S vaccination, among all patients and in particular among those who had a MCR. Because of the tolerance and excellent safety profile of galinpepimut-S, the immune response data in this and previous trials, and the observed survival patterns, the investigators have concluded that these results warrant additional randomized studies to help define the optimal use and benefit of galinpepimut-S in the treatment of MPM. A randomized phase II/III study of galinpepimut-S after multimodality therapy is planned.

References

1. Vogelzang NJ, Rusthoven JJ, Symanowski J, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2003; **21**(14): 2636-44.
2. Rimner A, Zauderer MG, Gomez DR, et al. Phase II Study of Hemithoracic Intensity-Modulated Pleural Radiation Therapy (IMPRINT) As Part of Lung-Sparing Multimodality Therapy in Patients With Malignant Pleural Mesothelioma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2016; **34**(23): 2761-8.
3. Mundlos S, Pelletier J, Darveau A, Bachmann M, Winterpacht A, Zabel B. Nuclear localization of the protein encoded by the Wilms' tumor gene WT1 in embryonic and adult tissues. *Development* 1993; **119**(4): 1329-41.
4. Amin KM, Litzky LA, Smythe WR, et al. Wilms' tumor 1 susceptibility (WT1) gene products are selectively expressed in malignant mesothelioma. *The American journal of pathology* 1995; **146**(2): 344-56.
5. Inoue K, Ogawa H, Sonoda Y, et al. Aberrant overexpression of the Wilms tumor gene (WT1) in human leukemia. *Blood* 1997; **89**(4): 1405-12.
6. Keilholz U, Menssen HD, Gaiger A, et al. Wilms' tumour gene 1 (WT1) in human neoplasia. *Leukemia* 2005; **19**(8): 1318-23.
7. Oji Y, Ogawa H, Tamaki H, et al. Expression of the Wilms' tumor gene WT1 in solid tumors and its involvement in tumor cell growth. *Japanese journal of cancer research : Gann* 1999; **90**(2): 194-204.
8. Rosenfeld C, Cheever MA, Gaiger A. WT1 in acute leukemia, chronic myelogenous leukemia and myelodysplastic syndrome: therapeutic potential of WT1 targeted therapies. *Leukemia* 2003; **17**(7): 1301-12.
9. Cheever MA, Allison JP, Ferris AS, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2009; **15**(17): 5323-37.
10. Pinilla-Ibarz J, May RJ, Korontsvit T, et al. Improved human T-cell responses against synthetic HLA-0201 analog peptides derived from the WT1 oncoprotein. *Leukemia* 2006; **20**(11): 2025-33.
11. Krug LM, Dao T, Brown AB, et al. WT1 peptide vaccinations induce CD4 and CD8 T cell immune responses in patients with mesothelioma and non-small cell lung cancer. *Cancer immunology, immunotherapy : CII* 2010; **59**(10): 1467-79.
12. May RJ, Dao T, Pinilla-Ibarz J, et al. Peptide epitopes from the Wilms' tumor 1 oncoprotein stimulate CD4+ and CD8+ T cells that recognize and kill human malignant mesothelioma tumor cells. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2007; **13**(15 Pt 1): 4547-55.
13. Maslak PG, Dao T, Krug LM, et al. Vaccination with synthetic analog peptides derived from WT1 oncoprotein induces T-cell responses in patients with complete remission from acute myeloid leukemia. *Blood* 2010; **116**(2): 171-9.
14. Byrne MJ, Nowak AK. Modified RECIST criteria for assessment of response in malignant pleural mesothelioma. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2004; **15**(2): 257-60.
15. Rice D, Rusch V, Pass H, et al. Recommendations for uniform definitions of surgical techniques for malignant pleural mesothelioma: a consensus report of the international association for the study of lung cancer international staging committee and the international mesothelioma interest group. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2011; **6**(8): 1304-12.

16. Rosenzweig KE, Zauderer MG, Laser B, et al. Pleural intensity-modulated radiotherapy for malignant pleural mesothelioma. *International journal of radiation oncology, biology, physics* 2012; **83**(4): 1278-83.
17. Gaiger A, Carter L, Greinix H, et al. WT1-specific serum antibodies in patients with leukemia. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2001; **7**(3 Suppl): 761s-5s.
18. Weder W, Kestenholz P, Taverna C, et al. Neoadjuvant chemotherapy followed by extrapleural pneumonectomy in malignant pleural mesothelioma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2004; **22**(17): 3451-7.
19. Rea F, Marulli G, Bortolotti L, et al. Induction chemotherapy, extrapleural pneumonectomy (EPP) and adjuvant hemi-thoracic radiation in malignant pleural mesothelioma (MPM): Feasibility and results. *Lung cancer* 2007; **57**(1): 89-95.
20. Weder W, Stahel RA, Bernhard J, et al. Multicenter trial of neo-adjuvant chemotherapy followed by extrapleural pneumonectomy in malignant pleural mesothelioma. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2007; **18**(7): 1196-202.
21. Batirel HF, Metintas M, Caglar HB, et al. Trimodality treatment of malignant pleural mesothelioma. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2008; **3**(5): 499-504.
22. Bolukbas S, Manegold C, Eberlein M, Bergmann T, Fisseler-Eckhoff A, Schirren J. Survival after trimodality therapy for malignant pleural mesothelioma: Radical Pleurectomy, chemotherapy with Cisplatin/Pemetrexed and radiotherapy. *Lung cancer* 2011; **71**(1): 75-81.
23. Krug LM, Pass HI, Rusch VW, et al. Multicenter phase II trial of neoadjuvant pemetrexed plus cisplatin followed by extrapleural pneumonectomy and radiation for malignant pleural mesothelioma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2009; **27**(18): 3007-13.
24. Van Schil PE, Baas P, Gaafar R, et al. Trimodality therapy for malignant pleural mesothelioma: results from an EORTC phase II multicentre trial. *The European respiratory journal* 2010; **36**(6): 1362-9.
25. Hasani A, Alvarez JM, Wyatt JM, et al. Outcome for patients with malignant pleural mesothelioma referred for Trimodality therapy in Western Australia. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2009; **4**(8): 1010-6.

Table 1

	All (N=41)		Galinpepimut-S (N=20)		Control (N=21)	
Characteristic	**N**	**%**	**N**	**%**	**N**	**%**
Age, years Median (range)	68 (34-84)		70 (34-84)		67 (48-79)	
Gender						
Male	35	85	17	85	18	86
Female	6	15	3	15	3	14
KPS at enrollment						
70%	4	10	1	5	3	14
80%	19	46	7	35	12	57
90%	17	41	11	55	6	29
100%	1	2	1	5	0	0
Smoking status						
Former	25	61	12	60	13	62
Current	0	0	0	0	0	0
Never	16	39	8	40	8	38
Histology						
Epithelioid	39	95	18	90	21	100
Mixed	2	5	2	10	0	0
Sarcomatoid	0	0	0	0	0	0
Surgery						
EPP	3	7	1	5	2	10
EPD	6	15	3	15	3	14
P/D	14	34	7	35	7	33
Partial PD	18	44	9	45	9	43
MCR						
Yes	20	49	10	50	10	48
No	21	51	10	50	11	52
Chemotherapy						
Pem/platinum	40	98	19	95	21	100
Other	0	0	0	0	0	0
None	1	2	1	5	0	0
Radiation						
Pleural IMRT	31	76	14	70	17	81
Other	3	7	2	10	1	5
None	7	17	4	20	3	14

Table 2

Event	Galinpepimut-S (N=20)		Control (N=21)	
	Any grade (%)	≥grade 3 (%)	Any grade (%)	≥grade 3 (%)
Injection site reaction	17 (85)	0 (0)	9 (43)	0 (0)
Fatigue	10 (50)	0 (0)	10 (48)	0 (0)
Fever	0 (0)	0 (0)	4 (19)	0 (0)
Arthralgias	0 (0)	0 (0)	2 (10)	0 (0)
Nausea	2 (10)	0 (0)	0 (0)	0 (0)
Rash, maculopapular	1 (5)	0 (0)	1 (5)	0 (0)
Lymphopenia	1 (5)	1 (5)	1 (5)	1 (5)

Table 3

	Vaccine N=10 (%)	**Control N=12 (%)**
CD4 ELISPOT		
***Positive**	4 (40)	1 (8)
***Negative**	4 (40)	8 (67)
***Not tested**	2 (20)	3 (25)
CD8 ELISPOT		
***Positive**	1 (10)	1 (8)
***Negative**	1 (10)	3 (25)
***Not tested**	8 (80)	8 (67)
Tetramer assay		
***Positive**	1 (10)	2 (17)
***Negative**	0 (0)	3 (25)
***Not tested**	9 (90)	7 (58)

Table 1. Patient, Disease, and Prior Treatment Characteristics

KPS = Karnofsky performance status; EPP = extrapleural pneumonectomy; EPD = extended pleurectomy decortication; P/D = pleurectomy decortication (removal of all gross tumor with a parietal and visceral pleurectomy but without diaphragmatic or pericardial resection); partial PD = partial pleurectomy decortication (partial removal of parietal and/or visceral and/or cases with residual gross tumor)

Table 2. Treatment-Related Adverse Events

Table 3. Immune Response Data

Figure Legends

Figure 1. Consort Diagram of the Phase II Randomized Study

Disposition of consented patients.

Figure 2. Progression-Free Survival

Kaplan-Meier plot of progression-free survival by treatment arm calculated from the time of randomization to progression, death, or censor date.

Figure 3. Overall Survival

Kaplan-Meier plot of overall survival by treatment arm calculated from the time of randomization to progression, death, or censor date.

Figure 4A. Progression-free survival among patients with macroscopic complete resection

Kaplan-Meier plot of progression-free survival by treatment arm among patients with macroscopic complete resections calculated from the time of randomization to progression, death, or censor date.

Figure 4B. Overall survival among patients with macroscopic complete resection

Kaplan-Meier plot of overall survival by treatment arm among patients with macroscopic complete resections calculated from the time of randomization to progression, death, or censor date.

Figure 1

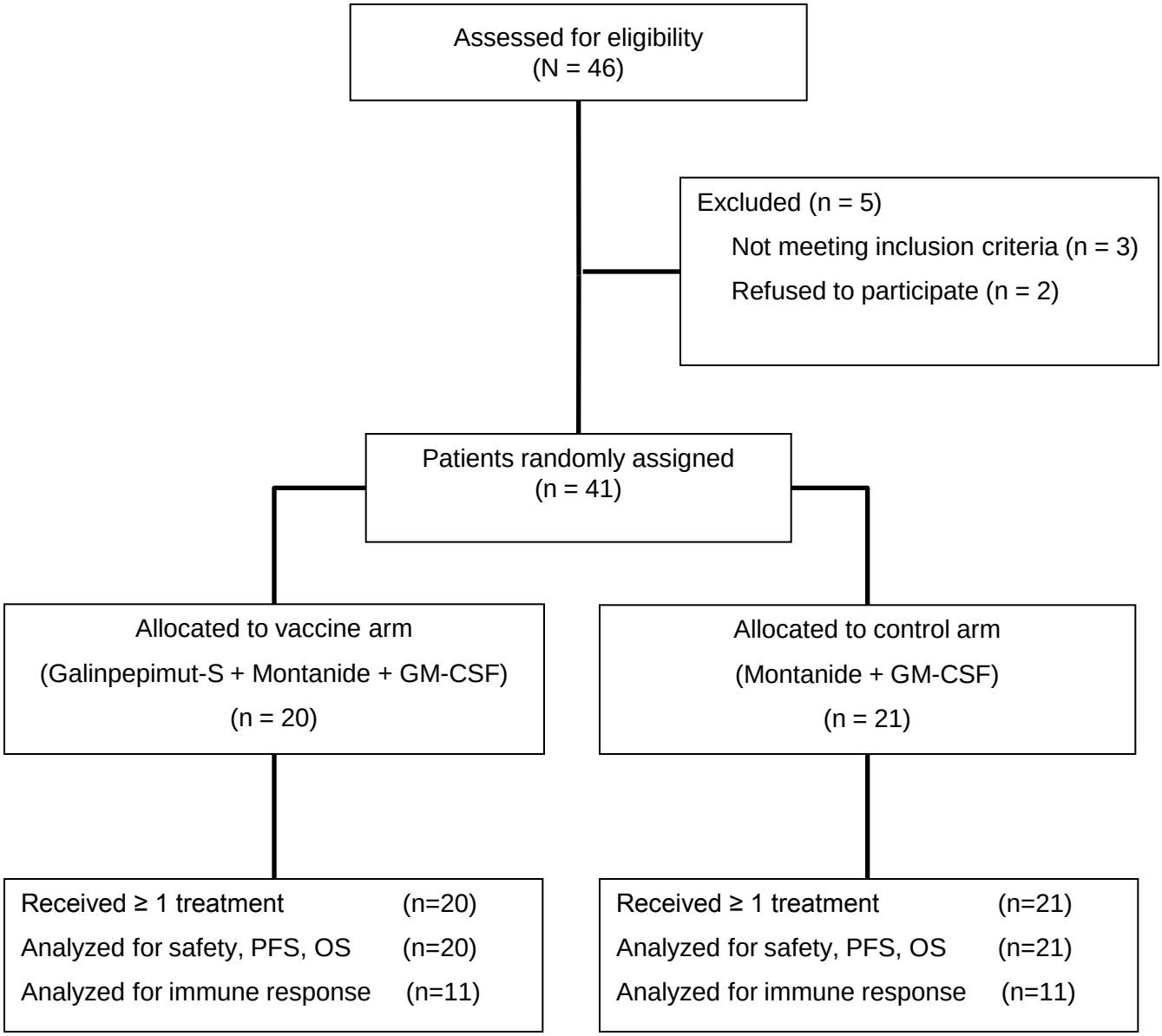

Assessed for eligibility
(N = 46)
Excluded (n = 5)
Not meeting inclusion criteria (n = 3)
Refused to participate (n = 2)
Patients randomly assigned
(n = 41)
Allocated to vaccine arm
(Galinpepimut-S + Montanide + GM-CSF)
(n = 20)
Allocated to control arm
(Montanide + GM-CSF)
(n = 21)
Received ≥ 1 treatment (n=20)
Analyzed for safety, PFS, OS (n=20)
Analyzed for immune response (n=11)
Received ≥ 1 treatment (n=21)
Analyzed for safety, PFS, OS (n=21)
Analyzed for immune response (n=11)

Figure 2

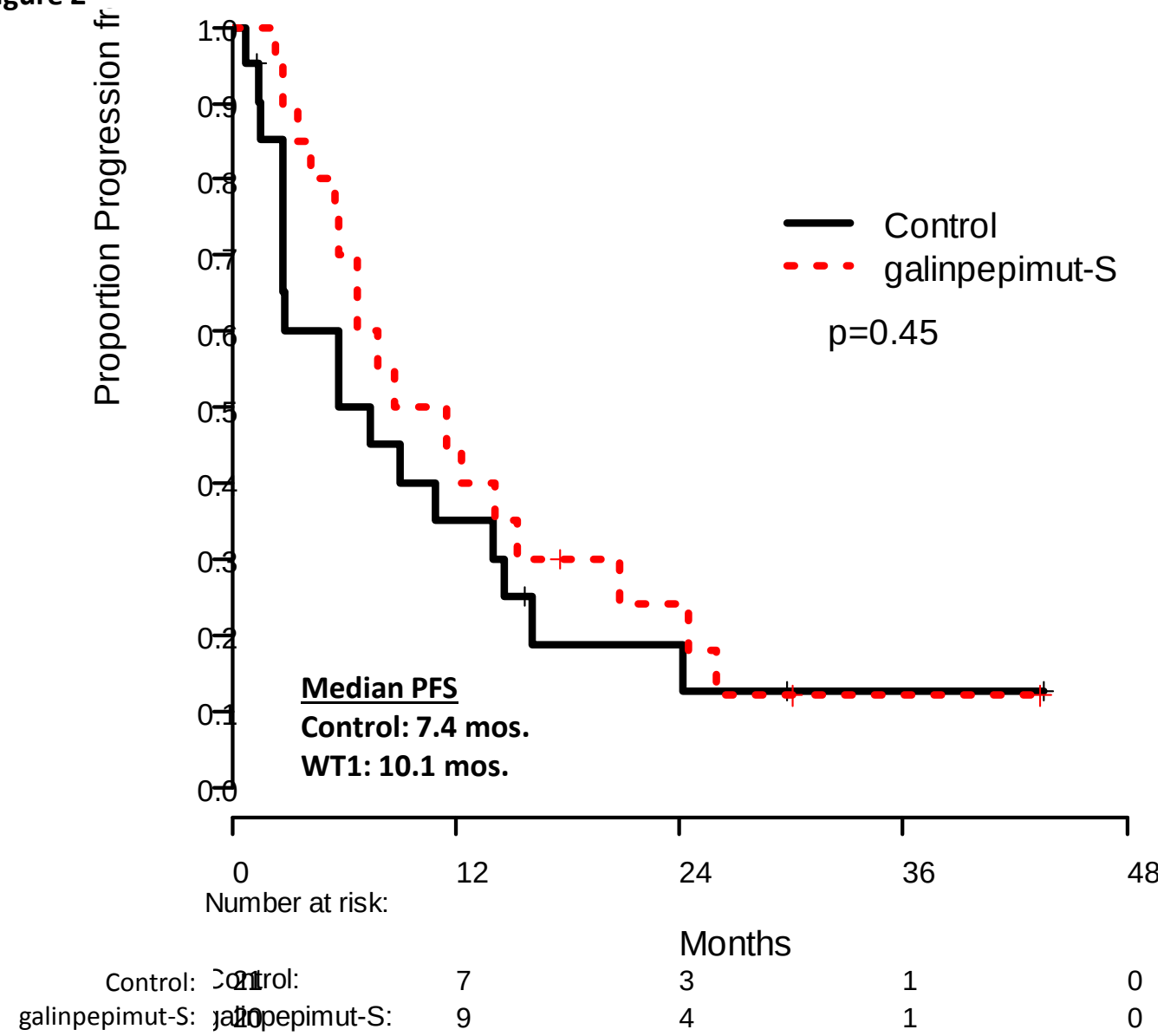

Proportion Progression fr
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Control
galinpepimut-S
p=0.45
Median PFS
Control: 7.4 mos.
WT1: 10.1 mos.
0
12
24
36
48
Months
Number at risk:
Control: 21 7 3 1 0
galinpepimut-S: 20 9 4 1 0

Figure 3

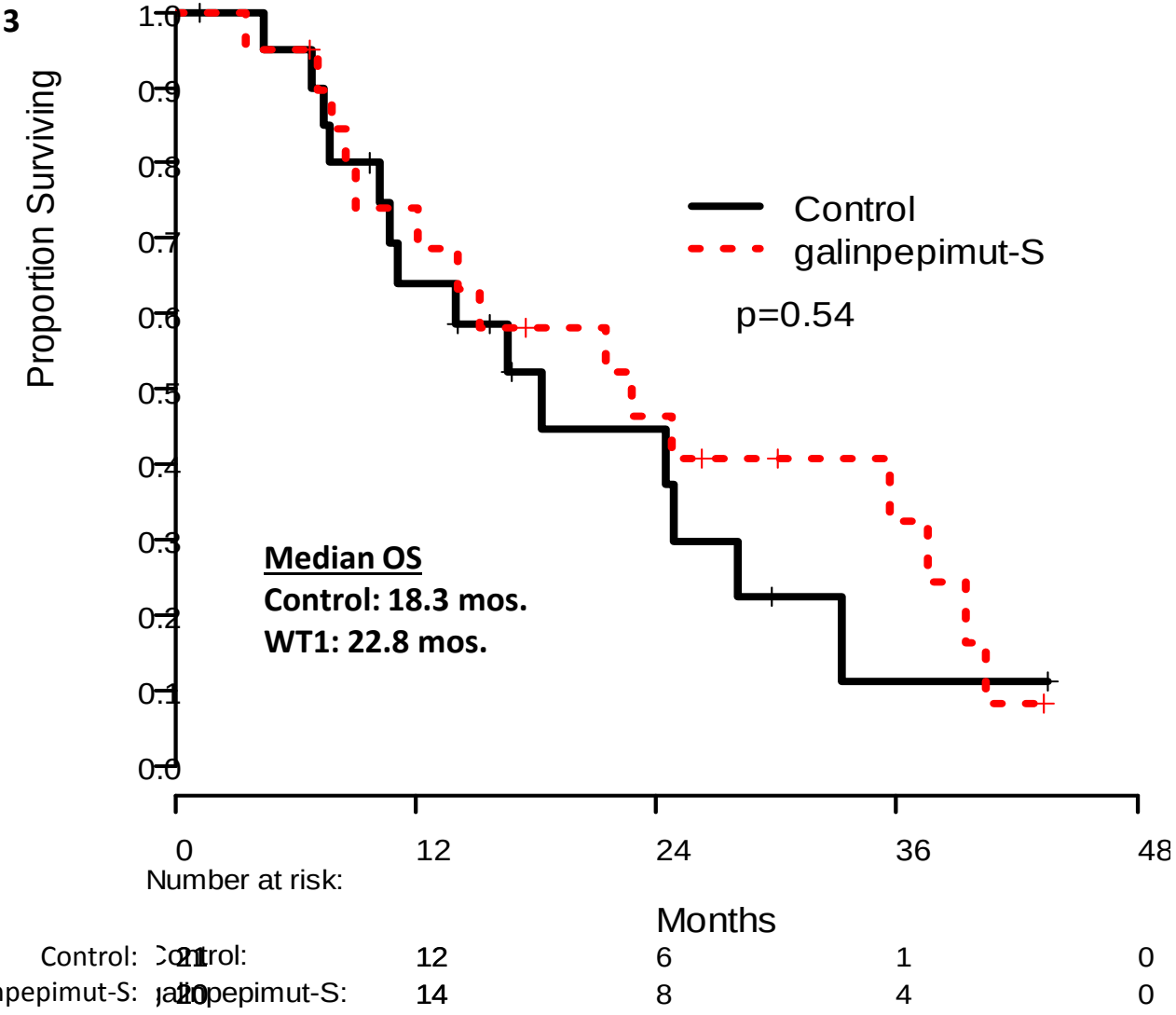

Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Control
galinpepimut-S
p=0.54
Median OS
Control: 18.3 mos.
WT1: 22.8 mos.
0
12
24
36
48
Months
Number at risk:
Control: 21 12 6 1 0
galinpepimut-S: 20 14 8 4 0

Figure 4a

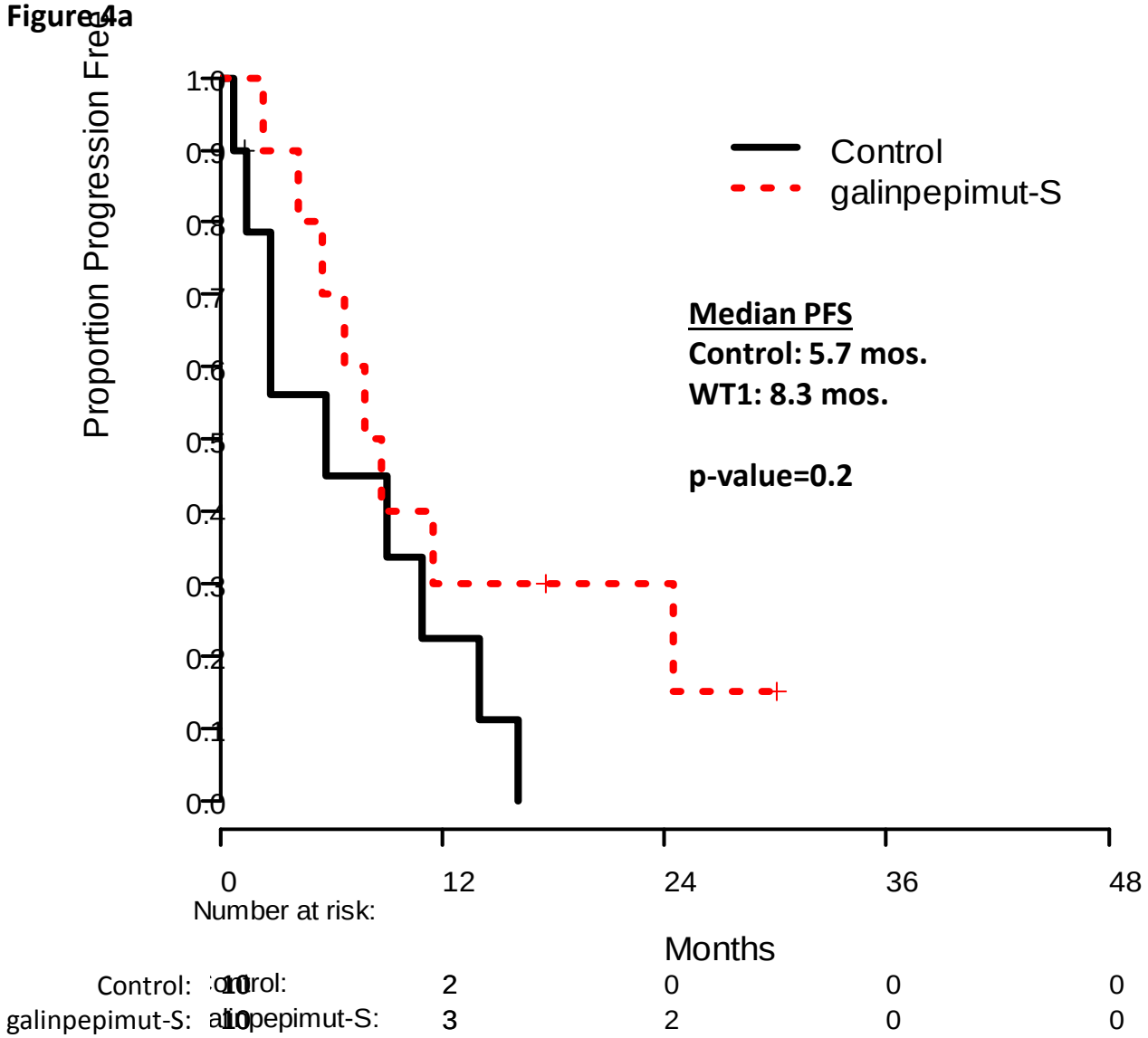
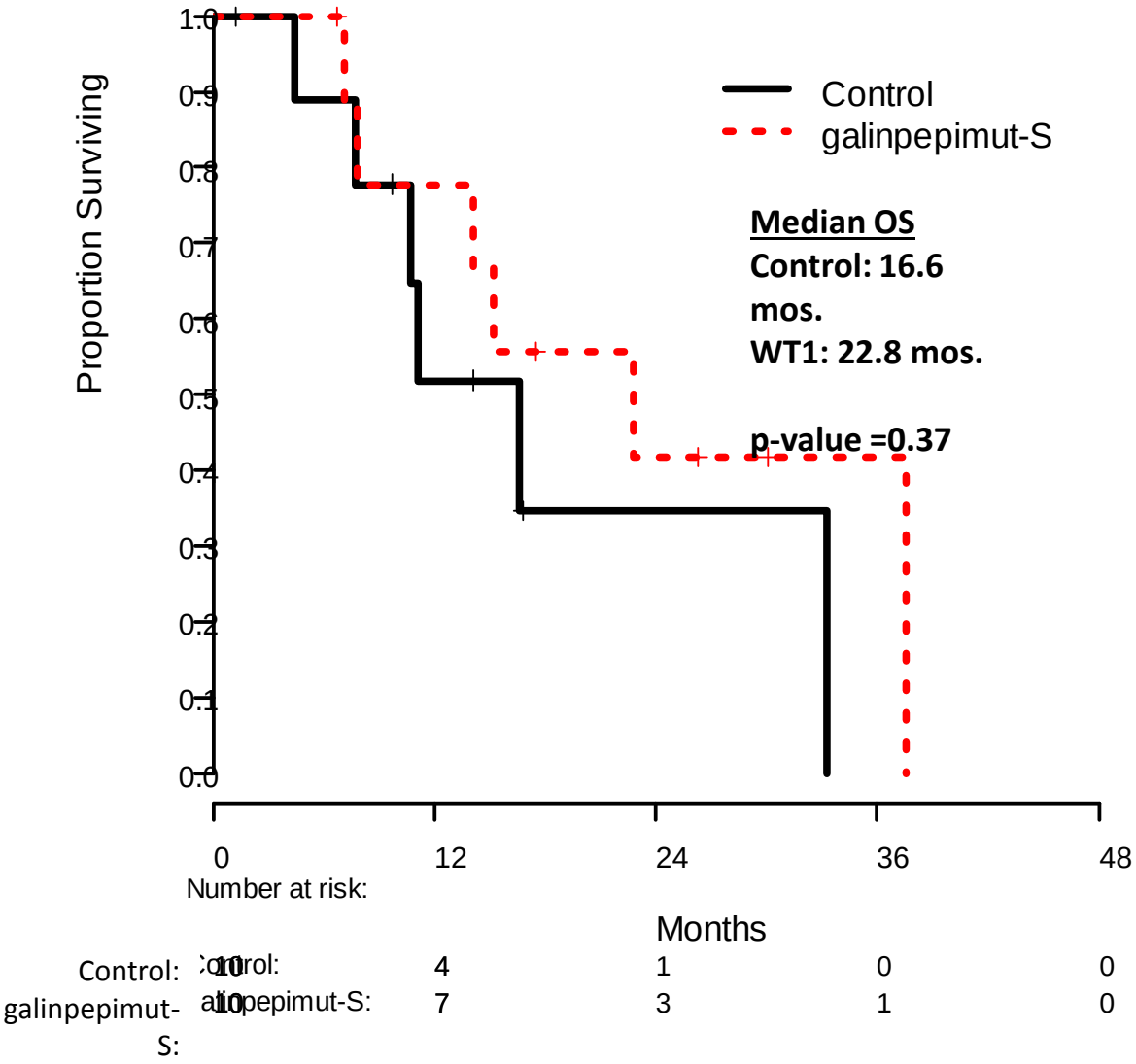

Proportion Progression Free
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Control
galinpepimut-S
Median PFS
Control: 5.7 mos.
WT1: 8.3 mos.
p-value=0.2
0
12
24
36
48
Months
Number at risk:
Control: 10 2 0 0 0
galinpepimut-S: 10 3 2 0 0

Figure 4b


Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Control
galinpepimut-S
Median OS
Control: 16.6 mos.
WT1: 22.8 mos.
p-value =0.37
0
12
24
36
48
Months
Number at risk:
Control: 10 4 1 0 0
galinpepimut-S: 10 7 3 1 0

Supplementary Table 1

Peptide name	HLA class	CD4/8 stimulation	Sequence
WT1-A1	I	CD8	***Y**MFPNAPYL
WT1-122A1 long	II	CD4 and CD8	*SGQA**Y**MFPNAPYLPSCLES
WT1-427 long	II	CD4	RSDELVRHHNMHQRNMTKL
WT1-331 long	II	CD4	PGCNKRYFKLSHLQMHSRKHTG

Supplementary Table 2

Author	Year	Number of patients	Median time to recurrence following surgery (months)
Weder[18]	2004	19	16.5
Rea[19]	2007	21	16.3
Weder[20]	2007	61	13.5
Batirel[21]	2008	20	10
Bolukbas[22]	2009	35	15.8
Krug[23]	2009	77	10.1
Van Schil[24]	2009	59	13.9
Hasani[25]	2009	36	12.5

Supplementary Table 1. Galinpepimut-S Peptide Sequences

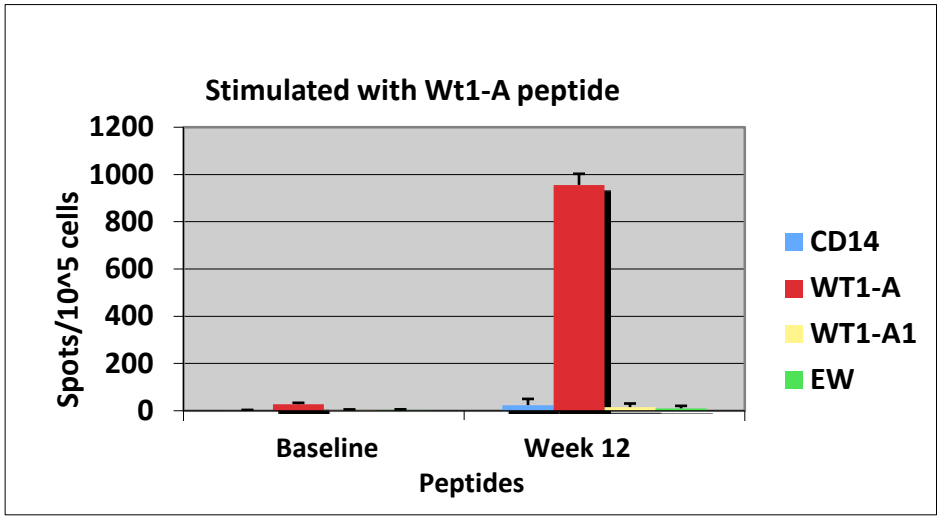
*Native peptide has R, not Y, at bolded location

The native sequences were mutated to increase affinity of these peptides to HLA molecules with longer binding of the epitopes in antigen-presenting cells and therefore higher stimulation of the T cells.

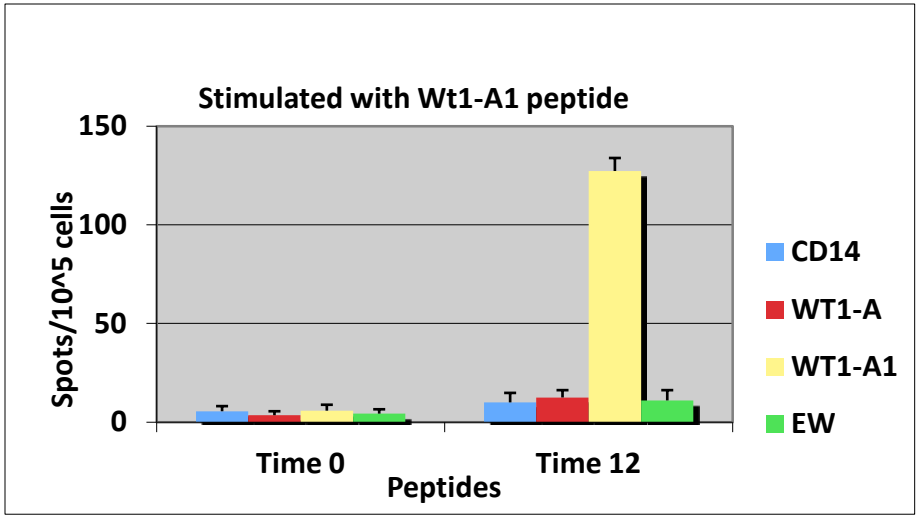
Supplementary Table 2. Prior Multicenter Trials of Neoadjuvant Chemotherapy, EPP, and Hemithoracic Radiation

EPP = extrapleural pneumonectomy

Supplementary Figure 1a



Supplementary Figure 1b



Supplementary Figure 2