Amendment

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** I have reviewed this research project and considered the NIH Policy for Inclusion of Women and Minorities in Clinical Research. Taking into account the overall impact that the project could have on the research field involved, I feel the current plans adequately includes both sex/ gender, minorities, children, and special populations, as appropriate. The current enrollment is in line with the planned enrollment report for inclusion of individuals on the basis of their sex/gender, race, and ethnicity and is appropriate and of scientific and technical merit.

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A Pilot/ Phase II Study of Pentostatin Plus Cyclophosphamide Immune Depletion to Decrease Immunogenicity of SS1P in Patients with Mesothelioma, Lung Cancer or Pancreatic Cancer

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A. Obtain information by intervening or interacting with living individuals for research purposes
B. Obtaining identifiable private information about living individuals
C. Obtaining the voluntary informed consent of individuals to be subjects
D. Makes decisions about subject eligibility
E. Studying, interpreting, or analyzing identifiable private information or data/specimens for research purposes
F. Studying, interpreting, or analyzing de-identified data or specimens for research purposes
G. Some/all research activities performed outside NIH

Drug Monitor:	Elad Sharon M.D., CTEP/DCTD/NCI
Drug Sponsor:	CTEP, NCI (status - administratively complete as of August 8, 2016)
NCI Supplied Agent:	SS1 (dsFv) PE38
IND #:	9052

PRÉCIS

Background:

Mesothelin is a cell surface glycoprotein present on normal mesothelial cells that is highly expressed in many human cancers including mesothelioma, lung and pancreatic adenocarcinoma. SS1 (dsFv) PE38 is a recombinant anti-mesothelin immunotoxin that has undergone phase I testing and has been evaluated in combination with pemetrexed and cisplatin for treatment of malignant pleural mesothelioma. SS1 (dsFv)PE38 is highly immunogenic and the majority of patients develop antibodies to it at end of one cycle. Pre-clinical studies demonstrate that SS1(dsFv)PE38 may be administered multiple times in combination with an immune-depleting regimen consisting of pentostatin and cyclophosphamide.

Objectives:

<u>Mesothelioma Pilot Objective</u>

- To assess the safety, tolerability, and feasibility of a conditioning regimen of pentostatin and cyclophosphamide in combination with SS1(dsFv)PE38
- To monitor antibody formation to SS1(dsFv)PE38 and to assess the impact of the conditioning regimen on the formation of these antibodies

<u>Mesothelioma Positive Cancers Dose De-escalation Pilot Objective</u>

- To determine the safety profile and recommended phase 2 dose of SS1P (dsFv)PE38 in drug lot FIL129J01 using dosing regimen A in patients with mesothelioma, lung and pancreatic adenocarcinoma

<u>Phase 2 and Lung and Pancreatic Adenocarcinoma Expansion Pilot Objective</u>

- To evaluate objective tumor response in subjects with pleural mesothelioma, peritoneal mesothelioma, lung and pancreatic adenocarcinoma using Regimen A

Eligibility:

- Patients with one of the following histologically confirmed malignancies:
  - malignant pleural or peritoneal mesothelioma with epithelial or biphasic tumors having less than a 50% sarcomatoid component who have previously been treated on at least one platinum-containing chemotherapy regimen with progressive disease documented prior to study entry
  - advanced (Stage IIIB/IV) lung adenocarcinoma who have had at least one prior chemotherapy for advanced disease. Patients who received an approved targeted therapy as first-line treatment should have also received chemotherapy prior to study entry.
  - recurrent, locally advanced unresectable or metastatic adenocarcinoma of the pancreas.
- Measurable disease by modified RECIST criteria for pleural mesothelioma or by RECIST criteria for peritoneal mesothelioma, lung adenocarcinoma and pancreatic adenocarcinoma

- Adequate renal, hepatic and hematopoietic function
- No major surgery, radiotherapy, chemotherapy or biologic therapy within 28 days of therapy

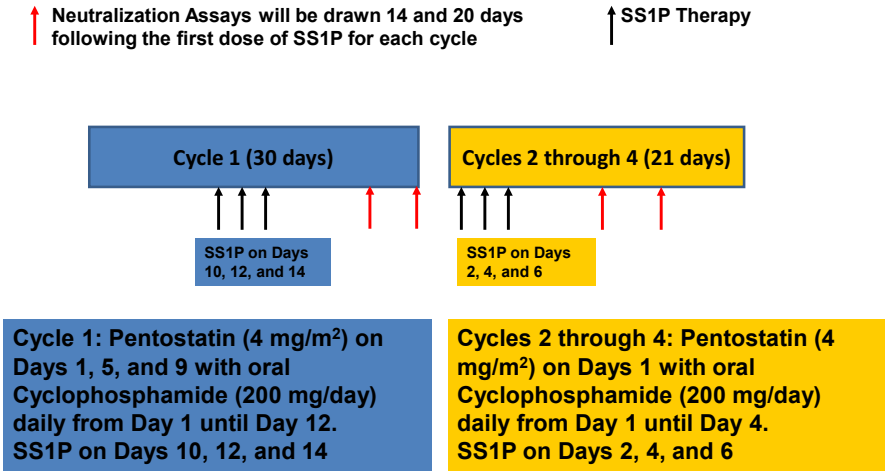
Design:

- During the mesothelioma pilot phase of this study, the first eleven mesothelioma patients enrolled in this study received a conditioning regimen of pentostatin on days 1, 5 and 9 of the first cycle and day 1 of subsequent cycles in combination with cyclophosphamide on days 1 through 12 of the first cycle and days 1 through 4 of subsequent cycles (Regimen A) while the next 8 mesothelioma patients received conditioning regimen of pentostatin on days 1, 5, 9, 13 and 17 of the first cycle and day 1 and 5 of subsequent cycles in combination with cyclophosphamide on days 1 through 20 of the first cycle and days 1 through 8 of subsequent cycles (Regimen B). SS1P was administered every other day for six days (3 doses) beginning on the day after the last pentostatin dose in each cycle for both regimens.
- In the mesothelin positive cancers dose de-escalation pilot study, a maximum of 12 patients with mesothelioma or lung or pancreatic adenocarcinoma will be enrolled in a 3+3 design to test up to 2 decreasing dose levels of SS1P administered in combination with cyclophosphamide and pentostatin on the Regimen A schedule for safety.
- In the phase 2 mesothelioma and pancreatic and lung adenocarcinoma pilot expansion portions of the study, a two-stage Minimax phase II trial design will be used to enroll up to 16 evaluable subjects with pleural mesothelioma (cohort 1), up to 10 evaluable subjects with peritoneal mesothelioma (cohort 2), up to 10 patients with lung adenocarcinoma (cohort 3) and up to 10 evaluable subjects with pancreatic adenocarcinoma (cohort 4) who will receive treatment on Regimen A.
- Treatment cycles will be repeated for up to four cycles if patients do not develop neutralizing antibodies, which will be assessed by a biological assay 14 and 20 days (+/- 2 days) following the first dose of SS1P in each cycle (corresponding to Days 24 and 30 of Cycle 1, and Days 16 and 22 of Cycles 2 through 4)
- Toxicity will be assessed by the CTEP Version 4.0 of CTCAE
- Tumor response assessments will be performed at the end of 2 cycles and at the end of treatment
- Tumor biopsies will be performed before treatment, after 2 cycles, and after the last cycle or at follow-up.

SCHEMA

REGIMEN A

Schema of a pilot study of pentostatin plus cyclophosphamide immune depletion to decrease immunogenicity of SS1P in patients with mesothelioma



Schedule of First Cycle

	Day 1 P C	Day 2 C	Day 3 C	Day 4 C	Day 5 P C	Day 6 C
Day 7 C	Day 8 C	Day 9 P C	Day 10 SS1P C	Day 11 C	Day 12 SS1P C	Day 13
Day 14 SS1P	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20
Day 21	Day 22	Day 23	Day 24 Neutralization Assay Drawn	Day 25	Day 26	Day 27
Day 28	Day 29	Day 30 Neutralization Assay Drawn				

Schedule of Cycles 2 through 4

			Day 1 **P** **C**	**Day 2** **SS1P** **C**	**Day 3** **C**	**Day 4** **SS1P** **C**
Day 5	**Day 6** **SS1P**	**Day 7**	**Day 8**	**Day 9**	**Day 10**	**Day 11**
Day 12	**Day 13**	**Day 14**	**Day 15**	**Day 16** **Neutralization Assay Drawn**	**Day 17**	**Day 18**
Day 19	**Day 20**	**Day 21**	**Day 22*** **Neutralization Assay Drawn**			

P=Pentostatin, C=Cyclophosphamide, SS1P=SS1(dsFv)PE38

*Note: Day 22 of Cycle 2 - 3 will correspond to Day 1 of Cycle 3 - 4 respectively.

TABLE OF CONTENTS

Précis ... 3
Schema ... 5
Regimen A ... 5
Table of Contents ... 7
1 OBJECTIVES ... 11
1.1 Mesothelioma Pilot Objectives ... 11
1.2 Regimen A Mesothelin Positive Cancers Pilot Dose De-Escalation Study (Lot FIL129J01) ... 11
1.3 Phase 2 Mesothelioma and Lung and Pancreatic Adenocarcinoma Pilot Expansion Cohorts Objectives ... 11
2 BACKGROUND ... 12
2.1 Peritoneal and Pleural Mesothelioma ... 12
2.2 Lung Adenocarcinoma ... 15
2.3 Pancreatic Adenocarcinoma ... 18
2.4 Host Immune Depletion With Pentostatin and Cyclophosphamide ... 20
2.5 Rationale for Increasing the Dose Intensity of Pentostatin and Cyclophosphamide (Regimen B) ... 27
2.6 Rationale for Discontinuation of Regimen B and expansion of Regimen A ... 29
2.7 Rationale for Dose De-Escalation Study of Drug Lot FIL129J01 ... 32
2.8 SS1(dsFv) PE38 lot transition ... 33
2.9 Correlative Studies Background ... 34
3 PATIENT SELECTION ... 34
3.1 Eligibility Criteria ... 34
3.2 Inclusion of Women and Minorities ... 37
3.3 Table 7: Accrual Targets ... 37
3.4 On-Study Research Evaluation ... 38
4 PATIENT REGISTRATION ... 39
4.1 Registration Process ... 39
5 TREATMENT PLAN ... 39
5.1 Overview ... 39
5.2 Agent Administration ... 45
5.3 SS1 (dsFv) PE38 ... 45

5.4 Pentostatin ... 45
5.5 Cyclophosphamide ... 47
5.6 Definition of Dose-Limiting Toxicity (DLT) ... 48
5.7 Staggering and Opening of New Cohorts (Mesothelioma Pilot only) ... 48
5.8 General Concomitant Medication and Supportive Care Guidelines ... 49
5.9 Duration of Therapy ... 51
5.10 Duration of Follow Up ... 52
5.11 Criteria for removal from Study ... 52
5.12 Off Protocol Therapy and Off-Study Procedure ... 52
6 DOSING DELAYS/DOSE MODIFICATIONS ... 52
6.1 General Guidance ... 52
6.2 Dose Modifications for Pentostatin and Cyclophosphamide ... 53
6.3 SS1(dsFv)PE38 dose modification ... 54
6.4 Re-Treatment and Dose Modification ... 55
7 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS ... 55
7.1 Definitions ... 55
7.2 Adverse Event Reporting to CTEP (NA as of CTEP status – administratively complete) ... 57
7.3 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs) ... 58
7.4 Adverse Event Characteristics ... 62
7.5 Expedited Adverse Event Reporting (NA as of CTEP status – administratively complete) ... 63
7.6 Routine Adverse Event Reporting (NA as of CTEP status – administratively complete) ... 65
7.7 NCI-IRB and Clinical Director (CD) Reporting ... 65
7.8 Adverse Event Follow-up Duration ... 66
7.9 Pregnancy ... 66
8 PHARMACEUTICAL INFORMATION ... 67
8.1 CTEP-Supplied Investigational Agents ... 67
8.2 CYCLOPHOSPHAMIDE (CTX, Cytoxan, NSC-26271) ... 69
8.3 FILGRASTIM (G-CSF, Neupogen®) ... 70
8.4 PENTOSTATIN (NIPENT®; 2'-deoxycoformycin) ... 71
8.5 HYDROXYZINE HYDROCHLORIDE ... 72

8.6 RANITIDINE ... 72
8.7 ACYCLOVIR (ZOVIRAX®) ... 73
8.8 TRIMETHOPRIM/SULFAMETHOXAZOLE (TMP/SMX, Cotrimoxazole, Bactrim, Septra) ... 73
8.9 Dexamethasone (Decadron® Oral Tablets) ... 74
8.10 Ondansetron (Zofran®) ... 75
9 CORRELATIVE/SPECIAL STUDIES ... 75
9.1 Laboratory Correlative Studies ... 76
9.2 Immune Laboratory Studies ... 80
9.3 Tumor Biopsy ... 81
9.4 Sample Storage, Tracking and Disposition ... 81
10 STUDY CALENDAR ... 82
11 MEASUREMENT OF EFFECT ... 82
11.1 Antitumor Effect ... 82
11.2 Peritoneal Mesothelioma, Lung Adenocarcinoma and Pancreatic Adenocarcionoma Measurements ... 83
11.3 Pleural Mesothelioma ... 87
11.4 Reporting of Results ... 89
11.5 Other imaging studies - PET Scans ... 89
12 DATA COLLECTION /REPORTING / REGULATORY CONSIDERATIONS ... 89
12.1 Data Collection ... 90
12.2 Data Reporting ... 90
12.3 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA) ... 90
13 STATISTICAL CONSIDERATIONS ... 90
13.1 Study Design/Endpoints ... 90
13.2 Sample Size/Accrual Rate ... 94
13.3 Analysis of Secondary Endpoints ... 95
13.4 Reporting and Exclusions ... 96
14 HUMAN SUBJECTS PROTECTIONS ... 96
14.1 Rationale for Subject Selection ... 96
14.2 Justification for Exclusions ... 97
14.3 Participation of Children ... 97

14.4 Participation of Subjects Unable to Give Consent 97

14.5 Adults unable to give consent are excluded from enrolling in the protocol and as study activities are now limited to long term follow-up from which subjects derive no direct benefit, they are excluded from continued participation in the event that they lose capacity to consent during the study.Evaluation of Benefits and Risks/Discomforts 97

14.6 Consent Process and Documentation 97

15 DATA AND SAFETY MONITORING PLAN 99

16 REFERENCES 100

17 APPENDICES 104

17.1 Appendix A: Performance Status Criteria 104

17.2 Appendix B: Staging System for Pleural Mesothelioma 105

17.3 Appendix C: Regimen A Study Calendar of Events 107

17.4 Appendix D: Estimated Creatinine Clearance by Cockroft-Gault Formula 111

17.5 Appendix E: Medication Diary for Cyclophosphamide 112

1 OBJECTIVES

1.1 MESOTHELIOMA PILOT OBJECTIVES

1.1.1 Primary Objectives

- To assess the safety, tolerability, and feasibility of a conditioning regimen of pentostatin and cyclophosphamide in combination with SS1(dsFv)PE38
- To monitor antibody formation to SS1(dsFv)PE38 and to assess the impact of the conditioning regimen on the formation of these antibodies

1.1.2 Secondary Objectives

- To evaluate the objective tumor response, duration of response, and progression-free survival
- To investigate the potential of soluble mesothelin levels to predict any therapeutic response
- To study the clinical pharmacology (pharmacokinetics) of SS1(dsFv)PE38
- To evaluate intra-tumoral immune response using tissue biopsies

1.2 REGIMEN A MESOTHELIN POSITIVE CANCERS PILOT DOSE DE-ESCALATION STUDY (LOT FIL129J01)

1.2.1 Primary

- To determine the safety profile and recommended phase 2 dose of SS1P (dsFv)PE38 in drug lot FIL129J01 using dosing regimen A in patients with mesothelioma, lung and pancreatic adenocarcinoma

1.3 PHASE 2 MESOTHELIOMA AND LUNG AND PANCREATIC ADENOCARCINOMA PILOT EXPANSION COHORTS OBJECTIVES

1.3.1 Primary Objective

- To evaluate objective tumor response (PR+CR) in subjects with pleural mesothelioma and with peritoneal mesothelioma.
- To evaluate objective tumor response (PR+CR) in subjects with pancreatic adenocarcinoma and lung adenocarcinomausing Regimen A of the study using drug lot FIL129J01

1.3.2 Secondary Objectives

- To assess duration of response, progression free survival and overall survival in subjects with pleural and with peritoneal mesothelioma on Regimen A
- To assess degree of tumor shrinkage by RECIST criteria in subjects with adenocarcinoma of the lung
- To evaluate response to post-study chemotherapy
- To evaluate intra-tumoral immune response using tissue biopsies

- To study the clinical pharmacology (pharmacokinetics) of SS1(dsFv)PE38
- To investigate the potential of soluble mesothelin levels to predict any therapeutic response
- To further characterize antibody formation to SS1(dsFv)PE38 and to assess the impact of the conditioning regimen on the formation of these antibodies

2 BACKGROUND

2.1 PERITONEAL AND PLEURAL MESOTHELIOMA

Malignant mesothelioma is a rare and lethal disease, which is known to arise in the serosal surfaces of the pleural and peritoneal cavities, and rarely, in the pericardial cavity or the tunica vaginalis. Mesothelioma accounts for 0.10% of deaths annually in the United States.[1] Malignant pleural mesothelioma is the most common of these, comprising of 80% of the cases and an annual incidence of about 2,500 in the United States. The median survival from diagnosis of pleural mesothelioma is approximately 12 months, whereas peritoneal mesothelioma can be considerably longer.[2,3] The most important risk factor for the development of pleural mesothelioma is asbestos exposure. As it was first noted among workers with occupational asbestos exposure three or four decades prior to disease occurrence, mesothelioma is known as a sentinel disease for asbestos exposure. However, mesothelioma can occur as the result of other mineral exposures (such as erudite), therapeutic radiation, and, possibly inflammation. According to an analysis of SEER data in 2008, about 58% of cases are estimated to have asbestos as a cause.[1]

In pleural mesothelioma, the majority of patients present with stage III or IV disease, and 85-90% of patients are considered unresectable at diagnosis. Males and patients with sarcomatoid histologic findings have worse prognoses, along with those presenting with extensive disease, poor performance status, elevated leukocyte counts, anemia, and thrombocytosis.[4,5] Treatment options for pleural mesothelioma include palliative surgery or radiotherapy, and chemotherapy. While such trimodality therapy has shown some long-term survival of selected patients, a recent case series examining trimodality therapy in Australia failed to show a benefit for patients receiving extrapleural pneumonectomy.[6] The approved first-line combination chemotherapy regimen in the U.S. is pemetrexed and cisplatin, a regimen that has been shown to increase survival time compared to cisplatin alone.[3] In a randomized phase III study, 226 patients received pemetrexed and cisplatin while 222 patients received cisplatin alone. The response rates were 41.3% in the pemetrexed/cisplatin arm versus 16.7% in the control arm ($p<.0001$). The median overall survival time in patients treated with pemetrexed and cisplatin was 12.1 months versus 9.3 months in patients treated with cisplatin alone ($P=.020$, two-sided log-rank test). Treatment with other chemotherapeutic agents, alone or in combination, also plays a significant role worldwide. However, to date, no additional regimen has shown a significant effect on survival.[7,8] There remains a need for second-line regimens to assist in improving outcomes for patients with this deadly disease.

As noted, peritoneal mesothelioma is a much rarer diagnosis, with perhaps 250 patients diagnosed in the United States every year.[9] Similar to ovarian cancer, patients can present with non-specific symptoms of abdominal distension, pain, bloating, changes in bowel habits, weight loss, ascites, and fever.[10] The non-specific nature of symptoms helps explain why patients can

have signs and symptoms for months before a proper diagnosis is made.[11] Case series at a variety of centers have shown improved survival and symptom reduction with operative cytoreduction and continuous hyperthermic peritoneal perfusion (CHPP).[12-15] Patients who relapse or are not considered candidates for surgical debulking are often treated with agents found to be useful in pleural mesothelioma, most significantly, pemetrexed and cisplatin.[11,16] Prognosis has been found to vary widely in peritoneal mesothelioma with a retrospective analysis of 25 patients exhibiting the differences. Amongst 25 patients analyzed, patients surviving less than four years had a median survival of around 12 months. However, patients surviving greater than four years had a median survival of 7 years.[17] Patients with resectable disease who undergo extensive cytoreductive surgery and CHPP have also been shown to have prolonged survival of greater than 5 years.[12,15] Despite this improved prognosis relative to pleural disease, patients undergoing therapy for peritoneal mesothelioma have few well-studied treatment options due in large part to the rare incidence of the disease. Nonetheless, an improved prognosis overall, coupled with treatment options, has led to an overall mesothelioma population enriched with peritoneal patients. The peritoneal mesothelioma population would thus benefit from research into agents with potential therapeutic benefits for this rare disease.

2.1.1 Rationale for Using SS1(dsFv)PE38 in Mesothelioma

Mesothelioma is an aggressive disease with poor prognosis. Combination treatment with cisplatin and pemetrexed is the only FDA approved regimen for this disease. Since almost all patients progress after initial treatment with this regimen they are candidates for second-line therapy. There is no clearly defined standard for treating these patients and therefore there is an urgent need to develop more efficacious treatments for patients with peritoneal and pleural mesothelioma who have already received chemotherapy.

Mesothelin is a 40-kDa cell surface glycoprotein that is highly expressed in mesothelioma, ovarian, pancreatic and some other cancers.[11,18,19] Within malignant pleural mesothelioma, the expression of mesothelin is closely related to the histological subtype of the tumor, being expressed on the epithelial but not sarcomatous tumor tissues. On biphasic tumors, mesothelin is expressed on the epithelial component. Small amounts of mesothelin shed into the circulation may be detected by sandwich ELISA. It has been suggested that elevated soluble mesothelin levels may correlate with advanced disease stage and total tumor burden. The biological function of mesothelin is unknown. A trans-intracellular binding activity with CA125, a tumor antigen routinely used in the diagnosis and monitoring of ovarian cancer has been noted.[20] Among normal human tissues mesothelin is expressed on mesothelial cells of the pleura, pericardium, and peritoneum, but is absent from vital organs including heart, liver, lung, kidney and nervous tissue.

SS1(dsFv)PE38 (SS1P) is a high affinity (KD = 0.72 nM) anti-mesothelin disulfide-stabilized murine-antibody Fv genetically combined with PE38.[21] PE38 is a fragment of the potently cytotoxic *Pseudomonas* exotoxin from which the native cell-binding domain and other unnecessary sequences have been removed. The chimeric recombinant immunotoxin, SS1(dsFv)PE38, kills mesothelin-expressing cells but not similar cells that do not express detectable levels of mesothelin. SS1(dsFv)PE38 was created at the U.S. National Cancer Institute's Laboratory of Molecular Biology (NCI). SS1(dsFv)PE38 has been studied as a single agent in two phase one cancer chemotherapy trials. The protocol whose regimen is being utilized is summarized below:

A phase I study of SS1(dsFv)PE38 was conducted to determine the toxicities, maximum tolerated dose (MTD) and pharmacokinetics of the recombinant immunotoxin SS1P (anti-mesothelin dsFv-PE38) in patients with mesothelin-expressing cancers. The study drug was given as a 30-min i.v. infusion every other day (QOD) for six or three doses and was administered to 34 patients with advanced mesothelioma (n = 20), ovarian (n = 12), and pancreatic (n = 2) cancer. The initial cohort of 17 patients received SS1P QOD x 6 doses and the MTD was 18 micrograms/kg/dose. Dose-limiting toxicities (DLT) included grade 3 urticaria (n = 1) and grade 3 vascular leak syndrome (n = 2). To allow further SS1P dose escalation, 17 patients were treated on the QOD x 3 schedule and the MTD was 45 microg/kg/dose. The DLT was grade 3 pleuritis and was seen in two of two patients treated at a dose of 60 microg/kg/dose and in one of nine patients treated at a dose of 45 microg/kg/dose. At the MTD of 45 micrograms/kg, the mean C(max) of SS1P was 483 ng/mL and half-life was 466 min. Ultimately, 33 of the patients were considered evaluable. Of those, 4 had minor responses, 19 had stable disease (including 2 with resolution of ascites), and 10 had progressive disease. Following that study, it was determined that SS1(dsFv)PE38 was well tolerated with pleuritis as the DLT at the highest dose level. Evidence of clinical activity was noted in a group of heavily pretreated patients. Of note, serum was collected during the trial to determine the prevalence of neutralizing antibodies at the start of the trial. According to those results, none of the patients had neutralizing antibodies before treatment. Immunogenicity was tested with Day 21 neutralization assays that showed 30 of 34 treated patients (88%) had at least 75% neutralization of the study drug. Of the 34 patients enrolled in the study, 32 (94.1%) tested positive for serum antibody to SS1(dsFv)PE38 at some point during the first two treatment courses. The majority of treated subjects 23 (67.6%) developed antibodies by Day 15 of treatment.[22]

Neutralizing antibody formation has limited the potential efficacy of SS1(dsFv)PE38, denying patients the possibility of benefit due to the effect of neutralization. While this is a limitation in treatment with SS1(dsFv)PE38, there are numerous protein-based therapies which may have a therapeutic effect for a wide variety of malignancies. However, in the early history of biologic therapy development, an immediate limitation was the development of an immune response to the protein. In particular, mouse antibodies led to a host immune response, known as human anti-mouse antibodies (HAMA) which limited the availability of therapies to the few that could evade this immune reaction. A partial solution has been the development of humanized antibodies, some of which are now available in clinical practice. However, most antibodies have limited intrinsic therapeutic activity. One important way to increase their activity would be to conjugate an antibody to a particular bacterial or plant toxins. Unfortunately, these antibody-drug-conjugates are also often immunogenic proteins, limiting the potential of this therapeutic avenue. A variety of methods have been proposed to help diminish the rapidity of the immune response. However, attempts at immune suppression through the use of single agent cyclophosphamide or rituxumab have so far been unsuccessful.[23,24] Rather than focus on B-cell depletion, preclinical research and previous clinical trials in transplant patients suggest that the immune response can be suppressed by focusing efforts on the destruction of the T-cells directed against the immunogenic protein. Antibody response to protein antigens requires T-cell and B-cell interaction and involvement. Targeting T-cell involvement helps attenuate the initial step in neutralizing antibody formation.

We intend to use a conditioning regimen to reduce the capacity of host immunity to respond to the non-human protein of the investigational agent, SS1(dsFv)PE38. As noted, in previous clinical trials with SS1(dsFv)PE38, neutralization assays have determined that between 80 to

90% of patients will develop neutralizing antibodies within three weeks of initiating single agent therapy. This has limited the use of SS1(dsFv)PE38 to one or two cycles prior to the neutralization of the agent's effect by the host immune response. If this neutralizing effect can be reduced by 50%, this would be a significant advance in the effort to utilize this and other antibody-drug-conjugates.

Clinical researchers at the National Cancer Institute have prior clinical experience with non-myeloablative conditioning regimens, which have been utilized to diminish the T-cell-dependent host-versus-graft rejection reaction that can occur in patients undergoing allogeneic stem cell transplant. The primary objective will be analyzed by neutralization assays, with a reduction in neutralization by 50% being considered significant. Further, given the extremely poor prognosis of patients with mesothelioma and limited treatment options, if we are able to treat ≥50% of patients with repeated cycles of SS1P that could result in potentially clinically significant benefit to these patients.

2.2 LUNG ADENOCARCINOMA

2.2.1 Overview of NSCLC

Non-small cell lung cancer (NSCLC) is the leading cause of cancer related death in Europe and in the USA. Lung cancer was responsible for 1.4 million deaths from cancer in 2008, accounting for 18% of all cancer deaths[25]. More than 70% of patients are diagnosed with advanced disease which is not amenable to curative therapy. In these patients and in those who have relapsed with advanced disease following prior definitive treatment, palliative systemic therapy is the primary treatment approach.

Therapy of advanced NSCLC is now individualized based upon somatic driver mutations found in the tumor. Patients with tumors harboring activating mutations in the tyrosine kinase domain of epidermal growth factor receptor (EGFR) gene are treated with EGFR tyrosine kinase inhibitors (TKI). To date three EGFR TKIs have been approved: gefitinib, erlotinib and afatinib. Compared with chemotherapy, in the first-line treatment of EGFR mutant tumors, EGFR TKIs produced objective response rates nearing 75% and improved progression-free survival[26]. Patients with tumors harboring translocations of anaplastic lymphoma kinase (ALK) gene are treated with crizotinib, an ALK TKI. Objective response rates to crizotinib are approximately 60% with a median progression-free survival of 8 to 10 months[27]. For patients with no identifiable tumor driver mutations, systemic therapy which usually consists of platinum-based cytotoxic chemotherapy doublet improves overall survival compared with supportive care alone[28]. However objective response rates to chemotherapy are generally in the range of only 20% to 30%, median survival in the range of 7 to 10 months, and 1-year survival rate less than 40%[29,30].

Treatments targeting the tumoral immunosuppressive mechanims have shown promise in NSCLC. Immune checkpoints are molecules expressed on the surface of immune cells including T lymphocytes that modulate the immune response to antigens via inhibitory or stimulatory signaling to T cells. Two most extensively studied immune checkpoints in lung cancer are cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed cell death-1 (PD-1). Activation of both receptors causes down-regulation and inhibition of immune responses.

Ipilimumab, an anti-CTLA-4 antibody was evaluated in a randomized phase II study in combination with chemotherapy[31]. Patients with chemotherapy-naive NSCLC (n=204) were

randomized to receive paclitaxel and carboplatin with either placebo or ipilimumab in one of the following two regimens: concurrent ipilimumab (four doses of ipilimumab plus paclitaxel and carboplatin followed by two doses of placebo plus paclitaxel and carboplatin) or phased ipilimumab (two doses of placebo plus paclitaxel and carboplatin followed by four doses of ipilimumab plus paclitaxel and carboplatin). The study met its primary objective of improving the immune-related progression-free survival (ir-PFS) for phased ipilimumab compared with control (HR 0.72; P =0.05), but not for concurrent ipilimumab (HR 0.81; P=0.13). Phased ipilimumab, concurrent ipilimumab, and control were associated with a median irPFS of 5.7, 5.5, and 4.6 months, respectively. There was no significant difference in OS between the three arms. In the phased ipilimumab arm, improvements in irPFS appeared to be greater in patients with squamous histology (HR 0.55; 95% CI, 0.27-1.12) than in patients with non-squamous histology (HR 0.82; 95% CI, 0.52- 1.28).

PD-1, another key immune-checkpoint receptor is expressed by activated T and B cells and is structurally similar to CTLA-4, but has distinct biologic functions and ligand specificity. PD-1 is engaged by its ligands, PD-L1 and PD-L2 within the tumor microenvironment. When activated T-cells expressing PD-1 encounter PD-L1 or PD-L2, T-cell effector functions are diminished. Nivolumab, an anti-PD-1 antibody was evaluated in a phase I trial where it was administered intravenously once every 2 weeks in 8-week cycles in patients with selected advanced cancers[32]. In the NSCLC expansion cohorts treated at 1, 3, and 10 mg/kg, response rates were 18% (14 of 76 patients). Responses were observed across squamous [objective response rates (ORR) 33%; 6 of 18] and non-squamous (ORR 12%; 7 of 56) histologies.

BMS-936559, an anti-PD-L1 antibody was evaluated in a phase I trial where it was administered intravenously once every 2 weeks in 6-week cycles in patients with selected advanced cancers[33]. In the NSCLC expansion cohorts treated at 3 mg and 10 mg/kg, response rates were 10% (5 of 49). Responses in the squamous and non-squamous histologies were 8% (1 of 13) and 11% (4 of 36) respectively. A number of other anti-PD-1 (e.g. pembrolizumab) and anti-PD-L1 (e.g. MPDL-3280A) antibodies have demonstrated preliminary anti-tumor activity in NSCLC. In a phase I study of 38 previously treated NSCLC patients, pembrolizumab 10 mg/kg administered intravenously once every 3 weeks resulted in immune-related response of 24[34]%.

Despite improved outcomes with targeted therapies and immunotherapies in the recent years, there remain a large proportion of patients with no targetable driver mutations. For example, EGFR mutations are observed only in approximately 15 percent of NSCLC adenocarcinoma in the United States. ALK translocations are found in an even smaller proportion: only about 4 percent of NSCLC adenocarcinoma. Furthermore, despite the success of genotype-directed therapies in EGFR-mutant and ALK-positive patients, resistance inevitably develops. The median PFS after treatment with EGFR or ALK inhibitors is generally less than 1 year. The need for effective treatment in NSCLC is more apparent in the second and third line settings where approved drugs pemetrexed and docetaxel result in objective response rates of less than 10%, median progression-free survival of 3 months and median overall survival of 8 months.[35]. Thus there is an urgent need for novel targeted therapies in NSCLC.

2.2.2 Mesothelin expression in lung cancer

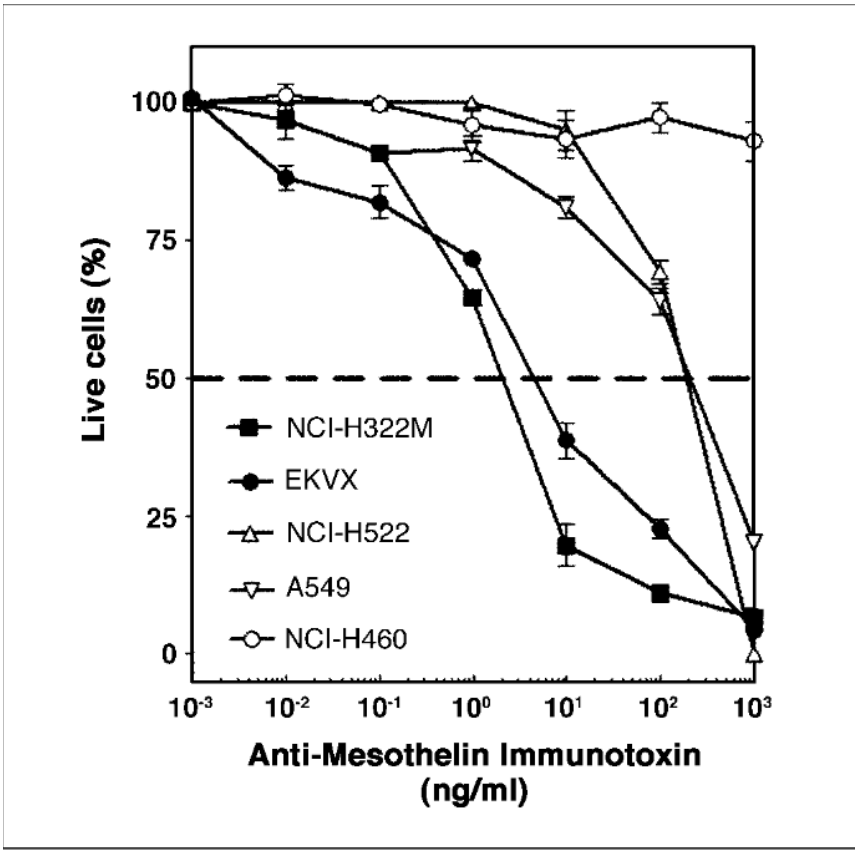
Mesothelin expression has been demonstrated in approximately 30-70% of lung adenocarcinoma[36-40]. We recently characterized mesothelin expression in lung adenocarcinoma using prospectively obtained clinical and pathological data from 99 patients. These patients

underwent tumor molecular profiling of potentially actionable genes using a multi-platform approach on a prior CTEP-sponsored protocol (11-C-0096). Our results (unpublished) demonstrate that in a cohort consisting predominantly of patients with advanced NSCLC, 51% of lung adenocarcinoma express mesothelin and high expression (mesothelin expression in > 25% cells) is seen in 24%. Mesothelin expression was independently and strongly associated with KRAS mutation, and mesothelin expression was associated with inferior survival. In early stage NSCLC, a recent retrospective study also showed that mesothelin expression is associated with KRAS mutation and confers a poor prognosis[41].

We have previously demonstrated that mesothelin mRNA and protein are present in a substantial number of lung adenocarcinoma cell lines.[42] We also determined the cytotoxicity of SS1P in five NSCLC cell lines (A549, EKVX, NCI-H322M, NCI-H460, and NCI-H522) by a WST cell death assay [42]. In NCI-H322M and EKVX, the two adenocarcinoma cell lines with the highest mesothelin expression on the cell surface, SS1P was very active with IC50 values ranging from 2 ng/Ml (NCI-H322M) to 5 ng/mL (EKVX) (**Figure 1**). In the A549 and NCI-H522 cell lines with low mesothelin expression, lower but significant cytotoxic activity (IC50= 200 ng/mL) was observed.

The threshold for mesothelin expression which would be predictive of response to anti-mesothelin therapy is not known. Hence we propose to enroll patients with any mesothelin expression in this pilot trial. Retrospective assessment of this data and correlation with outcomes would provide us preliminary data to plan potentially larger trials in the future where the association between mesothelin expression and tumor response in NSCLC can be better defined. Mesothelin expression will be determined by immunohistochemistry and interpreted by a pathologist with expertise in this method.

Figure 1: SS1(dsFv)PE38 cytotoxicity in various lung cancer cell lines



2.2.3 Rationale for using SS1(dsFv)PE38 in lung cancer

Mesothelin is an attractive antigen to target in lung adenocarcinoma for several reasons. It is frequently expressed in lung adenocarcinoma, but its expression in normal adult tissue is restricted to mesothelial cells lining the pleura, peritoneum and pericardium, as well as cells of the fallopian tubes and tonsils.[43] Previous pre-clinical studies shows cytotoxic activity of SS1P in mesothelin-expressing lung cancer cell lines.[42] This provides a strong rationale for exploring the activity of the anti-mesothelin immunotoxin SS1P in subjects with lung adenocarcinoma.

2.3 PANCREATIC ADENOCARCINOMA

2.3.1 Overview of pancreatic adenocarcinoma

Pancreatic adenocarcinoma is the fourth leading cause of death from cancer in the United States and accounts for nearly 43,000 deaths every year.[44] Pancreatic cancer carries a poor prognosis with an overall 5-year survival rate of 5-6%[45]. Surgery provides the only curative treatment option; however, only 10% of the patients present with operable disease[46]. For patients with recurrent, locally advanced unresectable or metastatic pancreatic adenocarcinoma, palliative systemic therapy is the primary treatment approach.

Systemic chemotherapy provides benefit to patients with advanced pancreatic cancer, improving disease-related symptoms and survival when compared to best supportive care alone. Gemcitabine has been considered the first line therapy for advanced pancreatic cancer for the last two decades due to its efficacy in alleviation of disease-related symptoms. The median overall

survival for patients with advanced pancreatic adenocarcinoma receiving gemcitabine is approximately 6 months[47]. Many attempts to increase survival efficacy by combining gemcitabine with other cytotoxic drugs, such as cisplatin, oxaliplatin, irinotecan, and capecitabine did not improve outcomes.[48]. Recently, addition of nab-paclitaxel to gemcitabine was shown to improve overall survival by 1.8 months, and the response rate by 16%, resulting in approval of this drug for treatment of pancreatic cancer. This improvement comes at the expense of increased peripheral neuropathy and myelosuppression.[49] The combination of gemcitabine with biologic agents has proven unsuccessful thus far, but addition of the EGFR inhibitor erlotinib to gemcitabine resulted in a minimal improvement in survival of about 2 weeks.[50] Recently the regimen of FOLFIRINOX (infusional 5-FU, leucovorin, oxaliplatin and irinotecan) showed superiority over gemcitabine. FOLFIRINOX is currently the standard treatment for patients with good performance status (ECOG 0 or 1). Patients who received FOLFIRINOX had a median overall survival of 11 months median progression-free survival of 6 months and an objective response rate of 32%[51]. FOLFIRINOX was associated with substantial toxicities including neutropenia, febrile neutropenia, thrombocytopenia, diarrhea, and sensory neuropathy.

There are limited systemic therapeutic options for patients with unresectable, recurrent or metastatic pancreatic adenocarcinoma and all are associated with severe toxicities. There is an urgent need for novel targeted therapies for this population.

Mesothelin expression has been demonstrated in nearly all pancreatic adenocarcinomas. Argani et al showed that mesothelin is consistently present in pancreatic cancer serial analysis of gene expression (SAGE) libraries but not in libraries from normal pancreas[52]. In addition, mesothelin mRNA was present in 4 of 4 resected primary pancreatic cancers and could be localized by immunohistochemistry in 60 of 60 resected primary adenocarcinomas. Additional studies have replicated these results, demonstrating that mesothelin is highly expressed in almost all pancreatic adenocarcinomas, and that it is not expressed in normal pancreas, islet cell tumors of the pancreas, pre-malignant PanIN lesions or chronic pancreatitis[53-56]. Mesothelin expression correlates with tumor aggressiveness in animal models of pancreatic cancer [57] and cancer cell growth in motility in pancreatic cancer cells in culture. In addition, high level expression of mesothelin expression in patient surgical specimens (as determined by immunohistochemistry analysis) was found to be highly predictive of decreased survival for patients with resectable pancreatic cancer.[58]

Mesothelin has been utilized as a target for new therapeutics because of this differential expression. In human studies, a live-attenuated Listeria vaccine expressing mesothelin (CRS-207) was evaluated in a dose-escalation study in subjects with mesothelioma, lung, pancreatic, or ovarian cancers. Seventeen subjects received up to 4 doses of 1×10^8, 3×10^8, 1×10^9, or 1×10^{10} cfu. Listeriolysin O and mesothelin-specific T-cell responses were detected and all 3 research subjects with pancreatic cancer lived ≥15 months. The vaccine was moved into Phase 2 testing for pancreatic cancer patients who had received at least one prior line of chemotherapy. It was given in combination with GVAX, an irradiated GM-CSF-secreting pancreatic cell line, to stimulate antigenic response, and with low-dose cyclophosphamide to inhibit regulatory T cells. Patients were randomized 2:1 to receive two doses of GVAX/ cyclophosphamide followed by four doses of CRS-207, or six doses of the GVAX/ cyclophosphamide adjuvant alone. At last report, the data were still maturing, but patients receiving CRS-207 had a statistically significant increase in survival with OS of 6.9 months, versus 3.9 months for patients who received GVAX/ cyclophosphamide alone ($p = 0.011$).[59] Clinical testing of CRS-207 is ongoing. These studies

demonstrate that targeting mesothelin may be a promising strategy when developing new therapeutics for pancreatic cancer.

Table 1 shows mesothelin expression and cytotoxicity of SS1P in pancreatic ductal adenocarcinoma cell lines. Cytotoxic activity of SS1P was observed in 4 out of the 6 cell lines tested with IC50 <100 ng/ml. These cells had 3,000-60,000 mesothelin binding sites/cell.

Table 1. Mesothelin expression and cytotoxicity of SS1P in PDAC cell lines

Cell lines	Mesothelin binding sites/cell (x 10^3)	IC50 ± SEM (ng/ml)
		SS1P
GUMC108	35	3.98 ± 0.49
KLM-1	60	23.90 ± 2.81
AsPC-1	30	14.76 ± 2.24
BxPC-3	3	24.4 ± 2.47
Panc 3.014	90	848.2 ± 151.8
PK-1	5	638.5 ± 11.9

Average data from at least three independent experiments. PDAC = pancreatic ductal adenocarcinoma, SEM = standard error of mean

2.3.2 Rationale for using SS1(dsFv)PE38in pancreatic adenocarcinoma

Mesothelin is an attractive antigen to target in pancreatic adenocarcinoma for several reasons. It is frequently expressed in pancreatic adenocarcinoma, and not expressed in non-malignant pancreatic tissue. Previous clinical studies and pre-clinical evaluations suggest efficacy of mesothelin-targeted therapeutics in pancreatic adenocarcinoma[60]. This provides a strong rationale for exploring the activity of the anti-mesothelin immunotoxin SS1P in subjects with pancreatic adenocarcinoma.

2.4 HOST IMMUNE DEPLETION WITH PENTOSTATIN AND CYCLOPHOSPHAMIDE

The rationale for using a non-myeloablative conditioning regimen was developed from two prior protocols for transplantation which have been performed at the Clinical Center of the NIH.

During bone marrow transplantation, host T cells present at the time of transplantation mediate a host-versus-graft rejection response that inhibits alloengraftment, contributes to mixed chimerism (and the resultant delay in onset of GVT effects), and can ultimately result in graft rejection. As such, the number and function of host T cells present at the time of transplantation is an important consideration with respect to alloengraftment. Similarly, the immunogenicity of various foreign proteins is dependent on a T and B cell response.

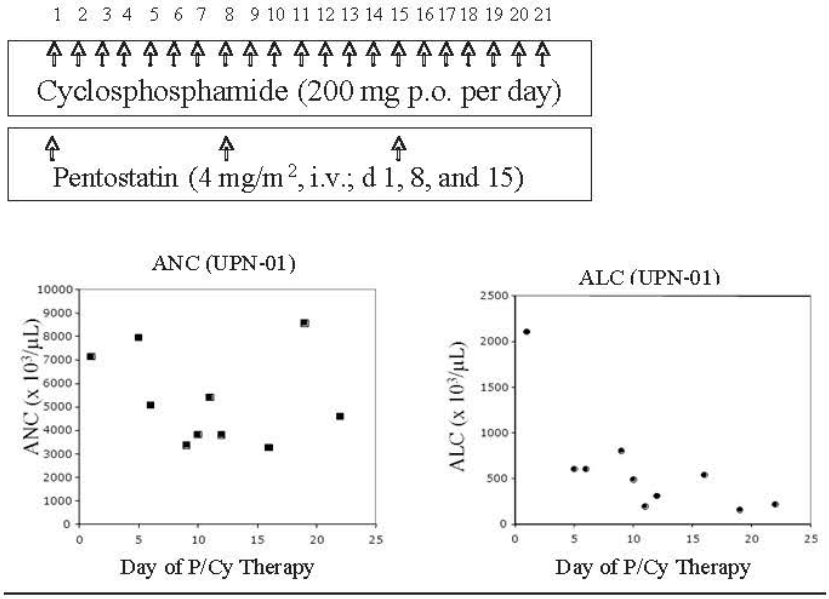
As such, in NCI Protocol 08-C-0088, Dr. Daniel Fowler and his co-investigators designed a regimen that was predicted to reduce the host's $CD3^+$ T cell count to < 200 cells/mcL prior to administration of the allogeneic hematopoietic stem cell and Th2 cell products. The rationale to develop a novel pentostatin and cyclophosphamide regimen to achieve host immune depletion is

based on published clinical data, as well as murine data that indicate pentostatin results in more profound immune T cell depletion than fludarabine.

In NCI Protocol 08-C-0088, subjects with metastatic Renal Cell Carcinoma (RCC) were enrolled, and predicted to be at higher risk for mixed chimerism and graft rejection relative to hematologic malignancy subjects due to their absence of prior exposure to conventional chemotherapy agents. Metastatic RCC subjects were thought to be likely to be chemotherapy-naïve, and therefore could be anticipated to have CD3 cell counts similar to age-matched controls (approximately 1400 CD3 cells/mcL)[61]. Therefore, the investigators needed, in the setting of metastatic RCC, to develop a new regimen that consistently and quickly achieves significant host T cell depletion (as defined by attainment of host CD3 cell number of ~ 200 cells/mcL in a treatment interval of three weeks). The three week time interval of host immune depletion was chosen as this was the amount of time required to harvest donor cell products and to generate *ex vivo* the investigational agent, Th2.rapa cells. The plan to administer Th2.rapa cells in the absence of a conventional Flu/Cy preparative regimen also stemmed from the investigators' desire to avoid the engraftment syndrome toxicity that was observed in that setting.

NCI Protocol 08-C-0088 is still an ongoing allograft experiment, but preliminary results show that the use of pentostatin plus cyclophosphamide has led to host immune depletion without myeloid cell suppression. Consistent results have been achieved in each of the eleven patients treated on NCI Protocol 08-C-0088, with all eleven treated patients having reached the goal of ALC<200 with no incidence of ANC<1000). Also, each of the eleven patients has achieved stable alloengraftment. A sample patient's absolute neutrophil count and absolute lymphocyte count are shown in **Figure 2**. Adverse events from all eleven patients were collected from the patients' conditioning period. A summary of all adverse events recorded during the conditioning period of the trial is shown in **Figure 3**.

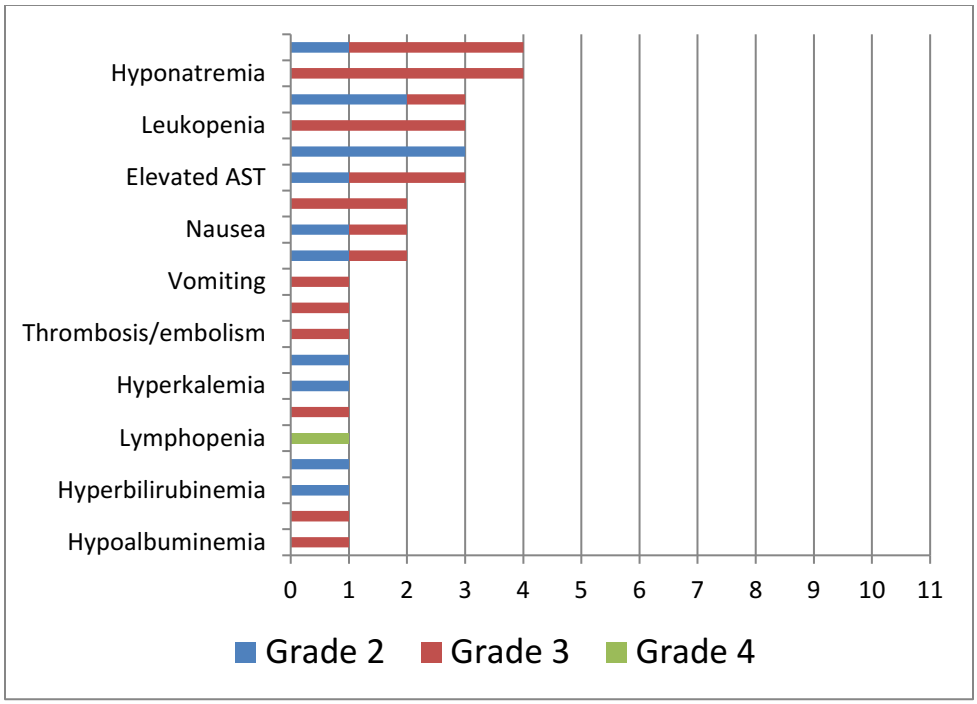
Figure 2:



Use of pentostatin plus cyclophosphamide to achieve host immune depletion without myeloid cell suppression. Schema shown is the preparative regimen of P/C utilized on low-intensity allogeneic HSCT protocol 08-C-0088. The lower panels show a representative patient result for stability of the ANC over the 21-day treatment interval (left panel) and for reduction in the ALC over the treatment interval. Consistent results have been achieved in each of the eleven patients treated on this protocol (that is, 11/11 patients have reached the goal of ALC<200 with no incidence of ANC<1000). Each of the eleven patients has achieved stable alloengraftment.

Figure 3. Summary of Adverse Events on Protocol 08-C-0088 during the Conditioning Regimen Period.

Events shown represent the highest grade event recorded for each patient. Thus, out of a total of eleven patients, four have experienced anemia, with three patients experiencing Grade 3 anemia and one experiencing Grade 2 anemia. Results show that the regimen was well tolerated.



Based upon the preliminary clinical findings of NCI Protocol 08-C-0088, as shown in the figures above, we will utilize a novel pre-transplant immune T cell depletion regimen that consists of intravenous pentostatin and daily oral, low-dose cyclophosphamide given over a fixed 12-day treatment interval. The rationale to truncate the 21-day PC regimen in protocol 08-C-0088 to the 12-day regimen in the current protocol stems from a desire to limit treatment delay in patients with refractory and progressive mesothelioma. The purpose of maintenance doses of PC is to limit neutralizing antibody formation from host immune cells that may have regenerated after the initial induction immune depletion. The immunotoxin SS1(dsFv)PE38 will be given on Days 10, 12, and 14 of Cycle 1 and Days 2, 4, and 6 of subsequent cycles. Cycles will be repeated every 21 days with the immunotoxin, pentostatin and cyclophosphamide. Neutralization assays will be taken 14 and 20 days (+/- 2 days depending on individual patient needs) following the first dose of SS1(dsFv)PE38. Study therapy was initially extended for up to 6 cycles but is reduced to 4 cycles as of in the phase 2 mesothelioma, mesothelin positive cancers dose de-escalation study and lung and pancreatic cancerexpansion cohorts provided there is no unacceptable toxicity, no disease progression or no evidence of SS1(dsFv)PE38 neutralization. The return to 4 cycles was based upon the observation that 80% of patients who were treated with Regimen A developed SS1P neutralizing antibodies by cycle 4. The rationale for the increase to 6 cycles was derived from the following observations:

1. Weekly i.v. pentostatin at a dose of 4 mg/m^2 is effective therapy for many sub-types of human lymphoma, particularly T cell lymphoma;[62]
2. Pentostatin depletes non-malignant human T cells prior to allogeneic transplantation; in the only study published on this topic, Dr. Pavletic while at the University of Nebraska found that a total dose of 12 mg/m^2 of single-agent pentostatin administered over a 3-day interval reduced host CD3$^+$ T cell number by approximately 50%;[61]
3. At a pentostatin dose of 4 mg/m^2, it has been determined that the adenosine deaminase (ADA) enzyme, which is the molecular target inhibited by pentostatin, is inhibited for at least one week;
4. Because the proposed patient population in this study is likely to have received prior cisplatin and pemetrexed therapy, eligible patients with creatinine clearance < 60 mL/min/m^2 will be started at a pentostatin dose of 2 mg/m^2
5. Analogous to results with fludarabine, more profound depletion of human lymphocytes occurs when ADA inhibition by pentostatin is combined with an DNA alkylation agent, such as cyclophosphamide;[63,64] and
6. In humans, a one month regimen of daily oral cyclophosphamide therapy (100 or 200 mg per day) reduces circulating T cell numbers by approximately 50%.[65]
7. Based on experience with three patients in the current study among whom one experienced a DLT (grade 3 pleuritis) after one cycle with no development of neutralizing antibodies; one had stable disease at restaging, but developed neutralizing antibodies at the end of cycle 2; and one has shown partial response through cycle 3 with no development of neutralizing antibodies, it is possible that patients may derive benefit from treatment extension beyond 3 cycles.

In recent murine experiments (see **Figure 4**, **Figure 5** and **Figure 6**), we have found that a combination of pentostatin and a relatively low-dose of cyclophosphamide can modestly reduce the myeloid cell lineage and result in severe host immune T cell depletion. Importantly, the pentostatin and cyclophosphamide regimen resulted in greater host T cell depletion and dramatically reduced neutralizing antibody formation to SS1(dsFv)PE38. These data further demonstrate the great degree of synergy that exists between pentostatin and cyclophosphamide, as only a nominal level of host T cell depletion was observed with single agent pentostatin therapy. Taken together, these data provide a rationale for developing a novel host immune depletion strategy that utilizes combination pentostatin and cyclophosphamide. Both drugs have been studied in combination for a variety of conditions, particularly chronic lymphocytic leukemia.[63,66]

Injection Schedule for 9-week Mouse Study

Weekly Immunotoxin (Weekly IT)

	Week 1							Week 2							Weeks 3-9
Day	1	2	3	4	5	6	7	1	2	3	4	5	6	7	Same as Week 2
						IT							IT		

4-day P/C with Weekly IT (4-Day P/C)

	Week 1							Week 2							Weeks 3-9
Day	1	2	3	4	5	6	7	1	2	3	4	5	6	7	Same as Week 2
	P			P											
	C	C	C	C											
						IT							IT		

4-day P/C with Weekly P/C and Weekly IT (Weekly P/C)

	Week 1							Week 2							Weeks 3-9
Day	1	2	3	4	5	6	7	1	2	3	4	5	6	7	Same as Week 2
	P			P							P				
	C	C	C	C							C				
						IT							IT		

4-day P/C with Weekly P/C+C and Weekly IT (Weekly P/C+C)

	Week 1							Week 2							Weeks 3-9
Day	1	2	3	4	5	6	7	1	2	3	4	5	6	7	Same as Week 2
	P			P							P				
	C	C	C	C							C	C			
						IT							IT		

Figure 4. To examine the immunogenicity of SS1P, Balb/c mice were immunized intraperitoneally weekly, with or without Pentostatin and Cyclophosphamide (P/C) treatment. The murine experiment involved 4 cohorts treated with immunotoxin and various means of conditioning, as shown below. Mice were injected with immunotoxin (IT) (5µg/day) on Day 6 of every week on a 9-week treatment cycle. One cohort was treated with IT alone. The other three treated groups were given a 4-day induction of pentostatin (P) (1mg/kg/day) and cyclophosphamide (C) (50mg/kg/day) depletion regimen. Two of those treated cohorts received maintenance P/C. One cohort received weekly injections of P/C alone. The other received P/C with an additional C injection. In addition, two control groups were included, one injected with saline, and another untreated.

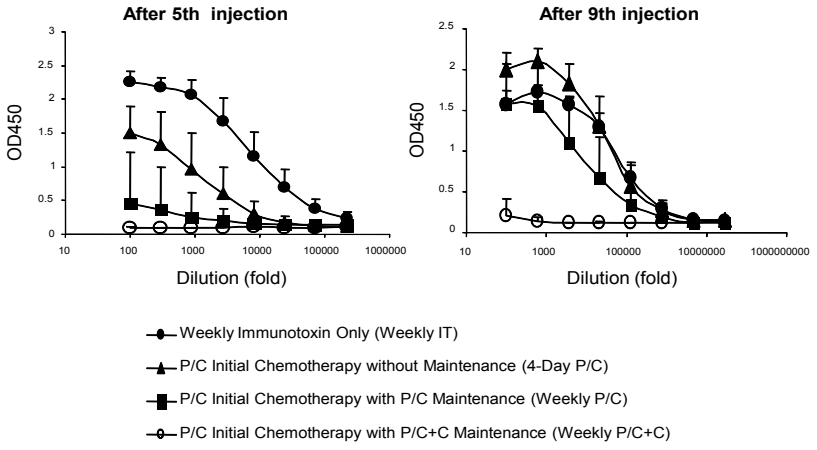


Figure 5. Shown are the serum samples from the final blood draw after 5 weeks and 9 weeks of Immunotoxin therapy in the four mouse cohorts treated with SS1(dsFv)PE38. The figure shows the results of Immune complex capture ELISA (ICC), which detects antibodies which recognize conformational epitopes of PE38. Cohorts included the groups treated with immunotoxin alone (Weekly IT), P/C initial chemotherapy alone (4-Day P/C), P/C initial chemotherapy with P/C weekly maintenance (Weekly P/C), and P/C initial chemotherapy with P/C+C weekly maintenance (Weekly P/C+C). The results suggest a significant and sustained decrease in antibody formation in the mouse cohort with P/C+C weekly maintenance relative to other groups treated with the immunotoxin.

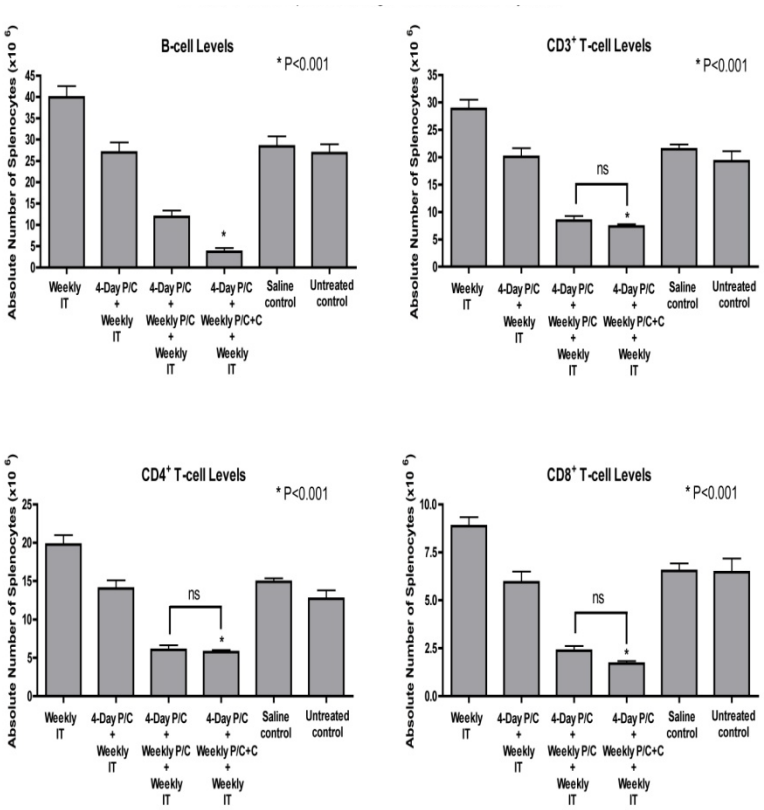


Figure 6. Murine model of pentostatin plus cyclophosphamide (P/C) for immune depletion without myeloablation prior to immunotoxin therapy. At the end of treatment, mouse spleens were harvested and total number splenocytes counted. Different subsets of lymphocytes were identified by flow cytometry. Values represent the mean +/- SEM of n=5 mice per cohort per time point. As previously shown, the cohort in which mice were treated with P/C initial chemotherapy with P/C+C weekly maintenance (Weekly P/C+C) had the most dramatic decrease in antibody formation to the immunotoxin. In the chart below, B-cell depletion is most significant in the Weekly P/C+C maintenance group. The Weekly P/C and Weekly P/C+C cohorts were similar in their levels of T-cell depletion.

In summary, this study will utilize a regimen of pentostatin (1-4 mg/m^2 [depending upon CrCl], intravenously on Days 1, 5, and 9 of Cycle 1 and Day 1 of subsequent cycles) and low-dose oral cyclophosphamide (200 mg daily on Days 1 through 12 of Cycle 1, and Days 1 through 4 of subsequent cycles). The goal of this regimen is to reduce host T cell numbers from study entry (estimated mean T cell number of 1400 cells/mcL) to a level of approximately 200 cells/mcL. Given the synergy between pentostatin and cyclophosphamide, we believe this target is achievable, as the additive effect of the immune depleting properties of the two agents would be expected to reduce the T cell count from 1400 to 350 (50% reduction each from pentostatin and cyclophosphamide).

2.5 Rationale for Increasing the Dose Intensity of Pentostatin and Cyclophosphamide (Regimen B)

Atthe completion of the mesothelioma pilot study eleven patients had been enrolled on this study of whom ten were evaluable for assessing the primary and secondary objectives of the trial. Preliminary results showed that the combination of pentostatin and cyclophosphamide with SS1(dsFv)PE38 was safe and feasible. None of the patients developed opportunistic infections.

Regimen A was effective in delaying the formation of antibodies to SS1(dsFv)PE38, the primary objective of this trial. As shown in **Table 2**, only 2 out of 10 patients assessed developed antibodies at the end of cycle 1. Regimen A was effective at delaying anti-SS1P antibody formation: only 2/10 patients developed anti-SS1P antibodies at the end of cycle 1, whereas 5/10 patients developed antibodies after cycle 2. One patient did not develop antibodies after 6 cycles, and another did not develop antibodies after 2 cycles. These results show that only 2 out of 10 (20%) patients developed antibodies by end of cycle 1 which is significantly better than 30 of 34 (88%) patients who developed antibodies at end of cycle 1 when treated with SS1(dsFv)PE38 alone.

Although, the ability of this regimen to delay development of anti-SS1(dsFv)PE38 antibodies so patients can get a second cycle of therapy was a significant improvement, seven out of nine patients had developed antibodies by end of cycle 2. The patients who received four and six cycles of therapy had a substantial tumor response maintained more than 12 months after completion of therapy. Therefore it appeared that the ability to administer more than two cycles of SS1(dsFv)PE38 could improve therapeutic efficacy.

Table 2: Development of Neutralizing Antibodies

Patient	C1 D1	C1 D15	C2 D1	C2 D15	C3 D1	C3 D15	C4 D1	C4 D15	C5 D1	C5 D15	C6 D1	C6 D15
2	-	-	-	+								
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	+									
5	-	-	-	-	-	-	-	+				
6	-	-	-	+								
7	-	-	-	+								

Patient	C1 D1	C1 D15	C2 D1	C2 D15	C3 D1	C3 D15	C4 D1	C4 D15	C5 D1	C5 D15	C6 D1	C6 D15
8	-	-	-	-*								
9	-	-	-	+								
10	-	-	-	+								
11	-	-	+									

* No further follow-up data available.

Although the conditioning regimen (Regimen A) of pentostatin and cyclophosphamide was able to transiently achieve the goal of suppression in absolute lymphocyte count < 200 / µL, the effect was not sustained with lymphocyte counts trending upward late in cycle 1. As is shown in **Figure 7** and **Figure 8**, the ALC count was > 200/uL in all patients when they received the first dose of SS1P in cycle 2.

Figure 7. **Absolute lymphocyte count for individual patients during cycle 1 and 2.**

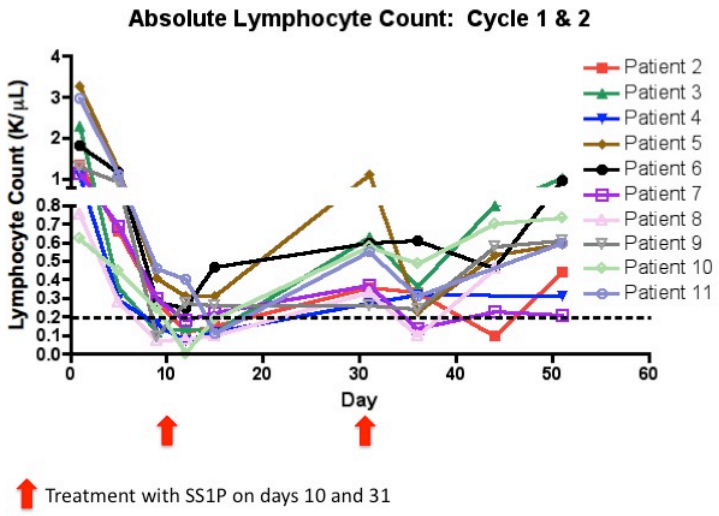
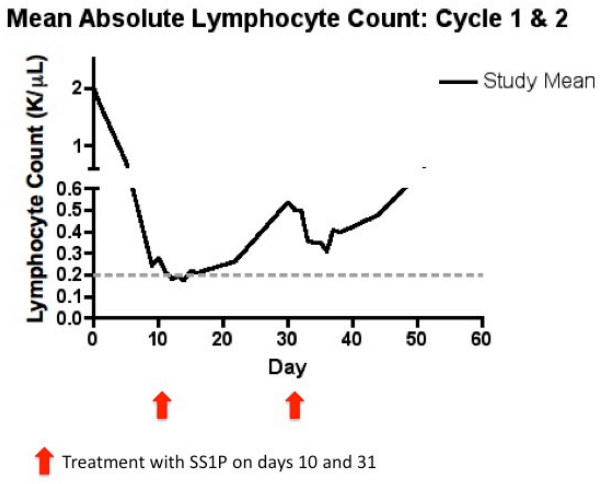


Figure 8. **Mean Absolute lymphocyte count for during cycle 1 and 2.**



Since patients were not able to achieve an ALC < 200/ uL at the time of initiation of cycle 2 using Regimen A it most likely contributed to antibody formation in majority of the patients. By intensifying both T and B cell suppression prior to and during SS1(dsFv)PE38 therapy we attempted further delay or prevent development of anti-SS1(dsFv)PE38 antibodies allowing administration of 3 or more cycles of therapy. Due to these results, we sought to determine if we could delay anti-SS1P antibodies before the start of cycle 3, and therefore achieve good SS1P blood levels during cycle 3 by adding Regimen B to the study.

2.6 RATIONALE FOR DISCONTINUATION OF REGIMEN B AND EXPANSION OF REGIMEN A

As indicated above, Regimen B was added to the study in order to delay the formation of anti SS1(dsFv)PE38 antibodies and thus allow prolonged administration of the agent. In regimen B, patients received conditioning regimen of pentostatin on days 1, 5, 9, 13 and 17 of the first cycle and day 1 and 5 of subsequent cycles in combination with cyclophosphamide on days 1 through 20 of the first cycle and days 1 through 8 of subsequent cycles. The first cycle consisted of 38 days, and each subsequent cycle consisted of 25 days. SS1P was given at a dose of 35 mcg/kg. Treatment cycles were repeated for up to six cycles if patients do not develop neutralizing antibodies.

Between 4/4/2013 and 8/2/2013, eight patients were treated on protocol. The demographic characteristics, site of primary tumor and prior therapies are summarized in **Table 3**.

Table 3: Patient demographics, treatment and response						
Pt	Age (y) /Sex	Tumor Site*	Number of Prior Therapies	No. of SS1P Cycles Received	Reason for off-treatment	Best Tumor Response
1	29/M	Peritoneum	2	1	Neutralizing Ab formation	Stable disease
2	75/F	Pleura	1	3	Fatigue, anorexia	Stable disease
3	70/M	Pleura	4	5	Patient decision	Stable disease
4	62/M	Pleura	2	4	Patient receiving treatment	Stable disease
5	72/M	Pleura	5	<1 (3 pento, 12 cytoxan)	Atrial fibrillation, hypoxia	Not evaluable
6	69/M	Pleura	3	<1 (4 pento, 14 cytoxan)	Early disease progression	Not evaluable
7	42/F	Peritoneum	4	2	Patient receiving treatment	Stable disease
8	63/M	Pleura	2	2	Patient receiving treatment	Not evaluated
*All patients had epithelial type mesothelioma.						

Out of 6 patients evaluable for neutralizing antibody formation, only one (17%) developed antibodies after cycle 1 suggesting that regimen B is effective at preventing or delaying development of anti-SS1P antibodies. Two patients were not evaluable for antibody formation: one patient had early rapid disease progression and was taken off treatment after only 4 doses of pentostatin and 14 doses of cytoxan; a second patient had intolerable toxicities and was taken off treatment after only 3 doses of pentostatin and 12 doses of cytoxan. None of the 4 evaluable patients developed anti-SS1P antibodies after cycle 2. Delaying anti-SS1P antibodies also

allowed administration of additional cycles of SS1P and resulted in higher serum SS1P levels (**Table 5**).

Table 4. SS1P-neutralizing antibody formation										
Pt no.	200 ng/ml					1000 ng/ml				
	Pre-C1	Pre-C2	Pre-C3	Pre-C4	Pre-C5	Pre-C1	Pre-C2	Pre-C3	Pre-C4	Pre-C5
1	59	100				1	92			
2	10	12	11	14		0	18	0	2.2	
3	0	0	12	0	2.7	0	0	0	0.5	1.1
4	3	6.6	0	25		0	0	0.5	3.1	
7	10	2.8				0	0			
8	52	35				2.4	3.4			

Six patients had at least one dose of SS1P. Serum concentrations of SS1P were significantly higher than those of patients who received regimen A (**Table 5**). Moreover, since antibody formation was delayed, SS1P concentrations continued to be high with subsequent cycles.

Table 5. SS1P Cmax ng/ml (mean of Cmax achieved with 3 doses during each cycle)					
Pt no.	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
1	341				
2	1030	868	882		
3	1031	1019	1041	848	732
4	858	675	598		
7	1194				
8	1534	1906			

Regimen B resulted in higher than expected frequency and grade of toxicities. Two out of the eight patients stopped treatment early due to intolerable toxicities: a patient had intolerable fatigue which precluded treatment after 3 cycles; a second patient developed atrial fibrillation and hypoxia following which treatment was discontinued. Overall 3 patients developed atrial fibrillation (all grade 3) including one patient who needed electrical cardioversion for rhythm control. Three patients developed hypoxia including one patient who required ventilatory support. Three patients developed rash presumably related to Bactrim. A majority of patients developed fatigue.

Five out of 8 patients were evaluable for response. One patient is still on treatment and two others are not evaluable for response. All five evaluable patients had stable disease as the best response.

In comparison, Regimen A in the pilot study was well tolerated: no grade 4 toxicities ascribed to SS1P were observed; Grade 3 toxicities included non-cardiac chest pain, pleuritic pain and back pain (9% each). Less serious SS1P-related adverse events included fatigue, non-cardiac chest pain, edema and hypoalbuminemia. Adverse events related to pentostatin or cyclophosphamide included Grade 4 lymphopenia (100%), Grade 3 anemia (9%), transaminitis (18%) and fever (9%). Despite immunosuppression, no patient developed bacterial, viral or fungal infections.

Of the ten evaluable patients treated on regimen A in the pilot, three had durable partial responses, three stable disease, and four progressive disease. All three patients had durable responses lasting 8 to 15 months from start of therapy. One patient with stable disease and one patient with initial progressive disease had marked tumor responses to salvage chemotherapy. The median overall survival was 8.8 months with a median potential follow-up of 12.7 months as of June 13, 2013.

In summary, although regimen B was effective at immune depletion and delaying or preventing SS1P-neutralizing antibody formation, it was associated with more toxicities. In addition, no responses were seen with Regimen B. In view of the above findings we propose an amendment to the existing protocol to confirm the response rates seen in the Regimen A pilot in larger cohorts of patients with both pleural and peritoneal mesothelioma. The underlying rationale on having 2 separate cohorts for pleural and peritoneal mesothelioma being the differing biology of the diseases as well as the variable response rates seen in both malignancies with regimen A during the pilot. In addition to confirming the clinical activity, treating additional patients on regimen A would also enable us to explore the mechanisms underlying responses using prospectively obtained paired tissue samples before and after treatment.

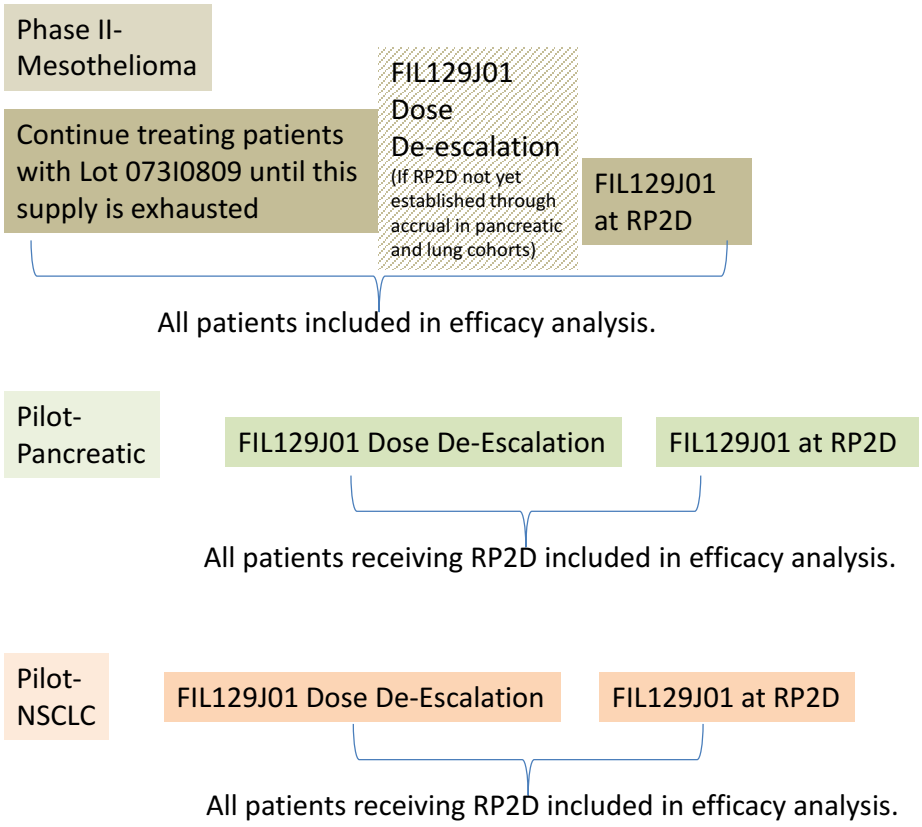
2.7 RATIONALE FOR DOSE DE-ESCALATION STUDY OF DRUG LOT FIL129J01

Based on our previous findings, we have concluded that SS1P lot FIL129J01results in serum SS1P concentrations that are higher than expected.

Briefly, patients on the phase II part of the study, received two different lots of SS1P namely 073I0809 and FIL129J01. We observed increased side effects such as weight gain, edema and pleuritis in patients receiving SS1P Lot FIL129J01. In addition, preliminary analyses of pharmacokinetic data showed higher SS1P blood levels in patients receiving SS1P Lot FIL129J01.

To account for the higher potency of lot FIL129J01 compared with lot 073I0809, we propose a dose de-escalation study to determine the recommended phase 2 dose of SS1(dsFv)PE38 lot FIL129J01 in patients with mesothelioma, pancreatic and lung adenocarcinoma when used in combination with pentostatin and cyclophosphamide. The schema of proposed studies are shown in **Figure 9** below.

Figure 9



2.8 SS1(DSFV) PE38 LOT TRANSITION

Note: As of the amendment dated 6/18/15, the inventory of SS1(dsFv)PE38, NSC 726388, lot 073I0809 is insufficient for further subject enrollment. Mesothelioma subjects enrolled prior to this date were and will continue to be treated with Drug lot 073I0809 until completion of treatment as follows:

35 mcg/kg/dose SS1 (dsFv) PE38 will be administered intravenously over 30 minutes, the dose determined as the MTD in the Regimen A 3+3 dose escalation study.

Table 6: Lot 073I0809 Dose Levels

Dose Level	Dose*		
	SS1(dsFv)PE38 (mcg/kg/dose)	**Pentostatin (mg/m2/dose)**	**Cyclophosphamide (mg)**
Level -1	25	See section **5.4.3**	200
Level 1 (starting level)	35	See section **5.4.3**	200

Newly enrolled (after amendment 6/18/15) mesothelioma patients will be treated on the dose de-escalation schedule or RP2D of lot FIL129J01. The same patient will not be treated with drug from 2 different lots.

2.9 CORRELATIVE STUDIES BACKGROUND

Mesothelin is a 40-kDa cell surface glycoprotein that is highly expressed in mesothelioma, ovarian, pancreatic, lung and some other cancers.[18,19,67] In malignant pleural mesothelioma, the expression of mesothelin is closely related to the histological subtype of the tumor, being expressed on the epithelial but not sarcomatous tumor tissues. On biphasic tumors, mesothelin is expressed on the epithelial component. Small amounts of mesothelin shed into the circulation may be detected by sandwich ELISA. It has been suggested that elevated soluble mesothelin levels may correlate with advanced disease stage and total tumor burden. The biological function of mesothelin is unknown. A trans-intracellular binding activity with CA-125, a tumor antigen routinely used in the diagnosis and monitoring of ovarian cancer has been noted.[20] Among normal human tissues mesothelin is expressed on mesothelial cells of the pleura, pericardium, and peritoneum, but is absent from vital organs including heart, liver, lung, kidney and nervous tissue.

Subjects' sera will be collected for retrospective determination of circulating mesothelin concentrations prior to each cycle and at the end of study. Soluble mesothelin levels, as measured by ELISA, may be a correlate of total tumor burden and may predict PK or therapeutic/toxic response. For details on sample collection procedure and storage instructions, please see Section 9. CA-125 is a routine clinical laboratory service, and CA-125 levels will be assessed by the Department of Laboratory Medicine at the NIH Clinical Center.

3 PATIENT SELECTION

3.1 ELIGIBILITY CRITERIA

3.1.1 Inclusion Criteria Mesothelioma Cohorts (Cohorts 1 and 2 Only)

3.1.1.1 Subjects must have histologically confirmed epithelial or biphasic mesothelioma not amenable to potentially curative surgical resection. However, patients with biphasic tumors that have a ≥ 50% sarcomatoid component will be excluded. The diagnosis will be confirmed by the Laboratory of Pathology / CCR / NCI.

3.1.1.2 Patients must have had at least one prior chemotherapy regimen, with the FDA-approved regimen of a platinum-based therapy in combination with pemetrexed being preferred unless there was a specific contraindication for an individual patient. There is no limit to the number of prior chemotherapy regimens received.

3.1.1.3 Total Bilirubin ≤1.5 X institutional upper limit of normal (ULN)

3.1.2 Inclusion Criteria Lung Adenocarcinoma Cohort (Cohort 3) Only

3.1.2.1 Subjects must have histologically confirmed advanced (Stage IIIB/IV) lung adenocarcinoma. The diagnosis will be confirmed by the Laboratory of Pathology/CCR/NCI.

3.1.2.2 Patients must have had at least one prior therapy for advanced disease [platinum-containing chemotherapy or one of the approved targeted therapies (an approved EGFR

TKI for EGFR mutant tumors or crizotinib and ceritinib for ALK translocated tumors)]. There is no limit to the number of prior chemotherapy regimens received.

3.1.2.3 Mesothelin expression in at least 5% of cells as assessed in archival tumor tissue samples, determined by the IHC assay performed at Laboratory of Pathology / CCR / NCI. Archival samples must be available for eligibility.

3.1.2.4 Total Bilirubin ≤1.5 X institutional upper limit of normal (ULN)

3.1.3 Inclusion Criteria Pancreatic Cancer Cohort (Cohort 4) Only

3.1.3.1 Subjects with recurrent, locally advanced unresectable or metastatic adenocarcinoma of the pancreas. The diagnosis will be confirmed by the Laboratory of Pathology/CCR/NCI.

3.1.3.2 Patients must have had at least one prior chemotherapy for advanced disease. There is no limit to the number of prior chemotherapy regimens received.

3.1.3.3 Total Bilirubin ≤2 X institutional upper limit of normal (ULN)

3.1.4 Inclusion Criteria All Subjects

3.1.4.1 Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm with conventional techniques or as >10 mm with spiral CT scan. See Section **11** for the evaluation of measurable disease.

3.1.4.2 Patients must not have had major surgery, radiation therapy, chemotherapy, biologic therapy (including any investigational agents), or hormonal therapy (other than replacement), within 4 weeks prior to entering the study and must have evidence of stable or progressive disease to be eligible.

3.1.4.3 Age ≥18 years. Since the study diseases are extremely rare in children they are excluded from this study.

3.1.4.4 Performance status (ECOG) ≤ 1 (**Appendix A**).

3.1.4.5 Patients must have adequate organ and marrow function (as defined below).

- leukocytes	≥3,000/mm^3
- absolute neutrophil count	≥1,500/mm^3
- hemoglobin	≥ 9 g/dL
- platelets	≥90,000/ mm^3
- total bilirubin	See guidelines for individual cohorts in sections **3.1.1.3**, **3.1.2.4** and **3.1.3.3**
- AST(SGOT)/ALT(SGPT)	≤3 X institutional ULN (5x if LFT elevations due to liver metastases)
- creatinine	≤1.5 X institutional ULN OR

- creatinine clearance	$\geq$ 45 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal, obtained through calculated (See **Appendix D**) or measured Creatinine Clearance

Patients may be transfused to obtain a hemoglobin of $\geq$9 g/Dl.

3.1.4.6 The effects of SS1(dsFv)PE38, pentostatin, and cyclophosphamide on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (barrier method of birth control; abstinence) for the duration of study therapy and for 3 months after the last dose of therapy. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. While hormonal methods of birth control are effective, we ask that female patients who are participating in the study cease hormonal forms of birth control, as these methods of birth control (birth control pills, injections, or implants) may affect the study drug. Patients must be off hormonal forms of birth control for at least 4 weeks prior to initiating the study.

3.1.4.7 Ability to comply with intravenous administration schedule, and the ability to understand and the willingness to sign a written informed consent document.

3.1.5 Exclusion Criteria (All Subjects)

3.1.5.1 Patients with symptomatic brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. However, patients who have had treatment for their brain metastases and whose brain metastatic disease status has remained stable for at least 4-6 weeks without steroids may be enrolled at the discretion of the principal investigator.

3.1.5.2 Uncontrolled medical illness including, but not limited to, ongoing or uncontrolled, symptomatic congestive heart failure (AHA Class II or worse), uncontrolled hypertension, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.1.5.3 HIV positive patients will be excluded due to a theoretical concern that the degree of immune suppression associated with the treatment may result in progression of HIV infection.

3.1.5.4 Patients with Hepatitis B and C will be excluded.

3.1.5.5 Serum neutralization antibody assay shows $\geq$ 75% neutralization of the SS1 (dsFv) PE38 activity at 200 ng/ml.

3.1.5.6 Patients may not be receiving any other investigational agents.

3.1.5.7 History of another invasive malignancy in the last two years. Adequately treated non-invasive, non-melanoma skin cancers as well as in situ carcinoma of the cervix will be allowed.

3.1.5.8 Prior treatment with drugs of the immunotoxin class.

3.1.5.9 Patients with tumor amenable to potentially curative therapy as assessed by the investigator.

3.1.5.10 Pregnant women are excluded from this study because SS1(dsFv)PE38, pentostatin, and cyclophosphamide have the potential for teratogenic or abortifacient effects. The agents in the trial may also potentially be secreted in milk and therefore breastfeeding women should be excluded. Because of the potential of teratogenic or abortifacient effects women of childbearing potential and men must agree to use adequate contraception (barrier methods) before, during the study and for a period of 3 months after the last dose of the investigational agent.

3.1.5.11 History of allergic reactions attributed to compounds of similar chemical or biologic composition to SS1(dsFv)PE38.

3.2 INCLUSION OF WOMEN AND MINORITIES

Both men and women and members of all races and ethnic groups are eligible for this trial. Every effort will be made to recruit women and minorities in this study.

Accrual targets for this study are as follows:

3.3 TABLE 7: ACCRUAL TARGETS

Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	7	+	10	=	17
Not Hispanic or Latino	20	+	38	=	58
Ethnic Category: Total of all subjects	27 (A1)	+	48 (B1)	=	75 (C1)
Racial Category					
American Indian or Alaskan Native	1	+	2	=	3
Asian	2	+	4	=	6
Black or African American	6	+	12	=	18
Native Hawaiian or other Pacific Islander	1	+	1	=	2
White	17	+	29	=	46
Racial Category: Total of all subjects	27 (A2)	+	48 (B2)	=	75 (C2)
	(A1 = A2)		(B1 = B2)		(C1 = C2)

Accrual Rate:	2	pts/month	**Total Expected Accrual:**	65	Min	75	Max

Projected Start Date of Study: March 1, 2011

3.4 ON-STUDY RESEARCH EVALUATION

3.4.1 History and Physical examination

Complete history and physical examination (including height, weight, vital signs and ECOG performance score) with documentation of

(i) measurable disease,

(ii) narcotic use and pain assessment and

(iii) prior therapies (surgical, radio therapeutic and cytotoxic) will be conducted within 2 weeks prior to starting therapy.

A complete medication history will be obtained prior to starting, including medications at baseline, over the counter medications, homeopathic remedies, vitamins, and alternative therapies, history of opportunistic infections (mucocutaneous candidiasis, oropharyngeal thrush, onychomycosis, disseminated varicella zoster as defined as >2 dermatomes or 2 noncontiguous dermatomes or other infections). Document whether any of the prior infections occurred in the presence of immunosuppressive therapy.

3.4.2 Imaging Studies (Baseline) -

3.4.2.1 A baseline clinical evaluation with CT scans of chest, abdomen and/or pelvis, and areas of known or suspected disease involvement will be completed within 28 days prior to commencement of therapy (i.e. Day 1 of Cycle 1).

3.4.2.2 An MRI may be included when appropriate.

3.4.2.3 FDG-PET scan will be performed to better define the extent of disease and response to therapy, within 28 days prior to the commencement of therapy (i.e. Day 1 of Cycle 1).

3.4.3 Laboratory Evaluation [baseline labs are to be obtained within one week prior to enrollment].

3.4.3.1 Hematological Profile:

CBC with differential and platelet count, prothrombin time, activated partial thromboplastin time.

3.4.3.2 Biochemical Profile:

Electrolytes, BUN, creatinine, glucose, AST, ALT, alkaline phosphatase, bilirubin, albumin, total protein, LDH, calcium, phosphorous, magnesium, and urinalysis.

3.4.3.3 EKG (baseline)

3.4.3.4 Pregnancy test for female patients of childbearing age and anatomic ability.

3.4.3.5 Serum neutralization assay to SS1(dsFv)PE38 at screening to determine the presence of antibodies.

3.4.3.6 Viral Markers Hepatitis Screen – HBsAg, anti-HCV, Anti-HAV IgM

3.4.4 Tissue samples

3.4.4.1 A block of primary tissue (or 10 unstained sections on charged slides) from the time of diagnosis will be required from each patient. Tissue blocks from a known recurrence will be accepted if original tumor samples are unavailable. Referring institutions will send the tumor block or 10 unstained sections on charged slides to CCR/NCI for correlative studies and confirmation of diagnosis. In the lung cancer cohort only, these samples will be tested for mesothelin expression. IHC analysis will be performed in all cohorts.

4 PATIENT REGISTRATION

4.1 REGISTRATION PROCESS

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm)) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail. Please note, it is very important for all registrars to acquire encrypted e-mail from NIH Help Desk, since the verification of registration includes patient's information. A recorder is available during non-working hours.

Questions about eligibility should be directed to the Study Coordinator and Research Nurse:

Yvonne Mallory, RN
10 Center Drive
Bldg 10, Rm 12N214
Bethesda, MD 20892
Telephone: 301-402-0255
Fax: 301-402-8801
Email: malloryy@mail.nih.gov

Technical questions about the form should be directed to the Central Registration Office (301-402-1732).

5 TREATMENT PLAN

5.1 OVERVIEW

5.1.1 Regimen A Mesothelioma Pilot

In this pilot dose escalation study, the first eleven patients received a 30-day initial cycle with pentostatin on days 1, 5, and 9 in combination with oral cyclophosphamide on days 1 through 12.

Patients will then receive SS1(dsFv)PE38 on days 10, 12, and 14. Subsequent cycles consisted of 21-day treatment cycles of pentostatin (Day 1), oral cyclophosphamide (Days 1 through 4) and SS1(dsFv)PE38 (Days 2, 4, and 6), for a maximum of six treatment cycles. Toxicity assessment will be continuous; tumor response assessments will be performed after every 2 cycles and at the end of treatment. Neutralization assays are performed on Days 24 and 30 (+/- 2 days depending on individual patient needs) of Cycle 1. Neutralization assays are performed on Days 16 and 22 (+/- 2 days depending on individual patient needs) of subsequent cycles. These dates correspond to 14 and 20 days respectively after the initial SS1(dsFv)PE38 dose for each cycle, in order to allow for comparisons to previous trials with SS1(dsFv)PE38.

5.1.2 Regimen B Mesothelioma Pilot

Eight additional patients were to be enrolled to receive an intensified pentostatin cyclophosphamide regimen at dose level 1 of SS1(dsFv)PE. These patients received a 38-day initial cycle with pentostatin on Days 1, 5, 9, 13, and 17 in combination with oral cyclophosphamide on days 1 through 20. Patients then received SS1(dsFv)PE38 on days 18, 20, and 22. Subsequent cycles consisted of 25-day treatment cycles of pentostatin (Days 1 and 5), oral cyclophosphamide (Days 1 through 8) and SS1(dsFv)PE38 (Days 6, 8, and 10), for a maximum of six treatment cycles. Toxicity assessment will be continuous; tumor response assessments will be performed after every 2 cycles and at the end of treatment. Neutralization assays were performed on Days 32 and 38 (+/- 2 days depending on individual patient needs) of Cycle 1. Neutralization assays will be performed on Days 20 and 26 (+/- 2 days depending on individual patient needs) of subsequent cycles. These dates correspond to 14 and 20 days respectively after the initial SS1(dsFv)PE38 dose for each cycle, in order to allow for comparisons to previous trials with SS1(dsFv)PE38.

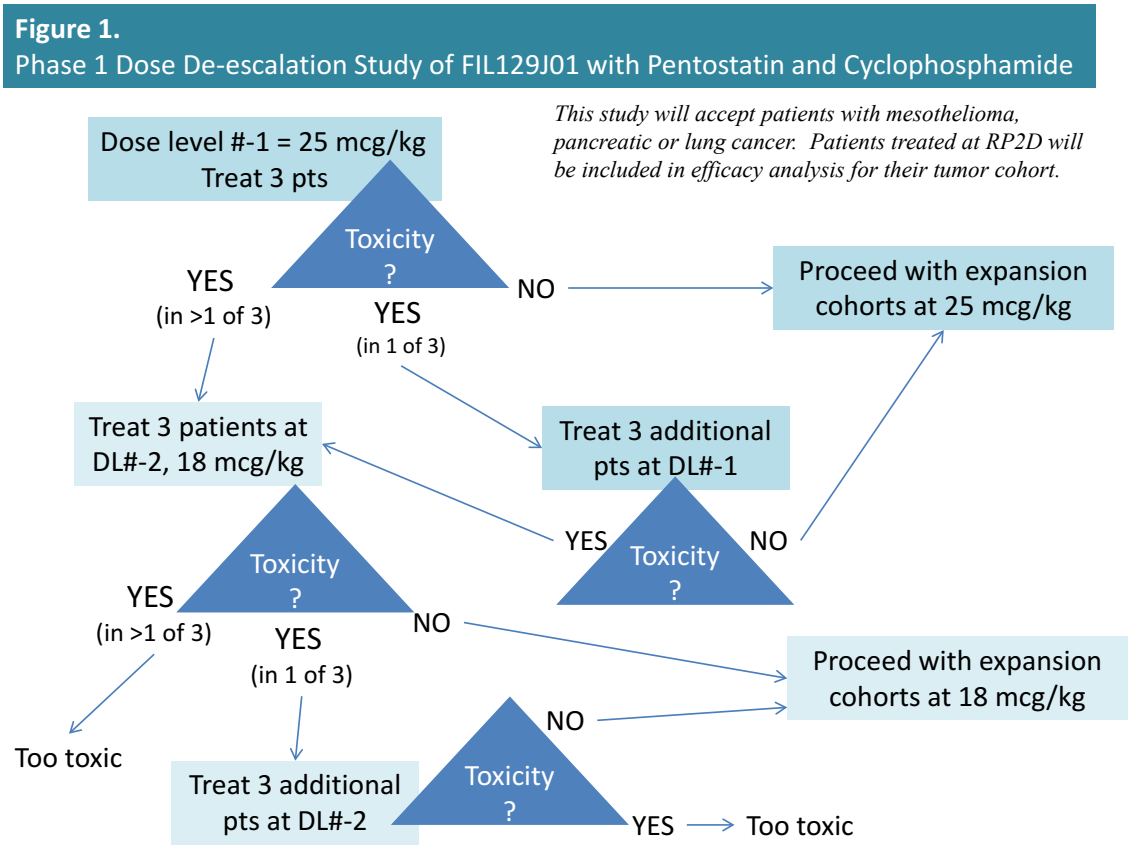
5.1.3 Regimen A Mesothelin Positive Cancers Dose De-escalation Pilot Study of SS1(dsFv)PE38 lot FIL129J01

All lung and pancreatic cancer subjects enrolling onto the study after the approval of the de-escalation study will take part in the dose de-escalation study to determine the recommended phase 2 dose (RP2D) of lot FIL129J01 of SS1(dsFv)PE38. As of amendment version 06/18/15, subjects with mesothelioma will be enrolled to Regimen A – Lot FIL129J01 as well. (Note, no patient will be switched from lot 073I0809 to lot FIL129J01 or vice versa). Initial doses of cyclophosphamide and pentostatin will be as given in **Table 8**.

5.1.3.1 Dose De-Escalation Schedule

Dose escalation will proceed in cohorts of 3–6 patients as shown in **Figure 10**. The recommended phase 2 dose is the first dose level at which no more than 1 of up to 6 patients experience DLT during 1 cycle of treatment, and the dose below that at which at least 2 (of ≤6) patients have DLT as a result of the drug. If a patient did not experience DLT and did not finish treatment, he or she will not be evaluable for toxicity and will be replaced in the dose level.

Figure 10: Dose De-escalation study of FIL129J01 with Pentostatin and Cyclophosphamide



A starting dose of 25 mcg/kg/dose which is about 70% of the current dosing (35 mcg/kg/dose) will be used. If unacceptable toxicity is observed at this dose level, the dose will be lowered to 18 mcg/kg. It is anticipated that the RP2D of SS1P lot FIL129J01 will be established after accrual of 9 patients or less. Patients with mesothelioma, pancreatic or lung adenocarcinoma will be enrolled in this dose de-escalation study.

5.1.4 Regimen A Mesothelioma Phase 2/Mesothelioma Postive Cancers Pilot Expansion Cohorts

If an RP2D is determined in the dose de-escalation study, subsequent patients with lung and pancreatic adenocarcinomas will be enrolled to the lung and pancreatic adenocarcionma pilot expansion study. Subjects with mesothelioma will be enrolled to the phase 2 mesothelioma study at the RP2D of lot FIL129J01 as of the amendment dated 6/18/15. Up to 16 evaluable patients with pleural mesothelioma (cohort 1), up to 10 evaluable patients with peritoneal mesothelioma (cohort 2), up to 10 evaluable patients with lung adenocarcinoma (cohort 3) and up to 10 patients with pancreatic adenocarcinoma (cohort 4) will be enrolled to Regimen A as described above, for a maximum of 4 cycles, in order to further help establish the efficacy of Regimen A. Cohorts 1 and 2 will be subject to an early stopping rule as described in section **13.1.3**. Patients in cohorts 3 and 4 will be accrued according to a 2 stage Minimax design as described in section **13.1.4**.

Table 8: Lot FIL129J01 Dose Levels			
Dose Level	**Dose***		
	SS1(dsFv)PE38 (mcg/kg/dose)	**Pentostatin (mg/m2/dose)**	**Cyclophosphamide (mg)**
Level -2	18	See section **5.4.3**	200
Level -1 (starting level)	25	See section **5.4.3**	200

Table 9: Cycle 1 - Treatment Regimen A

TREATMENT REGIMEN FOR CYCLE 1					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Pentostatin[a]	*Prior to infusion, infuse 1 L of 0.9% sodium chloride over 30-60 minutes.* *Premedicate:* *- Dexamethasone 12 mg IV, and ondansetron 8 mg IV 60 min prior to each dose* *-Dexamethasone 4 mg po daily for 2 days following pentostatin (i.e. Days*[c] *2, 3, 6, 7, 10, and 11 of Cycle 1)* *Ondansetron 8 mg po Q 12 h days*[b] *1 through 12*	See section **5.4.3** for starting dose See Section **6.2.2** for dose modifications	*IV over 30-60 minutes*	*Days 1, 5, and 9*	*30 days*
Cyclophosphamide	*Hydration: Drink 2-4 liters of fluid per day to maintain clear color urine. Empty bladder frequently and prior to sleeping*	200 mg	*PO in the a.m.*	*Daily from Day 1 through 12*	
SS1 (dsFv) PE38	*500 mL of 0.9% Sodium Chloride IV over 2-4 hours before and after each dose.* *Ranitidine 150 mg po, hydroxyzine 10-25 mg po, ONE hour prior to dose* *Dexamethasone 4mg po Q12h or QD as clinically indicated on days*[c] *10- 15*	See **Table 8**	*IV over 30 minutes (do not filter)*	*Days 10, 12 and 14*	

[a] If a subject develops evidence of renal insufficiency during the trial, then dose adjustments will be performed, as per Section **6**. A Creatinine Clearance may be measured or calculated (see **Appendix D**) if such dose adjustments are deemed necessary.

[b] One oral evening dose of ondansetron should be administered on each day of ondansetron IV dosing (Day 1, 5 and 9)

[c] On days 10 and 11 a single dexamethasone dose should count towards both pentostatin and SS1(dsFv)PE38 prophylaxis

Table 10: Cycles 2 through 4 - Treatment Regimen A

TREATMENT REGIMEN FOR CYCLES 2 THROUGH 4					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Pentostatin[a]	*Prior to infusion, infuse 1 L of 0.9% sodium chloride over 30-60 minutes.* *Premedicate:* *-Dexamethasone 12 mg IV, and ondansetron 8 mg IV 60 min prior to each dose* *-Dexamethasone 4 mg po daily for 2 days following pentostatin (i.e. Days*[c] *2 and 3)* *Ondansetron 8 mg po Q 12 h days*[b] *1 through 4*	See section **5.4.3** for starting dose See Section **6.2.2** for dose modifications	*IV over 30-60 minutes*	*Day 1, of each cycle*	*3 weeks (21 days)*
Cyclophosphamide	*Hydration: Drink 2-4 liters of fluid per day to maintain clear color urine. Empty bladder frequently and prior to sleeping*	*200 mg*	*PO in the a.m.*	*Daily from Day 1 through 4 of each cycle*	
SS1 (dsFv) PE38	*500 mL of 0.9% Sodium Chloride IV over 2-4 hours before and after each dose.* *Ranitidine 150 mg po, hydroxyzine 10-25 mg po, ONE hour prior to dose* *Dexamethasone 4mg po Q12h or QD as clinically indicated on days*[c] *2 - 7*	See **Table 8**.	*IV over 30 minutes (do not filter)*	*Days 2, 4 and 6 of each cycle*	

[a] If a subject develops evidence of renal insufficiency during the trial, then dose adjustments will be performed as per Section 6. A Creatinine Clearance may be measured or calculated (See **Appendix D**) if such dose adjustments are deemed necessary.

[b] One oral evening dose of ondansetron should be administered on each day of IV ondansetron dosing (Day 1)

[c] On days 2, and 3 a single dexamethasone dose should count towards both pentostatin and SS1(dsFv)PE38 prophylaxis

5.2 AGENT ADMINISTRATION

Treatment will be administered as outpatient or inpatient. Reported adverse events and potential risks for pentostatin, cyclophosphamide and SS1(dsFv)PE38 are described in Section 7. Appropriate dose modifications for SS1(dsFv)PE38 and pentostatin and cyclophosphamide are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

SS1 (dsFv) PE38 may be administered through either central or peripheral venous access.

5.3 SS1 (DSFV) PE38

5.3.1 Premedication

Patient will receive ranitidine, 150 mg tablet, and hydroxyzine 10 mg or 25 mg tablet, one time dose, at one hour before administration of SS1 (dsFv) PE38. Patient will receive dexamethasone 4mg PO Q12 hours or QD as clinically indicated on:

Regimen A: days 10 through 15 of cycle 1 and on days 2 through 7 of subsequent cycles.

5.3.2 Preparation

See Section 8 for preparation instructions.

5.3.3 Administration

All subjects will be dosed with SS1(dsFv)PE38 according to the Regimen A schedule below:

Regimen A: Days 10, 12, and 14 of Cycle 1 and Days 2, 4, and 6 of subsequent cycles.

Please see section **2.8** for treatment information on lot 07I0809.

5.3.3.1 Drug lot FIL129J01

As of amendment dated 6/18/15, all newly enrolled patients will be treated from drug lot FIL129J01. Subjects of the dose de-escalation cohort will be treated at the dose level determined by the dose de-escalation schedule. Once the RP2D is determined, newly enrolled patients receiving lot FIL129J01 will be treated at that dose.

5.4 PENTOSTATIN

5.4.1 Premedication

See anti-emetic therapy below.

5.4.2 Preparation

Pentostatin will be reconstituted by CC Pharmacy Department to a concentration of 2 mg/ml as per vial instructions. The appropriate patient specific dose will then be added to 0.9% sodium chloride to make up a total volume of 50 mL and infused intravenously over 30 to 60 minutes.

5.4.3 Administration

Each dose of pentostatin will be administered intravenously over 30-60 minutes. Based on published data[68], pentostatin dosing will initially be:

- 4 mg/m^2 of pentostatin if CrCl ≥ 60 mL/min/1.73 m^2
- 2 mg/m^2 of pentostatin if CrCl ≥ 45 but < 60 mL/min/1.73 m^2.

CrCl obtained by 24 hour urine or calculated by the Cockcroft-Gault formula (See **Appendix D**)

If a subject develops evidence of renal insufficiency during the induction interval of pentostatin and cyclophosphamide therapy (increase in serum creatinine level), then dosing will be modified as described in Section **6.2.2**.

5.4.4 Monitoring

Creatinine levels will be obtained prior to each dose of pentostatin and CrCl calculated. If a subject's creatinine rises to >1.5 X ULN during the conditioning regimen of pentostatin and cyclophosphamide or during therapy, then the CrCl (calculated – see **Appendix D**) should be repeated prior to subsequent dosing of pentostatin if there is a 33% increase in serum creatinine value from prior measurement.

Because pentostatin is rarely associated with neurologic toxicity (seizure, coma), special attention should be paid towards evaluating CNS toxicity. In the event that chemotherapy is associated with any neurologic toxicity of grade 2 or greater severity, the institutional PI should be contacted to discuss whether further pentostatin therapy and further protocol therapy is warranted.

5.4.5 Anti-emetic therapy:

Pentostatin can be emetogenic. Anti-emetic regimen guideline is described below. Variation of this regimen, as described below, is allowed at the discretion of the PI. (For detailed instructions, please refer to **Table 9** and **Table 10** for Regimen A)

5.4.5.1 Dexamethasone will be administered by IV infusion 60 minutes prior to each dose of pentostatin; the dose of dexamethasone will be 12 mg.

5.4.5.2 In addition, oral dexamethasone will be administered on the next two days following pentostatin therapy at a dose of 4 mg each day.

5.4.5.3 Ondansetron will be administered at a dose of 8 mg by IV infusion 60 minutes prior to each dose of pentostatin.

5.4.5.4 Ondansetron will be administered at an oral dose of 8 mg (tablets) every 12 hours on:

Regimen A: Days 1 through 12 (one evening dose will be administered on days 1, 5, and 9) during Cycle 1, and Days 1 through 4 for subsequent cycles (one evening dose will be administered on days 1).

5.4.5.5 Aprepitant may be added as needed to the anti-emetic regimen in patients with uncontrolled nausea and vomiting requiring additional anti-emetic therapy.

5.5 CYCLOPHOSPHAMIDE

5.5.1 Premedications

5.5.1.1 Hydration

Because cyclophosphamide can cause cystitis, it is important for patients to stay well hydrated. At a minimum, patients should drink at least 2 to 4 liters of fluid per day to maintain a clear color to the urine. It is also especially important to void the bladder prior to sleeping.

5.5.2 Dose and Schedule

5.5.2.1 Oral cyclophosphamide will be given on:

Regimen A: Days 1 through 12 of Cycle 1 and Days 1 through 4 of subsequent cycles

5.5.2.2 The dose of cyclophosphamide will be 200 mg each day (PO), with some provision for dose reduction as per Section **6.2.1**;

5.5.2.3 IV infusion of this same dose may be allowed if a patient is unable to tolerate oral therapy.

5.5.2.4 For IV infusion, cyclophosphamide will be reconstituted by CC Pharmacy Department to a concentration of 20 mg/ml as per vial instructions. A 200 mg dose will then be diluted in 100 ml of D5W or 0.9% sodium chloride and infused intravenously over 30 minutes. Patients will be instructed to drink an adequate amount of fluids and empty their bladders frequently during cyclophosphamide administration.

5.5.3 Monitoring

Patient compliance with the oral cyclophosphamide and other oral medications will be assessed by the research nurses at each patient's follow-up visit to NCI. The patient will be requested to maintain a medication diary of each dose of cyclophosphamide. The medication diary will be returned to clinic staff at the end of each cycle.

5.5.3.1 During the pentostatin/cyclophosphamide treatment interval of Cycle 1, CBC with differential, chem-14, should be repeated twice per week, on:

Regimen A: Days 1, 5, 9, and 12 (+/- 1 day). Subsequent cycles will have repeat evaluation of CBC with differential and chem-14 repeated on Days 1, 9, and 16 (+/- 2 days).

5.5.3.2 Urinalysis should be performed on:

Regimen A: Days 1, 5, and 9 (+/- 1 day) of Cycle 1. Urinalysis will be performed on Days 1 and 6 (+/- 1 day) of Subsequent cycles.

5.5.4 Toxicities

The primary toxicities anticipated with the pentostatin and cyclophosphamide regimen may include hematologic toxicity, nausea, mucositis, and toxicity relating to infectious complications. The regimen has been studied for use in chronic lymphocytic leukemia, with an acceptable toxicity level and decreased myelosuppression compared to other regimens.[63,66]

5.6 DEFINITION OF DOSE-LIMITING TOXICITY (DLT)

Only those adverse events deemed suspected of a relationship to SS1 (dsFv) PE38 or the combination of agents will be used in the definition of DLT. Adverse Events that are considered disease-related, and not suspected of relationship to SS1(dsFv)PE38 or the combination, will not be considered dose-limiting toxicities. Any toxicities occurring prior to day 10 of Cycle 1 will be attributed to the pentostatin/cyclophosphamide regimen. See Section **7.4** for definitions of adverse event characteristics.

The definition of DLT in these studies uses NCI's Common Terminology Criteria for Adverse Events (CTEP Version 4.0 of the CTCAE).

Dose limiting toxicity is defined as: Any ≥ Grade 3 toxicity with the following **additional criteria**:

5.6.1 Subjects with Normal or Grade 1 or Grade 2 Abnormal Hematologic Parameters at Baseline

- Only Grade 4 hematologic toxicity (with the exception of lymphopenia) lasting greater than five days will be considered a DLT. Lymphocyte count and subsets will not be considered in the definition of DLT.

5.6.2 Allergic Reactions

- Grade 2 allergic reactions of bronchospasm or urticaria, or any ≥ Grade 3 allergic reaction, in the presence of pre-medication, will be considered a DLT.

5.6.3 Any Grade 3 or greater, non-hematologic toxicity will be considered a DLT with the following <u>exceptions:</u>

5.6.3.1 Grade 3 electrolyte changes unless associated with clinically significant consequences or intervention. Any Grade 4 levels will be considered a DLT.

5.6.3.2 Hypocalcemia toxicity grade should be assigned based on the calcium level corrected for degree of hypoalbuminemia according to the following formula: For every albumin decrease of (↓) 1 gram/dL: a total calcium increase of (↑) 0.2 mmol/L is to be made

5.6.3.3 Grade 3 transaminase, alkaline phosphatase, or bilirubin elevation, provided there is resolution to values required for study entry prior to the start of the next cycle.

5.6.3.4 Grade 3 fever

5.6.3.5 Grade 3 infection unless relationship to SS1(dsFv)PE38 is suspected.

5.6.3.6 Grade 3 neutrophil count (decreased) associated with fever unless relationship to SS1(dsFv)PE38 is suspected.

5.6.3.7 Grade 3 hypoalbuminemia lasting no more than 7 days that occurs in the absence of vascular leak syndrome. In patients with baseline Grade 1 or 2 hypoalbuminemia, grade 3 hypoalbuminemia will not be considered a DLT regardless of duration.

5.7 STAGGERING AND OPENING OF NEW COHORTS (MESOTHELIOMA PILOT ONLY)

In the first cohort, the first two subjects will not be treated on the same day.

Opening the enrollment into any new cohort will be staggered so that no new cohort subject shall receive SS1 (dsFv) PE38 until the subjects of the previously treated cohort have completed days 1 to 30 of cycle 1 dosing without meeting DLT criteria. The first three subjects in each cohort must complete cycle 1. Patients who do not complete one cycle due to non-toxicity related reasons will be considered inevaluable and should be replaced.

The Study Chair (or his covering physician) will authorize the opening of cohorts, subsequent to the first cohort, and will notify the investigator and sponsor of his decision by e-mail or phone. This decision will be made when all subjects from the prior cohort have reached cycle 1 day 30 and the specific escalation criteria (see above) have been met.

5.8 General Concomitant Medication and Supportive Care Guidelines

No concomitant use of alternative, complementary therapies or over-the-counter agents will be allowed without approval of the PI. The following supportive care guidelines will be instituted regardless of dose cohort or cycle of therapy.

5.8.1 Neutropenia

In the event that grade 4 neutropenia develops during chemotherapy (absolute neutrophil count [ANC] < 500 cells per mm^3 and a first fever with T $\geq$ 38.3°C), filgrastim therapy will be instituted at a dose of 5 mcg/kg per day by subcutaneous injection. Filgrastim therapy should be discontinued if the neutrophil toxicity has resolved to grade 0 at the next CBC determination (bi-weekly determinations; ANC > 1500 cells per mm^3).

5.8.2 Infection Prophylaxis

(For a full description of infection prophylaxis please refer to the NIH BMT Consortium Supportive Care Guidelines at http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml)

Table 11: Infection Prophylaxis Regimen

Agent	***Indication***	***Dose***	***Schedule***
Sulfamethoxazole / Trimethoprim	PCP prophylaxis - all subjects	800 mg / 160 mg PO once daily on 3 days/week	Start on Day 1 of Cycle 1 and continue until a subject is completely off immune suppression therapy and the CD4 count is > 200 for 3 months.
Acyclovir[a]	HSV/VSV prophylaxis – all subjects	800 mg PO twice daily	Start on Day 1 of Cycle 1 and continue until a subject is completely off immune suppression therapy and the CD4 count is > 200 for 3 months.

[a] Requires dose or schedule modification in renally impaired patients.

5.8.2.1 All recipients will receive prophylaxis against Pneumocystis jirovecii pneumonia, beginning with the P/C treatment. PCP prophylaxis will continue until a subject is completely off immune suppression therapy and the CD4 count is > 200 for 3 months. Trimethoprim/sulfamethoxazole is the preferred regimen.

5.8.2.2 Subjects will receive prophylactic broad-spectrum antibiotics with ceftazidime in the setting of grade 4 neutropenia (ANC < 500/mcL) and a first fever with T ≥ 38.3°C). Recipients with allergy to cephalosporin antibiotics will receive an alternative regimen for prophylaxis as recommended by the infectious diseases consultant. Recipients with neutropenia and fever that persists for longer than 4 days despite broad-spectrum antibiotics will receive empiric antifungal therapy with caspofungin, liposomal amphotericin B, or voriconazole, in accordance with standard practices. Empiric antifungal therapy may be started at other times, if clinically indicated.

5.8.2.3 All subjects will receive acyclovir for prophylaxis against herpes simplex virus and varicella zoster virus infection/reactivation. This therapy will start on day 1 of cycle 1 and continue until the subject is completely off immune suppression therapy and the CD4 count is > 200 for 3 months. Because administration of acyclovir may further compromise renal function in addition to pentostatin, dosage adjustment will be required in the presence of decreased CrCl.

5.8.2.4 All recipients will undergo vaccinations beginning 6 months after the end of cycle 3, as further detailed in the NIH BMT consortium web-site.

5.8.3 Thrombocytopenia

Thrombocytopenia should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should only be given for a platelet count below 10,000/mcl. If invasive procedures are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with standard of practice, usually maintaining a platelet count ≥ 50,000/mcl.

5.8.4 Anemia

Symptomatic anemia should be treated with appropriate red blood cell support and transfusion is recommended if the hemoglobin falls below 8 g/dl. Patients will be allowed to use erythropoietin or analogs prior to entry and during the course of the study.

5.8.4.1 Recipients will receive packed red blood cells as needed to maintain Hb > 8.0 gm/dl (or higher, if clinically indicated). All blood products will be irradiated.

5.8.4.2 Leukocyte filters will be utilized for all blood and platelet transfusions to decrease the risk of CMV infection.

5.8.5 Central Venous Access (optional)

Central venous access devices such as a temporary internal jugular line, PICC lines via the brachial vein, semi-permanent HICKMAN®, GROSHONG®, or medi-port implanted devices are acceptable for this study. All devices will have nursing supervision to include patient self-care and cleaning/flushing of the devices.

5.8.6 Nutrition and Supportive Care

Nutritional assessment and psychological support: Neoplasms are commonly complicated by malnutrition. Patients with weight loss or evidence of wasting syndrome should have a nutritional consult. Patients who are having emotional difficulties dealing with their treatment, and disease, or those patients who request assistance, will be referred to a Social Worker for evaluation and support. If mucositis prevents adequate PO intake expected to last one week or more, parenteral hyperalimentation should be instituted. The dietary service will provide recommendations for macronutrient supplementation and the Pharmacy Department will provide support for the electrolytes and incompatibility issues. Oral intake will resume when clinically appropriate under the supervision of the dietary service of the Clinical Center Hospital.

5.8.7 Pleuritic pain

Pleuritic pain will be managed symptomatically and may involve the use of low-dose narcotic medications such as morphine sulfate. NSAIDs should be avoided, due to potential deleterious effects on kidney function.

5.8.8 Capillary Leak Syndrome

Capillary Leak Syndrome, which has been seen with SS1(dsFv)PE38, will be managed expectantly with intravenous fluids, albumin administration, or diuretics, as needed and determined by the clinical personnel.

5.9 DURATION OF THERAPY

In the absence of treatment delays due to adverse events, patients will receive up to 4 treatment cycles of SS1(dsFv)PE38 therapy, unless one of the following criteria applies:

- Serum neutralization assay (Day 24 (+/- 2 days) of Cycle 1 or Day 16 (+/- 2 days) of each subsequent cycle) shows ≥ 75% neutralization of the SS1 (dsFv) PE38 activity at 1000 ng/ml.
- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Delay of treatment of ≥ 3 weeks
- Dose limiting adverse events(s)
- Non compliance to therapy regimen as determined by the principal investigator or associate investigator
- Patient decides to withdraw, stops therapy, or starts a new treatment
- Deterioration of the patient’s condition that render further treatment unacceptable in the judgment of the investigator
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Death

The reason for treatment removal and the date the patient was removed must be documented in the Case Report Form.

5.10 DURATION OF FOLLOW UP

After four cycles, patients with responding or stable disease will be evaluated in clinic with physical examination, laboratory testing, and repeat imaging every 3 months until off study criteria are met. If a patient goes off treatment due to a drug related adverse event, the patient will be followed until the event resolves, returns to baseline or stabilizes. In addition, if the patient goes off treatment for other than disease progression, the patient will continue to be evaluated in clinic with physical examination, laboratory testing, and tumor restaging every 12 weeks +/- 1 week until Progressive Disease (PD). Patients who develop disease progression will be referred back to the local physician, but will be contacted at least yearly for survival.

5.11 CRITERIA FOR REMOVAL FROM STUDY

Patients will be removed from Study for the following reasons:

- Patient elects to cease participation and withdraws consent to the study
- Patient loses capacity to provide informed consent
- Patient non-compliance with protocol guidelines
- Death

Information will otherwise be collected from patients to better understand the effects of the study medication on progression-free survival, overall survival, and other statistical endpoints. The reason for Study removal and the date the patient was removed must be documented in the Case Report Form.

5.12 OFF PROTOCOL THERAPY AND OFF-STUDY PROCEDURE

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off study. An Participant Status Update Form from the website (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm)) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

6 DOSING DELAYS/DOSE MODIFICATIONS

6.1 GENERAL GUIDANCE

- Grading of AEs for dose modification is based on CTEP Version 4.0 of CTCAE
- The maximum delay of SS1(dsFv)PE38 for any reason, including toxicities, is 3 weeks.
- If a patient is discontinued from treatment due to treatment related toxicities, the patient should be followed for assessment of the toxicities until the AEs resolve or are deemed irreversible.

6.2 DOSE MODIFICATIONS FOR PENTOSTATIN AND CYCLOPHOSPHAMIDE

The goal of the pentostatin and cyclophosphamide regimen is to uniformly and safely reduce the number of host immune cells prior to SS1(dsFv)PE38 administration. The target goal for this immune depletion has been defined as an absolute lymphocyte count (ALC) of ≤ 200 cells/mcL. Given this immune depletion goal, and the desire to minimize toxicity related to cyclophosphamide, the following dose modifications of cyclophosphamide will be utilized. The goal of this dose adjustment schedule will be to ensure that each patient finishes chemotherapy with an ALC < 200, while limiting the chance that patients finish chemotherapy with severe reductions in absolute neutrophil count (ANC) < 250 cells/ mcL.

6.2.1 Cyclophosphamide

The dose of cyclophosphamide will be adjusted for ANC during Cycle 1, as follows:

Table 12: Cyclophosphamide Dose Adjustment for Cycle 1

Dose Adjustment for Cyclophosphamide			
Day of Cycle[1]	**ANC Value at time of Evaluation[2]**	**Platelet count at time of evaluation[2]**	**Cyclophosphamide Dose[3]**
1	≥1000	≥ 75,000	200
5	≥1000 500-999 <500	≥ 75,000 50,000-74,999 <50,000	200 100 0
9	≥1000 500-999 <500	≥ 75,000 50,000-74,999 <50,000	200 100 0

[1] Day of cycle: Days 1, 5, and 9 of Cycle 1 (all are on days which include pentostatin doses). A 24-hour difference in timing may be allowed for logistical purposes).

[2] ANC and platelet values expressed as cells per microLiter.

[3] Cyclophosphamide dose expressed as mg per day; the indicated dose will be administered daily until the next ANC and platelet measurement. Dosing will be based on whichever value (platelets or ANC) results in the lowest cyclophosphamide dose.

The dose of cyclophosphamide will be adjusted for ANC during subsequent cycles, as follows:

Table 13: Cyclophosphamide Dose for Cycles 2 through 4

Dose for Cyclophosphamide			
Day of Cycle[1]	**ANC Value at time of Evaluation[2]**	**Platelet count at time of evaluation[2]**	**Cyclophosphamide Dose[3]**
1	≥1000 <1000	≥75,000 <75,000	200 Delay cycle (see

			section **6.4.2**)

[1] Day of cycle: Day 1 for Cycles 2 through 4 (which are days of pentostatin doses). A 24-hour difference in timing may be allowed for logistical purposes).

[2] ANC and platelet values expressed as cells per microLiter.

[3] Cyclophosphamide dose expressed as mg per day; the indicated dose will be administered daily until the next ANC and platelet measurement. Dosing will be based on whichever value (platelets or ANC) results in the lowest cyclophosphamide dose.

6.2.2 Pentostatin

The dose of pentostatin will be adjusted for changes in kidney function as follows:

- If calculated CrCl* decreases during study implementation to between 40 and 59 ml/min, give 2 mg/m^2 of pentostatin.
- If calculated CrCl* decreases during study implementation to between 30 and 39 ml/min, give 1 mg/m^2 of pentostatin.
- If calculated CrCl* falls below 30 ml/min, pentostatin will be held until CrCl goes above 30. If dosing is delayed ≥ 3 weeks, the patients will be taken off treatment.

*CrCl will be calculated using the Cockroft-Gault formula (See **Appendix D**).

6.3 SS1(DSFV)PE38 DOSE MODIFICATION

If a patient experiences a toxicity defined as a DLT per section **5.6** of the protocol, the subject will be reduced 1 dose level. Only 1 dose reduction is allowed. In the dose de-escalation portion of the study, no dose reduction is permitted, and subjects that experience a DLT will not receive further treatment.

Each patient will have serum tests for neutralizing antibodies during eligibility evaluation and at Day 24 of Cycle 1 and Day 16 of subsequent cycles (+/- 2 days), which will be the equivalent of 14 days after the first dose of SS1(dsFv)PE38 administration during each respective cycle. Patients will receive a subsequent cycle of SS1(dsFv)PE38 only if the serum neutralization assay shows less than 75% neutralization of the SS1 (dsFv) PE38 activity at 1000 ng/ml. Patients whose lab tests show greater than or equal to a 75% neutralization of SS1(dsFv)PE38 activity will not be eligible to receive further SS1(dsFv)PE38.

6.3.1 Management of Expected SS1(dsFv)PE38 Toxicities which are not Dose-Limiting

Subjects will be premedicated with 10-25 mg hydroxyzine and 150 mg ranitidine orally 1 hour prior to each SS1(dsFv)PE38 dose.

To prevent renal insufficiency, subjects will receive 500 mL of 0.9% Sodium Chloride intravenously over two to four hours prior to and after administration of each dose of SS1(dsFv)PE38.

Pleuritic pain will be managed symptomatically and may involve the use of low-dose narcotic medications such as morphine sulfate. NSAIDs should be avoided, due to potential deleterious effects on kidney function.

Vascular Leak Syndrome, which has been seen with SS1(dsFv)PE38, will be managed expectantly with intravenous fluids, albumin administration, or diuretics, as needed and determined by the clinical personnel.

6.4 RE-TREATMENT AND DOSE MODIFICATION

Subjects without DLT will be eligible to receive a subsequent cycle of SS1(dsFv)PE38 at the same dose with at least 20 days between the first doses of the respective cycles for up to a total of up to 4 cycles. There will be no dose modifications to SS1(dsFV)PE38. The medication will be given or held according to the guidelines outlined in this protocol.

6.4.1 Suspension of Dosing within a Cycle

Subsequent doses during a cycle (i.e., Days 12, and 14 of Cycle 1 and Days 4 and 6 of subsequent cycles) for subjects who experience toxicity will be as follows:

6.4.1.1 Dosing will be suspended for subjects with a dose limiting toxicity that develops prior to receiving the last SS1(dsFv)PE38 dose of a cycle. Such subjects will be evaluated as if the cycle had been completed.

6.4.1.2 Dosing may be delayed for subjects with AE less than dose limiting prior to receiving the last dose of a cycle, at the discretion of the PI. In such a case, dosing may be delayed for up to 5 days.

6.4.2 Delay of Cycle

Cycles 2 - 4 may be delayed for up to two weeks to accommodate schedule conflicts, to avoid starting the cycle on a weekend or to permit resolution of an adverse event. ANC counts must be ≥1000 cells/μL and platelet counts must be ≥ 75,000 on day 1 of cycle 2 through 4 in order for subjects to proceed on the cycle. Delays of SS1(dsFv)PE38 during cycle 1 greater than or equal to three weeks from start of therapy (i.e., cycle 1 day 1) will result in subject discontinuation from therapy.

7 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

7.1 DEFINITIONS

7.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections 7.5 and 7.7.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 ADVERSE EVENT REPORTING TO CTEP (NA AS OF CTEP STATUS – ADMINISTRATIVELY COMPLETE)

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.3) and the characteristics of an observed AE (Section 7.4) will determine whether the event requires expedited reporting (via CTEP-AERS) **in addition** to routine reporting.

7.3 COMPREHENSIVE ADVERSE EVENTS AND POTENTIAL RISKS LISTS (CAEPRS)

7.3.1 CAEPRs for CTEP-Supplied Investigational Agent(s)

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
SS1(dsFv)-PE38 (NSC 726388)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. The CAEPR does not provide frequency data; refer to the Investigator's Brochure for this information. Below is the CAEPR for SS1(dsFv) PE38.

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 1.3, July 26, 2013[1]

Adverse Events with Possible Relationship to SS1(dsFv) PE38 (CTCAE 4.0 Term)		**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
CARDIAC DISORDERS		
	Pericardial effusion	
	Sinus tachycardia	
GASTROINTESTINAL DISORDERS		
	Abdominal pain	***Abdominal pain (Gr. 2)***
	Ascites	
	Diarrhea	
	Nausea	***Nausea (Gr. 2)***
	Vomiting	***Vomiting (Gr. 2)***

Adverse Events with Possible Relationship to SS1(dsFv) PE38 (CTCAE 4.0 Term)		**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
	Chills	***Chills (Gr. 2)***
	Edema limbs	***Edema limbs (Gr. 2)***
	Fatigue	***Fatigue (Gr. 3)***
	Fever	***Fever (Gr. 2)***
	General disorders and administration site conditions - Other (edema, NOS)	***General disorders and administration site conditions - Other (edema, NOS) (Gr. 3)***
	Non-cardiac chest pain	***Non-cardiac chest pain (Gr. 2)***
	Pain	
IMMUNE SYSTEM DISORDERS		
	Allergic reaction	***Allergic reaction (Gr. 2)***
INVESTIGATIONS		
	Alanine aminotransferase increased	***Alanine aminotransferase increased (Gr. 2)***
	Aspartate aminotransferase increased	***Aspartate aminotransferase increased (Gr. 2)***
	Weight gain	***Weight gain (Gr. 2)***
METABOLISM AND NUTRITION DISORDERS		
	Hypoalbuminemia	***Hypoalbuminemia (Gr. 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
	Arthralgia	***Arthralgia (Gr. 2)***
	Back pain	***Back pain (Gr. 2)***
	Myalgia	***Myalgia (Gr. 2)***
NERVOUS SYSTEM DISORDERS		
	Dizziness	
	Headache	***Headache (Gr. 2)***
RENAL AND URINARY DISORDERS		
	Proteinuria	***Proteinuria (Gr. 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS		
	Dyspnea	***Dyspnea (Gr. 2)***
	Hypoxia	
	Pleural effusion	***Pleural effusion (Gr. 1)***
	Pleuritic pain	***Pleuritic pain (Gr. 2)***

Adverse Events with Possible Relationship to SS1(dsFv) PE38 (CTCAE 4.0 Term)		**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
	Pruritus	***Pruritus (Gr. 2)***
	Rash maculo-papular	***Rash maculo-papular (Gr. 2)***
	Urticaria	***Urticaria (Gr. 2)***
VASCULAR DISORDERS		
	Capillary leak syndrome	***Capillary leak syndrome (Gr. 2)***
	Hypotension	***Hypotension (Gr. 2)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

Also reported on SS1(dsFv) PE38 trials but with the relationship to SS1(dsFv) PE38 still undetermined:

CARDIAC DISORDERS - Atrial fibrillation; Cardiac disorders - Other (dilated right heart); Myocardial infarction; Palpitations; Pericarditis; Supraventricular tachycardia

GASTROINTESTINAL DISORDERS - Constipation; Dyspepsia; Flatulence

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema trunk

INVESTIGATIONS - CPK increased; Cardiac troponin I increased; Cardiac troponin T increased; Creatinine increased

METABOLISM AND NUTRITION DISORDERS - Acidosis; Anorexia

NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Tumor pain

NERVOUS SYSTEM DISORDERS - Dysgeusia

PSYCHIATRIC DISORDERS - Confusion

RENAL AND URINARY DISORDERS - Hematuria

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Pneumonitis; Pulmonary edema

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Hyperhidrosis

VASCULAR DISORDERS - Flushing; Thromboembolic event; Vascular disorders - Other (peripheral vascular disorder)

Note: SS1(dsFv) PE38 in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.3.2 Adverse Event List(s) for Commercial Agent(s)

7.3.2.1 Pentostatin

Cardiovascular: hemorrhage, hypotension (3% to 10%); angina pectoris, arrhythmia, AV block, bradycardia, ventricular extrasystoles; heart arrest, heart failure, hypertension, pericardial effusion, phlebitis, pulmonary embolus, sinus arrest, tachycardia, thrombophlebitis, vasculitis (less than 3%).

CNS: Fatigue (42%); headache (17%); neurologic disorder, asthenia (12%); anxiety, confusion, depression, dizziness, insomnia, nervousness, paresthesia, somnolence (3% to 10%); abnormal thinking and dreaming, amnesia, ataxia, convulsions, dysarthria, emotional lability, encephalitis, hallucination, hostility, hyperkinesias, meningism, neuralgia, neuritis, neuropathy, neurosis, paralysis, syncope, twitching, vertigo (less than 3%); CNS toxicity (1%).

Dermatologic: Rash (43%); pruritus (21%); sweating (8%); skin disorder (4%); dry skin, urticaria (3% to 10%); acne alopecia, eczema, petechial rash, photosensitivity (less than 3%).

EENT: Abnormal vision, amblyopia, deafness, dry eyes, earache, labyrinthitis, lacrimation disorder, nonreactive eye, photophobia, retinopathy, tinnitus, watery eyes (less than 3%).

GI: Nausea/vomiting (63%); diarrhea (17%); abdominal pain (16%); anorexia (13%); stomatitis (12%); dental abnormalities, dyspepsia, flatulence, gingivitis (3% to 10%); constipation, dysphagia, glossitis, ileus, unusual taste (less than 3%).

Genitourinary: Abnormal kidney function, amenorrhea, breast lump, decreased libido, gout, impotence, nephropathy, renal failure, renal insufficiency, renal stone (less than 3%).

Hematologic-Lymphatic: Leukopenia (22%); anemia (8%); thrombocytopenia (6%); agranulocytosis (3% to 10%); acute leukemia, aplastic anemia, hemolytic anemia (less than 3%).

Hepatic: Hepatic disorder/elevated LFTs (2%).

Hypersensitivity: Allergic reaction (2%).

Lab Tests: Elevated creatinine (3% to 10%); hypercalcemia, hyponatremia (less than 3%).

Musculoskeletal: Myalgia (19%); arthralgia (6%); arthritis (less than 3%).

Respiratory: Coughing/increased cough (20%); upper respiratory tract infection (13%); dyspnea, rhinitis, pharyngitis (11%); asthma (3% to 10%); bronchospasm, laryngeal edema (less than 3%).

Miscellaneous: Fever (46%); chills (19%); pain, viral infection (8%); infection (7%); chest pain, death, face edema, peripheral edema (3% to 10%); flu-like symptoms, hangover effect, neoplasm (less than 3%).

7.3.2.2 Cyclophosphamide

Digestive System: Nausea and vomiting commonly occur; anorexia and, less frequently, abdominal discomfort or pain and diarrhea may occur. Isolated reports of hemorrhagic colitis, oral mucosal ulceration and jaundice occurring during therapy.

Skin and Its Structures: Alopecia occurs commonly Skin rash occurs occasionally; pigmentation changes and changes in nails can occur. Very rare reports of Stevens-Johnson syndrome and toxic epidermal necrolysis have been received during postmarketing.

Hematopoietic System: Leukopenia occurs in patients treated with Cyclophosphamide, is related to the dose of drug, and can be used as a dosage guide. Neutropenia and fever without documented infection has been reported in neutropenic patients.

Thrombocytopenia or anemia develops occasionally in patients treated with Cyclophosphamide. These hematologic effects usually can be reversed by reducing the drug dose or by interrupting treatment. Recovery from leukopenia usually begins in 7 to 10 days after cessation of therapy.

Urinary System: cystitis and urinary bladder fibrosis.

Hemorrhagic ureteritis and renal tubular necrosis have been reported to occur in patients treated with Cyclophosphamide. Such lesions usually resolve following cessation of therapy.

Infections: Infections.

Carcinogenesis.

Respiratory System: Interstitial pulmonary fibrosis has been reported in patients receiving high doses of Cyclophosphamide over a prolonged period.

Other: Anaphylactic reactions; death; possible cross-sensitivity with other alkylating agents has been reported. SIADH (syndrome of inappropriate ADH secretion); malaise and asthenia have been reported as part of the postmarketing experience.

7.4 ADVERSE EVENT CHARACTERISTICS

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the CTEP Version 4.0 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. The CTEP Version 4.0 of the CTCAE is identified and located on the CTEP website at (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. All appropriate treatment areas should have access to a copy of the CTEP Version 4.0 of the CTCAE.
- **'Expectedness'**: AEs can be 'Unexpected' or 'Expected' (see Section **7.4** above) for expedited reporting purposes only. 'Expected' AEs (the ASAEL) are ***bold and italicized*** in the CAEPR (Section **7.3.1**).

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.5 EXPEDITED ADVERSE EVENT REPORTING (NA AS OF CTEP STATUS – ADMINISTRATIVELY COMPLETE)

7.5.1 Method of Expedited Adverse Event Reporting

Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP home page (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" which can be downloaded from the CTEP home page (http://ctep.cancer.gov). These requirements are briefly outlined in the table below (Section **7.5.2**).

In the rare event when Internet connectivity is disrupted a 24-hour notification is to be made to NCI by telephone at: 301-897-7497, or 301-897-7402 for CIP studies. An electronic report MUST be submitted immediately upon re-establishment of internet connection. Please note that all paper CTEP-AERS forms have been removed from the CTEP website and will NO LONGER be accepted.

7.5.2 Expedited Reporting Guidelines

Table 14: Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted

within 10 calendar days of learning of the AE.
[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows: **Expedited 24-hour notification followed by complete report within 5 calendar days for:** • All Grade 3, 4, and Grade 5 AEs **Expedited 10 calendar day reports for:** • Grade 2 AEs resulting in hospitalization or prolongation of hospitalization [2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period. Effective Date: May 5, 2011

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

7.5.3 Expedited Adverse Event Reporting Exclusions

For this protocol, there are no AE reporting exclusions.

Note: All deaths on study must be reported using expedited reporting regardless of causality. Attribution to treatment or other causes should be provided.

7.6 ROUTINE ADVERSE EVENT REPORTING (NA AS OF CTEP STATUS – ADMINISTRATIVELY COMPLETE)

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through CTEP-AERS must <u>also</u> be reported in routine study data submissions.**

7.7 NCI-IRB AND CLINICAL DIRECTOR (CD) REPORTING

7.7.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems, and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations

- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.7.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:

    - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
    - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
    - All Grade 5 events regardless of attribution;
    - All Serious Events regardless of attribution.

    NOTE: Grade 1 events are not required to be reported.

7.7.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.8 ADVERSE EVENT FOLLOW-UP DURATION

All (either suspected or not suspected of relationship to SS1(dsFv)PE38) SAEs and AEs must be followed until one of the following occurs:

- Resolved or improved to baseline
- Severity improved to ≤Grade 2, if previously ≥ Grade 3
- Death
- Start of new anti-cancer regimen
- Investigator confirms that no further improvement can be expected
- Clinical or safety data will no longer be collected, or final database closure

The final outcome of each adverse event must be recorded on the Adverse Events CRF.

7.9 PREGNANCY

A female subject must be instructed to stop taking SS1 (dsFv) PE38 and immediately inform the investigator if she becomes pregnant during the study. The investigator should report all pregnancies within 24 hours to the sponsor. The investigator should counsel the subject, discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of

the subject should continue until conclusion of the pregnancy. Pregnancies occurring up to 90 days after the completion of SS1 (dsFv) PE38 must also be reported to the investigator.

Pregnancy occurring in the partner of a male subject participating in the study should be reported to the investigator and the sponsor. The partner should be counseled, the risks of continuing the pregnancy discussed, as well as the possible effects on the fetus. Monitoring of the subject should continue until conclusion of the pregnancy.

8 PHARMACEUTICAL INFORMATION

8.1 CTEP-SUPPLIED INVESTIGATIONAL AGENTS

8.1.1 CTEP IND Agent SS1 (dsFv) PE38 (NSC 726388)

8.1.1.1 Classification: Immunotoxin

8.1.1.2 Other Names: SS1P(dsFv)PE-38, Immunotoxin SS1P

8.1.1.3 Molecular Weight: 63 kDa

8.1.1.4 Description:

SS1 (dsFv) PE38 is a high affinity (KD = 0.72 nM) chimeric recombinant immunotoxin, in which the anti-mesothelin disulfide-stabilized murine-antibody Fv is genetically fused with PE38, a 38 kDa fragment of the potent *Pseudomonas* exotoxin A.

8.1.1.5 Mode of Action:

In SS1 (dsFv) PE38, the native cell-binding domain and other unnecessary sequences of the *Pseudomonas* exotoxin A have been removed and SS1 (dsFv) PE38 enters cells via endocytosis after binding of the mesothelin-specific Fv moiety to mesothelin on cell surface. After endocytosis and intracellular processing of SS1 (dsFv) PE38, a 38-kDa fragment of the exotoxin A enters the cytosol, inactivates the elongation factor 2, arrests protein synthesis and induces programmed cell death. SS1 (dsFv) PE38 kills mesothelin-expressing cells but not similar cells that do not express detectable levels of mesothelin.

8.1.1.6 How Supplied:

SS1 (dsFv) PE38 is a colorless liquid supplied in glass vials in phosphate-buffered saline (PBS). SS1 (dsFv) PE38 is available at a concentration of 250 mcg/mL. **The concentration of SS1 (dsFv) PE38 may vary from lot to lot. Check the vial label for the correct concentration prior to compounding your dose.**

8.1.1.7 Preparation:

Warm vials in the hand for 10 to 20 seconds before thawing vials at room temperature. Inspect vials visually after thawing. Do not use if material appears turbid. Do not shake; proteins can foam and may denature. Do not filter.

Dilute the required dose of SS1 (dsFv) PE38 with 0.2% HSA in 0.9% sodium chloride to a total volume of 50 mL in a sterile non- PVC or di-(2 ethylhexyl) phthalate (DEHP)-free bag (i.e PAB® bag). Add the constituents in the following order to an empty PAB® bag:

1. Human Serum Albumin (final concentration 0.2%). The container should be gently inverted several times to coat the interior after the human serum albumin has been added.

2. 0.9% sodium chloride
3. SS1 (dsFv) PE38

The total volume should equal 50 mL. Agitate gently to disperse.

Note: Significant absorption to glass and plastic surfaces has been observed for another PE38 immunotoxin. Therefore, SS1 (dsFv) PE38 must be diluted in the presence of 0.2% HSA. The 0.9% sodium chloride must be added to the HSA prior to adding SS1 (dsFv) PE38. Studies show no significant loss by absorption to the PAB® IV container and administration set of SS1 (dsFv) PE38 in the presence of HSA.

8.1.1.8 Storage:

Store intact vials at or below -70ºC. Do not refreeze thawed vials. Store SS1 (dsFv) PE38 infusions in the refrigerator for up to 4 hours. Allow infusions to equilibrate to room temperature for at least 1 to 2 hours prior to use.

8.1.1.9 Stability:

Shelf life surveillance of the intact vials is on-going. Use SS1 (dsFv) PE38 within 4 hours once further diluted in 0.2% HSA in 0.9% sodium chloride.

Note: Vials contain no preservatives and are intended for single use only. Discard opened vials within 4 hours after initial entry. Unopened vials may be stored at room temperature for up to 4 hours.

8.1.1.10 Route of Administration:

Intravenous

8.1.1.11 Method of Administration:

Administer over 30 minutes. Do not filter.

8.1.1.12 Availability

CTEP IND Agent SS1 (dsFv) PE38 (NSC 726388) is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

8.1.2 Agent Ordering

NCI supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application < https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx >. Access to

OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account < https://eapps-ctep.nci.nih.gov/iam/ > and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

8.1.3 Agent Accountability

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.2 CYCLOPHOSPHAMIDE (CTX, CYTOXAN, NSC-26271)

8.2.1 Supply

Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied in both tablet form and in white crystalline formulation for intravenous injection, in vials containing 100 mg, 200 mg, 500 mg, 1gm, and 2 gm.

Please refer to the FDA-approved package insert for additional information.

8.2.2 Preparation –

- Tablets – no additional preparation required
- Cyclophosphamide for injection
  - Reconstitute with appropriate amounts of 0.9% NaCl to produce a final concentration of 20 mg/ml. Discard solution after 24 hours at room temperature. Stable up to 6 days if refrigerated (2-8°C).
  - 200 mg of cyclophosphamide will be diluted in 100 mL of D5W or 0.9% NaCl and infused over 30 minutes. Patients will be instructed to drink an adequate amount of fluids and empty their bladders frequently during cyclophosphamide administration.

8.2.3 Storage and Stability –

The vials of cyclophosphamide containing the treatment doses are stable when stored at room temperature.

8.2.4 Route of Administration –

The cyclophosphamide used in this protocol will be given by oral administration on a daily basis on:

Regimen A: days 1 through 12 of cycle 1 and on days 1 through 4 during subsequent cycles.

IV infusion of the equal dose may be allowed if a patient is unable to tolerate oral therapy. The dose of cyclophosphamide will initially be 200 mg per day.

8.2.5 Mechanism of action:

DNA alkylation.

8.2.6 Toxicities:

Nausea and vomiting - variable; symptomatically improved with standard anti-emetics and/or benzodiazepines [e.g., lorazepam].

Cytopenias.

Hemorrhagic cystitis.

Mucositis.

Less common but serious complications include pulmonary fibrosis and secondary malignancies. Less common but reversible toxicities include alopecia and skin rash.

8.3 FILGRASTIM (G-CSF, NEUPOGEN®)

8.3.1 Supply

Commercially available as filgrastim injection in a concentration of 300µg/ml in 1ml (300µg) and 1.6ml (480µg) vials, and will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. Please refer to the FDA-approved package insert for additional information.

8.3.2 Preparation

For subcutaneous administration, the appropriate prescribed dose is drawn up from the vial with no further dilution prior to administration. For intravenous administration, the commercial solution for injection should be diluted prior to administration. It is recommended that the prescribed dose be diluted with dextrose 5% in water to a concentration greater than 5µg/ml just prior to administration; storage of the diluted filgrastim is not recommended. <u>Dilution of filgrastim to a final concentration of less than 5µg/ml is not recommended at any time. Do not dilute with saline at any time; product may precipitate</u>. Filgrastim diluted to concentrations between 5 and 15µg/ml should be protected from absorption to plastic materials by the addition of Albumin (Human) to a final concentration of 2mg/ml. When diluted in 5% dextrose or 5% dextrose plus Albumin (Human), filgrastim is compatible with glass bottles, PVC and polyolefin IV bags, and polypropylene syringes. The dose may be "rounded down" to within 10% of patient's calculated dose to use the drug cost-effectively.

8.3.3 Storage and Stability

Filgrastim for injection should be stored in the refrigerator at 2° to 8°C (36° to 46°F). Avoid shaking.

8.3.4 Administration

Subcutaneous injection is preferred. If clinically indicated, filgrastim may be administered as an intravenous infusion over 4 or 24 hours.

8.3.5 Toxicities

Medullary bone or skeletal pain is the most commonly reported toxicity. In addition, reversible elevations in uric acid, lactate dehydrogenase, and alkaline phosphatase are common laboratory abnormalities. Four cases of splenic rupture have been reported in healthy donors when given filgrastim or other myeloid growth factors for peripheral blood stem cell mobilization; 1 of these cases resulted in fatality. Five additional cases of splenic rupture have been reported in cancer patients undergoing chemotherapy or peripheral blood stem cell mobilization; splenic rupture may have contributed to deaths in 2 of these cases. One additional death due to splenic rupture after filgrastim therapy was reported to the manufacturer without additional information. According to the manufacturer, the reporting rate for splenic rupture with filgrastim is less than 1 in 486,000.

8.4 PENTOSTATIN (NIPENT®; 2'-DEOXYCOFORMYCIN)

8.4.1 Supply

Commercially available, and will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. The lyophilized powder will be resuspended according to manufacturer instructions, into a solution of 2 mg/ml concentration (10 mg. vial). Please refer to the FDA-approved package insert for additional information.

8.4.2 Preparation

The pentostatin dose will be determined by the creatinine clearance value (as determined by the 24 hour urine or calculation using the Cockroft-Gault formula (See **Appendix D**). The appropriate dose of the reconstituted pentostatin solution will be further diluted in 0.9% sodium chloride to a volume equal to 50 mL and infused over 30 to 60 minutes.

8.4.3 Storage and Stability

Upon reconstitution, the pentostatin can be stored at room temperature but should be used with 8 hours of reconstitution.

8.4.4 Administration

Subjects will receive one liter of 0.9% sodium chloride by intravenous infusion prior to the pentostatin delivery.

8.4.5 Mechanism of action:

Inhibition of adenosine deaminase, thereby increasing lymphocyte susceptibility to apoptosis.

8.4.6 Toxicities

Pentostatin is cleared by a renal mechanism (90%). As such, the pentostatin dose must be reduced for renal insufficiency (see Section **6.2.2**). The primary toxicity is related to opportunistic infection due to T cell depletion. At higher doses, CNS toxicity may include seizures, coma, and death. Interstitial pulmonary toxicity has also been described. Other toxicities include nausea, vomiting, and skin rash.

8.5 HYDROXYZINE HYDROCHLORIDE

8.5.1 Supply

Commercially available, and will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. Each table for oral administration contains 10 mg, and 25 mg, white, round, film coated tablets of hydroxyzine HCL. Please refer to the FDA-approved package insert for additional information.

8.5.2 Preparation

Hydroxyzine HCl will be given as an oral tablet.

8.5.3 Storage and Stability

Store commercially available product at controlled room temperature.

8.5.4 Administration

In this study, hydroxyzine HCl will be administered orally (10-25 mg) approximately 1 hour prior to each dose of SS1 (dsFv) PE38.

8.5.5 Toxicities

Side effects reported are usually middle and transitory. Anticholinergic effects: Dry mouth

Central Nervous system effects: Drowsiness is usually transitory and may disappear in a few days of continued therapy or upon dose reduction. Involuntary motor activity including rare instances of tremor and convulsions has been reported with higher than recommended doses. Confusion and oversedation may occur in the elderly.

8.6 RANITIDINE

8.6.1 Supply

Commercially available as tablets for oral administration from multiple manufacturers, and will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

8.6.2 Storage

Store oral tablets at controlled room temperature.

8.6.3 Administration

Oral. For gastric acid inhibition, antiulcer and histamine H2 antagonist activities. In this study, 150 mg will be administered orally approximately 1 hour prior to each dose of SS1 (dsFv) PE38.

8.6.4 Toxicities

No toxicities are anticipated to result from single doses of ranitidine administered as pre-medication for SS1 (dsFv) PE38. Common side effects with longer use than anticipated in this trial include drowsiness, dizziness, headache, abdominal discomfort, nausea, vomiting, diarrhea, and constipation.

8.7 ACYCLOVIR (ZOVIRAX®)

8.7.1 Supply

Commercially available as 400mg tablets and 800mg tablets, and will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. Dose adjustment is necessary in patients with significant renal impairment (refer to the manufacturer's labeling for dose adjustment guidelines.)

8.7.2 Pharmacology

Acyclovir is a synthetic purine nucleoside analogue with in vitro and in vivo inhibitory activity against herpes simplex virus types 1 (HSV-1), 2 (HSV-2), and varicella-zoster virus (VZV).

8.7.3 Storage and Stability

Oral tablets should be stored at 15° to 25°C (59° to 77°F) and should be protected from moisture.

8.7.4 Administration

Oral.

8.7.5 Toxicities

Nausea and/or vomiting, diarrhea, headache, acute hypersensitivity reactions; sweating; hematuria, hypotension, thrombocytopenia elevations in liver enzyme laboratory values (e.g. AST) have been reported Renal failure and CNS symptoms have been reported in patients with renal impairment who received acyclovir at greater than the recommended dose. Dose reduction is recommended in this patient population (refer to the manufacturer's labeling for dose adjustment guidelines).

8.8 TRIMETHOPRIM/SULFAMETHOXAZOLE (TMP/SMX, COTRIMOXAZOLE, BACTRIM, SEPTRA)

8.8.1 Supply

Commercially available as a single strength tablet containing trimethoprim 80mg and sulfamethoxazole 400mg and a double strength (DS) tablet containing trimethoprim 160mg and sulfamethoxaole 800mg, and will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. It is also available in an oral suspension at a concentration of 40mg of trimethoprim and 200mg sulfamethoxazole per 5ml. Parenteral TMP/SMX is available in a solution for injection at a concentration of 80mg of trimethoprim and 400mg of sulfamethoxazole per 5ml. Please refer to the package insert for complete drug information.

8.8.2 Preparation

For parenteral administration, the commercial solution for injection must be diluted prior to administration. It is recommended that each 5ml of the solution for injection be diluted with 100-125 ml or, if fluid restriction is required, in 75ml of dextrose 5% in water. 0.9% sodium chloride, Inj. may be substituted as a diluent but the resulting solutions have reduced stability. Consult with pharmacy for questions regarding diluent, volume, and expiration.

8.8.3 Storage and Stability

Oral tablets and oral suspension should be stored at 15º to 30ºC (59º to 86ºF) in a dry place and protected from light. TMP/SMX for injection should be stored at room temperature between 15º to 30ºC (59º to 86ºF) and should not be refrigerated. Stability of intravenous doses after final dilution is dependent on concentration and diluent. Consult with pharmacy for questions regarding stability and expiration dating.

8.8.4 Administration

Oral and parenteral. Parenteral doses should be administered by an intravenous infusion over 60 to 90 minutes.

8.8.5 Toxicities

The most common adverse effects from TMP/SMX are gastrointestinal disturbances (nausea, vomiting, anorexia) and allergic skin reactions (such as rash and urticaria). Fatalities associated with the administration of sulfonamides, although rare, have occurred due to severe reactions, including Stevens-Johnson syndrome, toxic epidermal necrolysis, fulminant hepatic necrosis, agranulocytosis, aplastic anemia and other blood dyscrasias. For TMP/SMX injection, local reaction, pain and slight irritation on IV administration are infrequent. Thrombophlebitis has rarely been observed.

8.9 DEXAMETHASONE (DECADRON® ORAL TABLETS)

8.9.1 Supply

For patient administration, oral tablets will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. Please refer to the package insert for complete drug information.

8.9.2 Storage and Stability

Oral tablets should be stored 20-25 C° (68-77 F°).

8.9.3 Administration:

60 minutes prior to each dose of pentostatin, dexamethasone oral tablets will be administered at a total dose of 12 mg. In addition, oral dexamethasone will be administered on the next two days following pentostatin therapy at a dose of 4 mg each day. (Please refer to **Table 9** and **Table 10** for Regimen A instructions)

8.9.4 Toxicities:

Dexamethasone is relatively contra-indicated in patients with systemic fungal infection. Rarely, anaphylaxis can occur as a result of dexamethasone therapy. Other toxicities include exacerbation of hypertension, fluid retention, and suppression of the hypothalamic-pituitary-adrenal axis.

8.9.5 Drug Interactions:

Concomitant use of dexamethasone and non-steroidal anti-inflammatory agents can increase risk of gastro-intestinal bleeding.

8.10 ONDANSETRON (ZOFRAN®)

8.10.1 Supply

For patient administration, oral tablets will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. Please refer to the package insert for complete drug information.

8.10.2 Storage and Stability

Oral tablets should be stored 20-25 C° (68-77 F°).

8.10.3 Administration:

Ondansetron will be administered at a dose of 8 mg by IV infusion 60 minutes prior to each dose of pentostatin.

Ondansetron will also be administered at an oral dose of 8 mg (tablets) every 12 hours on:

> *Regimen A:* Days 1 through 12 (one evening dose will be administered on days 1, 5, and 9) during Cycle 1, and Days 1 through 4 for subsequent cycles (one evening dose will be administered on days 1).

8.10.4 Toxicities:

Ondansetron should not be administered to individuals who have a known hypersensitivity to the agent or to other selective 5-HT_3 receptor antagonists.

8.10.5 Drug Interactions:

No significant interactions.

9 CORRELATIVE/SPECIAL STUDIES

Recognizing the difficulties in obtaining new and repeat tumor samples in study diseases, two main principles for assessments of laboratory correlates will be followed:

- utilization of already existing tumor biopsies (mainly from the initial diagnosis, and/or surgery), and in rare instances use of newly obtained pre-treatment biopsies
- evaluation of surrogate markers which can be assessed via peripheral blood samples

9.1 LABORATORY CORRELATIVE STUDIES

9.1.1 SS1 (dsFv) PE38 Pharmacokinetics study

Instructions for Pharmacokinetic Samples

Table 15: Sample Schedule for Cycles 1 and 2 -4

Dose	Sample No.	Sampling Time Post Dose Start	Blood volume
		(hour)	(mL)
1	1	Predose[1]	2
	2	EOI[2]	2
	3	1	2
	4	1.5	**2**
	5	2	2
	6	4	2
	7	8	2
	8	12	2
2	9	Predose	2
	10	EOI[2]	2
3	11	Predose	2
	12	EOI[2]	2
4	13	Predose	2
	14	EOI[2]	2
5	15	Predose	2
	16	EOI[2]	2
6	17	Predose	2
	18	EOI[2]	2
Total	18		**36**

All sampling times are calculated from the **start of infusion**.

1 This sample must be drawn before SS1(dsFv)PE38 administration.

2 EOI=end of infusion. This sample must be drawn at the end of the 30 minute infusion and the actual time should be recorded.

9.1.1.1 Collection and Handling of SS1 (DSFV) PE38 Serum Samples

Blood should not be drawn from the line being used for SS1(dsFv)PE38 administration

All blood samples will be taken by either direct venipuncture or an indwelling venous access. At each sample collection time, blood (2mL) will be drawn into a 3.5-mL serum separator tube (tiger top tube).

9.1.1.2 SS1 (dsFv) PE38 Pharmacokinetic Samples: Processing

Each sample should be processed in the following manner:

Allow blood to clot for 10 minutes and centrifuge to separate the serum within 30 minutes of collection. If unable to process within 30 minutes, then whole blood tubes may be stored upright in refrigerator (4-8°C) for up to 48 hours prior to processing at the Leidos Biomedical, Inc. lab. Stability studies will establish if degradation of soluble mesothelin in whole blood during 0.5 to 48 hours is significant and therefore if the data from these samples should be included in the analysis. If whole blood is refrigerated, a comment should be entered in the CRF comments field. Pre-labeled tubes will be provided, see study manual for details.

9.1.1.3 SS1 (dsFv) PE38 Pharmacokinetic Samples (Sera): Storing

All sera samples will be stored frozen at approximately –70°C at the Leidos Biomedical, Inc. Lab in Frederick, MD.

9.1.1.4 Pharmacokinetic Samples: Labeling

Table 16: The samples will be labeled as follows

Study code	**SS1(dsFv)PE38-**
Subject ID Number	
Visit number	**Cycle _____ Day _____**
Nominal Collection Interval (eg. Pre-dose, 0.5, 1 h)	**as applicable**
Date of collection	**dd/mm/yy**
Actual time of collection	**24 hr. clock**

The collection time of these samples must be documented in the Pharmacokinetic Blood Collection CRF pages. The sample number should correspond to that indicated above for a given time period. Please ensure that you are using waterproof ink, attaching it to the tube, and covering with clear tape before refrigerating. Pre-printed labels and tubes will be provided.

The exact clock time of dosing, as well as actual sample collection time will be entered on the CRF. Sampling problems will be noted on the comments section of the CRF.

9.1.1.5 Pharmacokinetic Samples: Shipment

All samples will be refrigerated until pick-up by courier and then delivered to David Waters, Ph.D. at the following address: (Please notify the vendor prior to shipment)

Leidos Biomedical, Inc.
Bldg 560, Lab 11/09

Attention Dave Waters, Ph.D.
1050 Boyles St.
Frederick, MD 21702
Phone: 301-846-5831
Email: david.waters@nih.gov

9.1.2 Levels of Circulating Mesothelin in peripheral blood

9.1.2.1 Mesothelin Serum Samples: Collection

Samples will be obtained prior to the first dose of each cycle, prior to the C1D14 SS1(dsFv)PE38 dose and at the end of treatment. All blood samples will be taken by either direct venipuncture or an indwelling venous access. At each sample collection time, blood (2mL) will be drawn into a 3.5-mL serum separator tube (tiger top tube) labeled as follows:

- Subject ID Number

- Study Number

- Time and date of collection

9.1.2.2 Mesothelin Serum Samples: Processing

Each sample should be processed in the following manner:

Allow blood to clot for 10 minutes and centrifuge to separate the serum within 30 minutes of collection. If unable to process within 30 minutes, then whole blood tubes may be stored upright in refrigerator (4-8°C) for up to 48 hours prior to processing. Processing of samples within 30 minutes is strongly preferred. Stability studies will establish if degradation of soluble mesothelin in whole blood during 0.5 to 48 hours is significant and therefore if the data from these samples should be included in the analysis. If whole blood is refrigerated, a comment should be entered in the CRF comments field. Pre-labeled tubes will be provided, see study manual for details.

Transfer the serum into two pre-labeled cryotubes and immediately freeze by placing on dry ice. Transfer frozen serum samples into a – 70°C freezer for storage.

9.1.2.3 Mesothelin Serum Samples Storing

All serum samples will be stored in Dr. Raffit Hassan's Lab at NCI, Bldg 37, Bethesda, MD.

9.1.2.4 Site(s) Performing Correlative Study.

Leidos Biomedical, Inc.
Attention: Dave Waters, Ph.D.
Bldg 560, Lab 11/09
1050 Boyles St.
Frederick, MD 21702
Phone: 301-846-5831
Email: david.waters@nih.gov

9.1.3 HLA Typing

Differential immune responses are often seen based on HLA typing. In order to determine if there is any differential antibody formation based on HLA typing, patients will be requested to have an optional HLA typing performed at any point during enrollment in the trial.

9.1.4 Immunohistochemistry analysis of Mesothelin for correlative studies

9.1.4.1 Collection of Specimen:

9.1.4.1.1 Archival material: A block of archival tumor material will be requested from each patient. A recent resection sample or blocks of tissue from the original resection are requested. Where a block cannot be released by the governing pathology department, 10 x 8µm re-cuts on charged slides will be requested.

9.1.4.2 Methods:

Immunohistochemistry will be performed in collaboration with Pathology Branch, Bldg 10/2N212. Mesothelin IHC will be performed by the NIH Clinical Center Department of Pathology as part of routine staining in their initial review of patient's tumor specimens.

9.1.5 Immunogenicity to SS1 (dsFv) PE38

Serum specimens will also be analyzed for immunogenicity to SS1(dsFv)PE38 using a serum neutralization assay similar to that used in the SS1(dsFv)PE38 phase I clinical trial.[22] Samples from Regimen A subjects will be collected at screening, Day 24 of Cycle 1 and Day 16 of subsequent cycles (+/- 2 days), which will be the equivalent of 14 days after the first dose of SS1(dsFv)PE38 administration during each respective cycle. Presence of anti-SS1(dsFv)PE38 antibodies in the subjects' sera will be evaluated using a serum neutralization assay. Serum samples will be shipped to Dave Waters, Ph.D. laboratory in Frederick, MD.

9.1.5.1 Immunogenicity Serum Samples: Collection

All blood samples will be taken by either direct venipuncture or an indwelling venous access. At each sample collection time, blood (4mL) will be drawn into a 10-mL serum separator tube (tiger top tube) labeled as follows:

- Subject ID Number
- Study Number
- Time and date of collection

9.1.5.2 Immunogenicity Serum Samples: Processing

Each sample should be processed in the following manner:

Allow blood to clot for 10 minutes and centrifuge to separate the serum within 30 minute of collection. If necessary whole blood may be stored in the collection tubes upright and refrigerated at 4-8°C for up to 24 hours prior to centrifugation and processing. Processing within 30 minutes is strongly preferred. Transfer the serum into two (2) pre-labeled cryotubes and immediately freeze by placing on dry ice. Transfer frozen serum samples into a – 70°C freezer for storage.

9.1.5.3 Immunogenicity Serum Samples: Storing

All serum samples will be stored frozen at approximately –70°C at Leidos Biomedical, Inc. Lab, Frederick, MD.

9.1.5.4 Immunogenicity Serum Samples: Shipping

Immunogenicity serum samples should be shipped (samples will be picked up by courier) Monday-Thursday to David Waters, Ph.D. at the following address:

Leidos Biomedical, Inc.

Attention: Dave Waters, Ph.D.
Bldg 560, Lab 11/09 1050 Boyles St.
Frederick, MD 21702
Phone: 301-846-5831
Please notify the vendor prior to shipment

9.1.6 Serum cytokine levels and C-reactive protein

To understand whether cytokine dysregulation plays a role in the anti-tumor effects and adverse effects, serum level of C-reactive protein, TNF-a, IL1b, IL6, IL7, IL15 at different time points are tested. Milliplex MAP KIT-HYTOMAG-60K mutli-cytokine detection kit commercially available from Millipore.

Samples will be obtained prior to the first dose of each cycle and prior to each dose of SS1P (days 10, 12, 14 in cycle 1 and days 2, 4, 6 in subsequent cycles). All blood samples will be taken by either direct venipuncture or an indwelling venous access. At each sample collection time, blood (5mL) will be drawn into a serum separator tube (tiger top tube) labeled as follows:

- Subject ID Number
- Study Number
- Time and date of collection

C-reactive protein testing will be performed on blood drawn for chemistries at different time points.

9.2 IMMUNE LABORATORY STUDIES

Blood will be drawn just prior to initial chemotherapy, prior to chemotherapy in cycles 2 through 4, and then at each long-term follow-up appointment (for patients who are still being followed on protocol for disease response evaluation after immunotoxin therapy). Patients will be assessed for lymphopenia throughout the study. If ALC $\geq$ 200 cells/microliter, blood will be further collected and evaluated as follows:

3 mL of blood in a lavender tube will be sent to NIH Clinical Pathology for assessment of a "TBNK" panel, which will determine absolute number of circulating $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and B cells.

24 mL of blood will be collected in red or green cell preparation tubes and sent to Dr. Fran Hakim, Building 10, 12th Floor Pre-Clinical Core of the NCI Experimental Transplantation and Immunology Branch. Such blood will be used to study more detailed aspects of immune effects of pentostatin/cyclophosphamide, such as: determination of further cell surface markers of immune cells (memory vs. naïve markers, Treg cell markers) and determination of T cell function (specifically, T cells will be co-stimulated and evaluated for their effector function, including cytokine secretion capacity). In this way, we seek to characterize both the immune depleting and immune suppressive effects of the pentostatin plus cyclophosphamide regimen.

9.3 TUMOR BIOPSY

9.3.1 Collection

Tissue biopsies will be encouraged but done strictly on a voluntary basis for newly enrolled patients as well as patients who have been treated on this protocol after they sign a new informed consent detailing the potential risks of the procedure. Biopsies will be performed pre-treatment, after 2 cycles of treatment, and at the end of the last cycle of treatment or at follow-up. Standard techniques will be used for pre-treatment and post-treatment percutaneous biopsies based on the results of imaging studies such as CT and PET scans that have been previously acquired. When clinically feasible and judged minimal risk by Interventional Radiology, the same total number biopsies will be prospectively acquired from areas of tumor that may exhibit imaging features of higher or lower cellularity, such as higher PET scan activity, or evidence solid features or viability on CT scan. This will allow biopsy specimens to be marked as "high imaging cellularity" or "low imaging cellularity", which could enhance the ability to obtain quality tissue biomarkers, immunohistochemistry or mRNA, and could facilitate the identification of reliable biomarkers for response.

Biopsies will be obtained from primary tumor sites and/or metastatic sites (if applicable). No more than 4 cores may be obtained per site. Half of the biopsy specimen will be used for immunohistochemistry (IHC) analysis. The other half will be snap frozen for later use.

9.3.2 Processing and Storage

The fixed tissue samples that will be used for IHC analysis will be processed and embedded in paraffin. Tissue blocks will be made from the paraffin-embedded tissue. Tissue blocks will be stored at room temperature.

The tissue that is snap frozen will be stored by Dr. Raffit Hassan's research Laboratory (Building 37, Room 5116).

IHC analysis will be performed at the Laboratory of Pathology at NCI to determine mesothelin expression within the tumor. Furthermore, collected tumor tissue will be assessed for CD4+ and CD8+ T cell infiltration as well as the presence of regulatory T cells (FoxP3+). Based on these results, additional immunologic markers of the tumor may be assessed.

9.4 SAMPLE STORAGE, TRACKING AND DISPOSITION

Blood and tissue collected during the course of this study will follow storage, handling and labeling procedures to ensure that security, confidentiality and sample integrity are maintained. All samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. Thus samples will be de-identified of personal data, with access to personal data restricted to the study investigators.

All cryopreserved samples are tracked for freezer location and storage criteria. All Samples are stored in a locked freezer at -70°C according to stability requirements. These freezers are located offsite at NCI-Frederick, at the Leidos Biomedical, Inc. Lab in Frederick, MD. Samples will be stored until requested by a researcher named on the protocol. All use and requests for use will be recorded by the Leidos Biomedical, Inc. Lab. Any unused samples must be returned.

Some samples as indicated below may be stored in monitored freezers/refrigerators in the investigator's laboratory at specified temperatures with alarm systems in place.

At the termination of this protocol, samples will remain in storage as detailed above. If additional studies are to be performed on any samples retaining patient identifiers, obtained during the conduct of this trial, a Request to Conduct Research for Stored Human Samples Specimens, or Data Collected in a Terminated NCI-IRB Protocol will be submitted. Otherwise, access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material. If specimens are to be discarded at any point, they will be disposed of in accordance with the environmental protection laws, regulations and guidelines of the Federal Government and the State of Maryland.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Any new uses of samples collected during the course of this trial must be reviewed and approved by the NCI IRB. Any loss or unintentional destruction of the samples will be reported to the IRB.

10 STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

See **Appendix C** for Study Calendar.

11 MEASUREMENT OF EFFECT

11.1 ANTITUMOR EFFECT

For the purposes of this study, patients should be re-evaluated for response after every 2 cycles and at the conclusion of treatment. Responses should be confirmed at least 4 weeks following initial documentation of objective response.

For peritoneal mesothelioma, lung adenocarcinoma and pancreatic adenocarcinoma response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Committee. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

For pleural mesothelioma, response and progression will be evaluated using the modified RECIST criteria for mesothelioma, which will be described below.

11.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with SS1 (dsFv) PE38.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Progression Free Survival (PFS) is defined as the time interval from the start of treatment to documented evidence of disease progression.

Overall Survival (OS) is defined as the time interval from the start of treatment to the date of death.

11.2 PERITONEAL MESOTHELIOMA, LUNG ADENOCARCINOMA AND PANCREATIC ADENOCARCIONOMA MEASUREMENTS

11.2.1.1 Peritoneal Mesothelioma, Lung Adenocarcinoma and Lung Adenocarcinoma Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm with conventional techniques (CT, MRI, x-ray) or as ≥10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. *If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the protocol.*

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

Target lesions. All measurable lesions up to a maximum of 5 lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 10 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness

recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

11.2.2 Peritoneal Mesothelioma, Lung Adenocarcionma and Pancreatic Adenocarcinoma Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US) When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in reference centers. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained.

Tumor markers Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific additional criteria for standardized usage of prostate-specific antigen (PSA) and CA-125 response in support of clinical trials are being developed.

Cytology, Histology These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is

mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.2.3 Peritoneal Mesothelioma, Lung Adenocarcinoma and Pancreatic Adenocarcinoma Response Criteria

11.2.3.1 Peritoneal Mesothelioma, Lung Adenocarcinoma and Pancreatic Adenocarcinoma Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum on study LD (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

11.2.3.2 Peritoneal Mesothelioma, Lung Adenocarcinoma and Pancreatic Adenocarcinoma Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.2.3.3 Peritoneal Mesothelioma, Lung Adenocarcinoma, Pancreatic Adenocarcinoma Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Table 17: Best Overall Response

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Response for this Category Also Requires:**
CR	CR	No	CR	≥4 wks. confirmation
CR	Non-CR/Non-PD	No	PR	≥4 wks. confirmation
PR	Non-PD	No	PR	
SD	Non-PD	No	SD	documented at least once ≥4 wks. from baseline
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD*	Yes or No	PD	
Any	Any	Yes	PD	

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration*". Every effort should be made to document the objective progression even after discontinuation of treatment.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

11.2.3.4 Peritoneal Mesothelioma, Lung Adenocarcinoma, Pancreatic Adenocarcinoma Confirmation

The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.

In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol

11.2.3.5 Peritoneal Mesothelioma, Lung Adenocarcinoma, Pancreatic Adenocarcinoma Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

11.3 PLEURAL MESOTHELIOMA

The assessment tool selected for use in this protocol to determine success in meeting the primary and major secondary endpoints is the EORTC modified RECIST criteria. The RECIST criteria were initially considered for this purpose; however, the application of RECIST criteria could be variably interpreted by different investigators in mesothelioma, leading to unsatisfactory results.[69] According to the authors, the modified RECIST criteria were designed to address the unique growth pattern of pleural mesothelioma.

Below is an excerpt from an article written by Byrne and Nowak[69] that describes the recommended modifications to the RECIST Criteria developed by the EORTC. For the purpose of assessing efficacy for this clinical trial, these modified criteria will be used in conjunction with the RECIST Quick Reference tool, a copy of which has also been provided on the following pages, and is available on the NCI web site: http://ctep.cancer.gov/guidelines/recist.html.

"The Modified RECIST criteria we have developed were as follows:

Tumor thickness perpendicular to the chest wall or mediastinum was measured in 2 positions at 3 separate levels on transverse cuts of CT scan. The sum of the 6 measurements defined a pleural unidimensional measure. Transverse cuts at least 1 cm apart and related to anatomical landmarks in the thorax were chosen to allow reproducible assessment at later time points. If measurable tumor was present, transverse cuts in the upper thorax, above the level of division of the main bronchi were preferred. At reassessment, pleural thickness was measured at the same position at the same level and by the same observer. This was not necessarily the greatest tumor thickness at that level.

Nodal, subcutaneous and other bidimensionally measurable lesions were measured unidimensionally as per the RECIST criteria. Unidimensional measurements were added to obtain the total tumor measurement. CR was defined as the disappearance of all target lesions with no evidence of tumor elsewhere, and PR was defined as at least a 30% reduction in the total tumor measurement. A confirmed response required a repeat observation on 2 occasions 4 weeks apart. Progressive disease (PD) was defined as an increase of at least 20% in the total tumor measurement over the nadir measurement, or the appearance of 1 or more new lesions. Subjects with stable disease (SD) were those who fulfilled the criteria for neither PR nor PD."

11.3.1 Pleural Mesothelioma Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest

measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Table 18: Best Overall Response

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Response for this Category Also Requires:**
CR	CR	No	CR	≥4 wks. confirmation
CR	Non-CR/Non-PD	No	PR	≥4 wks. confirmation
PR	Non-PD	No	PR	
SD	Non-PD	No	SD	documented at least once ≥4 wks. from baseline
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD*	Yes or No	PD	
Any	Any	Yes	PD	

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration*". Every effort should be made to document the objective progression even after discontinuation of treatment.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

11.3.2 Pleural Mesothelioma Confirmation

The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.

In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol

11.3.3 Pleural Mesothelioma Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

11.4 REPORTING OF RESULTS

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data).

All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients.

Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

The 95% confidence intervals should be provided.

11.5 OTHER IMAGING STUDIES - PET SCANS

FDG-PET scanning is of interest as part of this study as changes may correlate with clinical activity.

FDG-PET scans will be performed as part of this study. Primary response and treatment decisions, however, will be based on CT scan results. Patients enrolled on the study will undergo FDG-PET scan at baseline and at the time of restaging every 6 weeks after start of therapy.

12 DATA COLLECTION /REPORTING / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7 (Adverse Events: List and Reporting Requirements).

12.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

12.2 DATA REPORTING

12.2.1 Monitoring Method

This study will be monitored by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis, either by FTP burst of data or via the CDS web application. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site (http://ctep.cancer.gov/reporting/cdus.html).

12.2.2 Responsibility for Submissions

Data will be collected prospectively and entered into C3D.

12.3 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)/CLINICAL TRIALS AGREEMENT (CTA)

N/A.

13 STATISTICAL CONSIDERATIONS

13.1 STUDY DESIGN/ENDPOINTS

13.1.1 Mesothelioma Pilot

Following a determination of safety, the primary objective of this study is to determine if the use of a conditioning regimen prior to administration of SS1(dsFv)PE38 will be associated with a decrease in the immunogenicity of the regimen in patients with mesotheliomas.

The current estimate of the fraction of patients who develop neutralizing antibodies to SS1(dsFv)PE38 is approximately 80-95% by day 21 of the first treatment with SS1(dsFv)PE38.[22] Demonstration that the proposed regimen can be associated with a fraction substantially below that amount would be the primary goal.

Initially, in this pilot trial, 10 evaluable patients were to be enrolled at the 35 mcg/kg dose using the initially planned pentostatin/cyclophosphamide regimen (Regimen A) and if 0-6/10

developed antibodies, this would be considered acceptable. Eleven patients have actually been enrolled of whom ten are evaluable for the primary objective of determining if the regimen of pentostatin and cyclophosphamide can delay formation of antibodies to SS1(dsFv)PE38. Only 2 of 10 (20%) patients developed antibodies by end of cycle 1 which is much better than the historical results of using SS1(dsFv)PE38 alone, when 30 of 34 (88%) of patients developed anti-SS1(dsFv)PE38 antibodies. However, 7 out of 9 evaluable patients had anti-SS1(dsFv)PE38 antibodies by end of cycle 2. The goal of this protocol amendment is to determine if intensification of the pentostatin and cyclophosphamide regimen can delay development of anti-SS1(dsFv)PE38 antibodies by end of cycle 2, allowing administration of 3 or more cycles of SS1(dsFv)PE38. A total of 10 patients will be enrolled onto this study with the intensified pentostatin and cyclophosphamide regimen (Regimen B) at the 35 mcg/kg dose of SS1P and will receive the proposed conditioning regimen. If there are 0 to 6 of the 10 patients who develop neutralizing antibodies at the end of cycle 2, this will be considered a successful outcome. This calculation will be based on patients who receive two or more cycles of SS1(dsFv)PE38 treatment. If a patient does not receive a second cycle of SS1(dsFv)PE38 treatment due to toxicity or other reasons (such as disease progression), that patient will be replaced.

The following table shows the probability of obtaining from 0 to 6 out of 10 treated patients having neutralizing antibodies, as a function of the true probability of having neutralizing antibodies:

Table 19: Probability of Neutralizing Antibodies

True probability (neutralizing antibodies)	**Probability of 0-6 out of 10 treated patients having neutralizing antibodies**
0.40	0.945
0.45	0.898
0.50	0.838
0.80	0.121
0.85	0.050

Thus, if the true probability that patients will develop neutralizing antibodies despite the conditioning regimen is 80% or greater, there is only 12% probability or less of having 0-6 with neutralizing antibodies, while if the true probability is 45% or less, there is approximately 90% or greater probability of observing 0-6 with neutralizing antibodies. Thus, observing 0-6 with neutralizing antibodies is more likely to be associated with a regimen with a much lower risk of neutralizing antibodies than is the case without conditioning. A 95% confidence interval will also be formed about the observed proportion of patients who develop neutralizing antibodies.

Another primary objective is to assess the safety, tolerability, and feasibility of a conditioning regimen of pentostatin and cyclophosphamide in combination with SS1(dsFv)PE38. Toxicities will be assessed and documented from enrollment until 30 days after the last dose of treatment drug, or until toxicities resolve to baseline or stabilization. Should any 2 within the first 3 to 6 patients experience treatment limiting toxicity requiring cessation of treatment prior to the conclusion of the first cycle, the maximum tolerated dose will have been exceeded and patients will be enrolled to the next lower dose as defined in Section **5.1**.

As this is a small pilot study, the secondary objectives will be addressed in an exploratory manner and any findings will be reported without any adjustments for multiple comparisons. Any decrease in neutralization will be compared to timepoints of historical controls with the phase I trial referenced above.

13.1.2 Mesothelin positive cancers dose de-escalation pilot

The primary purpose of the dose de-escalation study is to establish the recommended phase 2 dose of SS1(dsFv)PE38 manufactured in lot FIL129J01. A maximum of 12 evaluable subjects will be enrolled at up to two dose levels. Patients with mesothelioma who are enrolled on the dose de-escalation study will count towards accrual in the Phase 2/Expansion portions and be used for analysis regardless of the dose level that they receive. Patients with lung and pancreatic cancers who are enrolled on the dose de-escalation study will count towards accrual in the Phase 2/ Expansion portion and be used for analysis *only* if they receive the recommended Phase II dose of the drug.

13.1.3 Mesothelioma Phase 2

13.1.3.1 Mesothelioma (Cohorts 1 and 2)

The primary objective of the phase 2 mesothelioma portion of the trial will be to determine if the combination of the initial regimen, Regimen A, is able to be associated with an acceptable proportion of patients experiencing a clinical response (PR+CR) with either pleural or peritoneal mesothelioma.

Data from the initial portion of the trial using regimen A demonstrated that 2/2 patients with peritoneal mesothelioma had a partial response as did 1/8 with pleural mesothelioma. The objective will be to determine if modest response rates may be obtained in each group with the individual response goals determined taking into consideration the results seen initially. As such, this phase II portion provides a replicate cohort for the initial results and will help to further establish the response rate of the regimen. However, the original patients on regimen A will not be counted towards evaluation in the phase 2 portion.

Mesothelioma patients enrolling in the dose de-escalation study will count towards the ceilings listed below and will be included in the analysis.

Patients with pleural and peritoneal mesothelioma will both be accrued to the trial using separate two stage Simon Minimax designs as follows:

In patients with pleural mesothelioma, the study will be conducted using a two-stage Minimax phase II trial design in order to rule out an unacceptably low 5% PR+CR rate (p0=0.05) in favor of an improved PR+CR response rate of 30% (p1=0.30). Both PR and CR will be considered a 'response' for purposes of this study. With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.10 (probability of rejecting a good treatment=0.10), this cohort will initially enroll 13 evaluable patients and if 0 of the 13 have a response, then no further patients will be accrued. If 1 or more of the first 13 patients has a response, then accrual would continue until a total of 16 patients with pleural mesothelioma have been enrolled. As it may take several weeks to determine if a patient has experienced a response, a temporary pause in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If there are 1 to 2 responses in 16 patients, this would be an uninterestingly low response rate. If there were 3 or more responses in 16 patients (18.8%), this would be sufficiently interesting to

warrant further study in later trials. Under the null hypothesis (5% response rate), the probability of early termination is 51.3%.

In patients with peritoneal mesothelioma, the study will be conducted using a two-stage Minimax phase II trial design in order to rule out an unacceptably low 10% PR+CR rate (p0=0.10) in favor of an improved PR+CR response rate of 45% (p1=0.45). With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.10 (probability of rejecting a good treatment=0.10), this cohort will initially enroll 8 evaluable patients and if 0 of the 8 have a response, then no further patients will be accrued. If 1 or more of the first 8 patients has a response, then accrual would continue until a total of 10 patients with peritoneal mesothelioma have been enrolled. As it may take several weeks to determine if a patient has experienced a response, a temporary pause in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If there are 1 to 2 responses in 10 patients, this would be an uninterestingly low response rate. If there were 3 or more responses in 10 patients (30.0%), this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (10% response rate), the probability of early termination is 43.1%. Upon progression, patients may be offered chemotherapy to determine if a clinical response may be obtained. In the prior use of Regimen A, 3 of 4 patients treated with subsequent chemotherapy were able to produce clinical responses. In this amendment, based on a fixed maximum of 26 patients, the fraction of the total number of patients treated with chemotherapy upon progression will be determined and presented separately by disease type as well as for all patients combined, in each case with a 95% confidence interval around the observed response rate. These results may then be evaluated informally relative to the 3 of 4 who responded earlier in the trial to chemotherapy, as well as to any other published results from patients with the same disease.

Based upon the need to do a dose de-escalation study with patients treated in these cohorts, there is a possibility that patients may be treated at a combination of 35 mcg/kg (lot 073I0809), 25 mcg/kg (lot FIL129J01) and 18 mcg/kg (lot FIL129J01) rather than being treated homogeneously within the cohorts. As a result, depending on the responses obtained it is possible that the cohorts may end accrual after stage 1 without all patients in stage 1 having been treated with the same drug doses. This may lead to stopping accrul to those cohorts early without having had the opportunity for all patients to be treated at the same, higher, dose level. In addition, if accrual is permitted to the second stage and patients are treated at 2 different dose levels, the responses obtained need to be interpreted in this context.

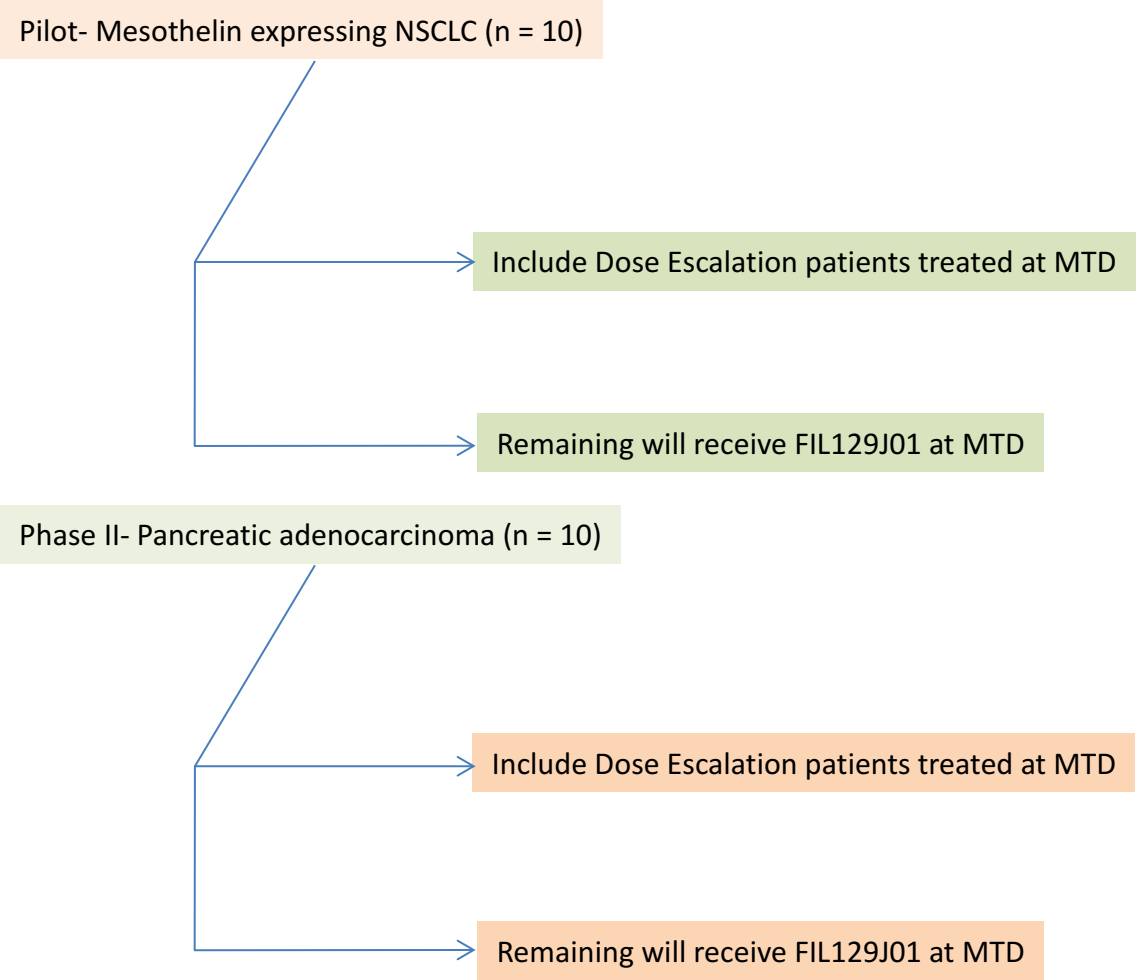
13.1.4 Lung (Cohort 3) and Pancreatic (Cohort 4) Adenocarcinoma

The primary objective of this pilot is to establish in a small number of patients if the response rate in lung adenocarcinoma or pancreatic cancer is adequate for further development of SS1P.

The design of the pancreatic and lung adenocarcinoma cohorts is shown in **Figure 11**. In these separate cohorts, we propose a Minimax two-stage phase II trial design in order to rule out an unacceptably low response rate of 5% (p0=0.05) in favor of an improved response rate of 35% (p1=0.35). In each cohort, with alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.10 (probability of rejecting a good treatment=0.10), this first stage will initially enroll 7 evaluable patients, and if 0 of the 7 have a response, then no further patients will be accrued in this cohort. If 1 or more of the first 7 patients has a response, then accrual would continue until a total of 10 patients have been enrolled in that cohort. If there is only 1 response in 10 patients, this would be an uninterestingly low response rate. If there were 2 or more responses in 10

patients in a cohort this would be sufficiently interesting to warrant further study in later trials. Patients with pancreatic or lung adenocarcinoma who are treated in the dose de-escalation phase (**Figure 10)** at the recommended phase 2 dose will be included in the efficacy analysis **Figure 11**.

Figure 11: Response analysis of pancreatic and lung adenocarcinoma cohorts



13.2 Sample Size/Accrual Rate

If 10 evaluable patients were treated at the 35 mcg/kg SS1P dose level of Regimen A, we planned to analyze all the data and determine if we should escalate the dose of SS1P, modify the pentostatin/cytoxan schedule to further decrease anti-SS1P antibody response or expand the cohort of patients being treated at the 35 mcg/kg SS1P dose level. As of amendment January 2013, 10 evaluable patients of 11 enrolled had been treated at the indicated dose level.

We then enrolled 8 additional evaluable patients under Regimen B, a modified pentostatin/cytoxan schedule, before abandon the regimen due to increased toxicity.

The 19 patients enrolled during the mesothelioma pilot, 46 evaluable patients planned to be enrolled during the phase 2/expansion portions of the study, up to 6 patients in the dose de-escalation study that may not be included in the pancreatic or lung expansion cohort ceilings and allowing for up to 4 patients being inevaluable with respect to immunogenicity or toxicity in the dose de-escalation study would mean that the accrual ceiling would be increased to 75.

Cohort	**Status as of amendment dated 6/18/15**	**Accrual ceiling (for open cohorts) or # enrolled (for closed cohorts)**
Regimen A Mesothelioma Pilot	closed	11
Regimen B Mesothelioma Pilot	closed	8
Regimen A pleural mesothelioma expansion	open	16
Regimen A peritoneal mesothelioma expansion	open	10
Dose de-escalation study[+]	open	6[ǂ]
Regimen A lung adenocarcinoma	open	10
Regimen A pancreatic cancer	open	10
Allowance for inevaluable subjects	NA	4
Total		75

ǂ Includes only the pancreatic and lung adenocarcinoma cohorts that may not be treated at the RP2D

It is anticipated that approximately 2 patients per month with mesothelioma, lung adenocardinoma and pancreatic adenocarcinoma may be enrolled onto the study. At this rate, accrual should be completed within approximately 3 – 4 years.

13.3 ANALYSIS OF SECONDARY ENDPOINTS

Durations of response, progression free survival, and overall survival will be determined actuarially using the Kaplan-Meier method.

A variety of immunohistochemistry evaluations, plasma studies, and other correlative studies will be undertaken as exploratory objectives during the conduct of this trial.

Immunohistochemistry results after treatment will be compared to baseline using McNemar's test for paired categorical data (or a marginal homogeneity test if appropriate). Continuously distributed data will be compared between responders and non-responders using a Wilcoxon rank sum test because of the potentially limited number of responders which may be identified.

Other descriptive, exploratory methods will be used as appropriate, and any p-values determined for analyses performed as secondary objectives will be presented without any formal adjustment for multiple comparisons, but will be described in the context of the secondary nature of the analysis and the number of such analyses performed.

13.4 REPORTING AND EXCLUSIONS

13.4.1 Evaluation of toxicity.

All patients will be evaluable for toxicity from the time of their first treatment with SS1(dsFv)PE38.

13.4.2 Evaluation of response.

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with exception of those who have not received at least one cycle of treatment and those with lung and pancreatic cancer in the dose de-escalation phase who do not receive the RP2D) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

14 HUMAN SUBJECTS PROTECTIONS

14.1 RATIONALE FOR SUBJECT SELECTION

This study will be open to all individuals with relapsed or refractory pleural or peritoneal mesothelioma, mesothelin expressing lung adenocarcinoma or pancreatic adenocarcinoma regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met. For safety reasons, pregnant women and children are excluded from this study. This study will be recruited through internal referral, our local physician referral base, and will be posted on clinicaltrials.gov. All individuals with relapsed or refractory pleural or peritoneal mesothelioma, pancreatic or mesothelin expressiong lung adenocarcinoma that have progressed after one cycle of standard care chemotherapy are eligible according to the eligibility criteria within section 3. This is a pilot/phase 2 study designed to evaluate the efficacy of a conditioning regimen of pentostatin and cyclophosphamide in reducing the immunogenicity of SS1(dsFv)PE38 in patients with advanced pleural or peritoneal mesothelioma, pancreatic or mesothelin expressing lung adenocarcinoma the side effect profile of the conditioning regimen in combination with SS1(dsFv)PE38, and the assessment of several biological endpoints. Subjects from both genders and all racial /ethnic groups are eligible for this study if they meet the

eligibility criteria outlined in section 3. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one ethnic group compared to another. Efforts will be made to extend accrual to each representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore racial/ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

14.2 JUSTIFICATION FOR EXCLUSIONS

Due to lack of knowledge of the effects of SS1(dsFv)PE38, cyclophosphamide and pentostatin on the fetus or on infants, as well as the possibility of teratogenic effects, pregnant and nursing women will be excluded from this trial. Patients with HIV will be excluded because information is unknown on the theoretical risk of the conditioning regimen on the progression of HIV, as pentostatin and cyclophosphamide are intended to target T cells, which is the target for HIV as well. Patients with unstable or serious medical conditions (ongoing or active infection, symptomatic congestive heart failure (AHA Class II or worse), unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements) are excluded due to the possibility that the combination regimen may worsen their condition and the likelihood that the underlying condition may obscure the attribution of adverse events with respect to the combination regimen of SS1(dsFv)PE38, pentostatin, and cyclophosphamide.

14.3 PARTICIPATION OF CHILDREN

Patients under the age of 18 years will be excluded from study. Since the study diseases are extremely rare in children and the toxic effects are yet unknown they are excluded from the study.

14.4 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

14.5 ADULTS UNABLE TO GIVE CONSENT ARE EXCLUDED FROM ENROLLING IN THE PROTOCOL AND AS STUDY ACTIVITIES ARE NOW LIMITED TO LONG TERM FOLLOW-UP FROM WHICH SUBJECTS DERIVE NO DIRECT BENEFIT, THEY ARE EXCLUDED FROM CONTINUED PARTICIPATION IN THE EVENT THAT THEY LOSE CAPACITY TO CONSENT DURING THE STUDY.EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The potential benefit to a patient who enters study is a reduction in the bulk of his/her tumor, which may or may not have a favorable impact on symptoms and/or survival. Potential risks include the possible occurrence of any of a range of side effects that are listed in the pharmaceutical section and the consent document. The procedure for protecting against or minimizing risks will be to medically evaluate patients on a regular basis, provide premedications and supportive therapies as described earlier.

14.6 CONSENT PROCESS AND DOCUMENTATION

An associate or principal investigator on the trial will inform patients of the purpose, alternatives, treatment plan, research objectives and follow-up of this trial. The patient will be provided an IRB-approved consent for review and signature and his/her questions will be answered. After a

decision is made to enroll into the study, a signature will be obtained from the patient at a subsequent visit. The original of the signed informed consent will be placed in the patient's medical record and a copy will be held in the research record.

All patients must have a signed informed consent form and an on-study (confirmation of eligibility) form filled out and signed by a participating investigator before entering on study.

Consent for optional biopsy will be obtained at the time of the procedure using the procedure consent. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

14.6.1 Telephone Re-Consent Procedure

Re-consent on this study may be obtained via telephone according to the following procedure: the informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone. A fully executed copy will be returned via mail for the subject's records. The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

14.6.2 Informed Consent of non-English Speaking Subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OSHRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed originals will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

15 DATA AND SAFETY MONITORING PLAN

15.1.1 Principal Investigator/Research Team

Any new significant finding that may affect the patient's willingness to continue in the study will be shared with patients. Data will be monitored regularly by the principal investigator and research staff in order to identify significant toxicity trends. Confidentiality will be maintained as much as possible, consistent with applicable regulations. Names of participants or identifying material will not be released without patient permission, except when such release is required by law. No patient's name or identifying information will be released in any publication or presentation. Records are maintained according to current legal requirements, and are made available for review according to the requirements of the Food and Drug Administration (FDA) or other authorized user, only under guidelines established by the Federal Privacy Act.

15.1.2 Sponsor Monitoring Plan

Please see section **12.2** for sponsor monitoring information.

15.1.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NCI-IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

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17 APPENDICES

17.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

17.2 APPENDIX B: STAGING SYSTEM FOR PLEURAL MESOTHELIOMA

International Mesothelioma Interest Group (IMIG) Staging system for Pleural Mesothelioma (1995)

Primary tumor (T)

TX: Primary tumor cannot be assessed

T0: No evidence of primary tumor

T1: Tumor involves same side pleura of the chest wall, with or without focal involvement of the pleura on the outer side of lung

T1a: Tumor involves same side pleura of the chest wall, no involvement of pleura on the outer surface of lung

T1b: Tumor involves same side pleura of the chest wall with focal involvement of pleura on the outer surface of lung

T2: Tumor involves same side pleura of the chest wall with at least one of the following features:

- Confluent tumor on the outer surface of the lung
- Involvement of the muscles of the diaphragm
- Involvement of the lung tissue deeper to the mesothelium covering the lung

T3: Tumor involves same side pleura of the chest wall with at least one of the following features:

- Involvement of the endothoracic fascia
- Involvement of the mediastinal fat
- Single focus of tumor involving the soft tissue of the chest wall
- Involvement of pericardium just on the out aspect (without penetration of pericardium)

T4: Tumor involves same side pleura of the chest wall with at least one of the following features:

- Diffuse or multi-focal involvement of the soft tissue of the chest wall
- Involvement of the rib
- Invasion through the diaphragm to the peritoneal cavity
- Invasion of any mediastinal organ
- Direct extension to the pleura on the other side
- Invasion into spine
- Penetration of the pericardium
- Pericardial effusion which is positive for cancer cells
- Involvement of heart muscle
- Involvement of the nerves of brachial plexus

Lymph node involvement (N)

NX: Regional lymph nodes cannot be assessed

N0: No regional lymph node involvement

N1: Involvement of same side broncho-pulmonary and or hilar lymph nodes only

N2: Involvement of subcarinal lymph node(s), and or same side or opposite side internal mammary or mediastinal lymph node(s)

N3: Involvement of opposite side mediastinal, internal mammary, or hilar lymph node(s) and or same side or opposite side supraclavicular or scalene lymph node(s)

Distant metastasis (M)

Mx: Distant metastasis cannot be assessed

M0: No distant metastasis

M1: Distant metastasis present

Staging

Stage 1

- T1 N0 M0

Stage IA

- T1a N0 M0

Stage IB

- T1b N0 M0

Stage II

- T2 N0 M0

Stage III

- T1, T2 N1 M0
- T1, T2, N2, M0
- T3, N0, N1, M0

Stage IV

- T4 Any N M0
- Any T N3 M0
- Any T, Any N, M1

17.3 APPENDIX C: REGIMEN A STUDY CALENDAR OF EVENTS

		Cycle 1 (30 days)										***Cycles 2 through 4 (21 days)***									
	Pre-Study	D 1	D 2	D 3	D 5	D 9	D 10	D 12	D 14	D 24	Day 30	D1	D2	D3	D 4	D 6	D 9	D 16	D 22	After every 2 cycles and end of treatment[g]	Long Term/Off Therapy Follow-up[h]
SS1 (dsFv) PE38 [a]							X	X	X				X		X	X					
Pentostatin[b]		X			X	X						X									
Cyclophosphamide		X	.....	.....	.....	.....		X				X	.....	.....	**X**						
Acyclovir		X	.....	.....	.....	.....	.....	.....	.....	.....	X	X	.....	.....	.....	.....	.....	.....	X	X	X[i]
TMP/SMX																		M, W, F*		M, W, F*	M, W, F*
Vaccines(Influenza, pneumococcal 13 valent & 23 valent, DTaP, Hib, IPV, Hep A & B, MMR, VZV, Meningococcal																					X[m]
Informed consent	X																				
Demographics &history	X																				
Physical exam & Perf status	X	X					X					X								X	X
Vital signs	X	X			X	X	X	X	X			X	X		X	X				X	X
Height	X																				
Weight	X	X					X					X				X				X	X
CBC w/diff	X	X			X	X		X				X					X	X		X	X
Acute Care Panel, Hepatic Panel, calcium, albumin, total protein [c]	X	X			X	X		X				X					X	X		X	X
EKG	X																				

		Cycle 1 (30 days)										***Cycles 2 through 4 (21 days)***									
	Pre-Study	D 1	D 2	D 3	D 5	D 9	D 10	D 12	D 14	D 24	Day 30	D1	D2	D3	D 4	D 6	D 9	D 16	D 22	After every 2 cycles and end of treatment[g]	Long Term/Off Therapy Follow-up[h]
Urinalysis	X	X			X	X						X				X				X	X
TBNK panel		X										X									X
immune studies [k](Dr. Hakim)		X										X									X
Serum neutralizing antibody testing	X[l]									X[l]	X[l]							X[l]	X[l]		
Hepatitis Screening Panel	X																				
SS1 (dsFv) PE38 PK study[d]							X[d]	X[d]	X[d]				X[d]		X[d]	X[d]					
Serum C-reactive protein		X[j]					X[n]	X[n]	X[n]			X[j]	X[n]		X[n]	X[n]					
Serum for cytokine evaluation		X[j]					X[n]	X[n]	X[n]			X[j]	X[n]		X[n]	X[n]					
Circulating Mesothelin Levels		X[j]							X[n]			X[j]								X	
Tissue evaluation (lung CA patients only for mesothelin expression)	X																				
Mesothelin IHC of archived tissue		X																			
Radiologic evaluation • CT (C/A/P) • FDG-PET	X																			X	X (if indicated)
Tumor measurements	X																			X	X (if indicated)
Urine or serum pregnancy test[e]	X																				

		Cycle 1 (30 days)										***Cycles 2 through 4 (21 days)***									
	Pre-Study	D 1	D 2	D 3	D 5	D 9	D 10	D 12	D 14	D 24	Day 30	D1	D2	D3	D 4	D 6	D 9	D 16	D 22	After every 2 cycles and end of treatment[g]	Long Term/Off Therapy Follow-up[h]
HLA typing[f]	X																				
Concurrent meds	X	X					X														X
Adverse Event evaluations	X						X														X

* It is recommend that subjects take TMP/SMX on Monday, Wednesday and Friday to promote consistency. However, any 3 days in a given week is sufficient.

a. SS1(dsF v)PE38 Premedicate: Ranitidine 150 mg po, hydroxyzine 10-25 mg po, ONE hour prior to SS1PE38; Dexamethasone 4 mg po Q12 h or QD as clinically indicated on days 10 - 15 of cycle 1 and on days 2-7 of cycles 2 through 6. **Note** PO dexamethasone taken prophylactically for SS1(dsFv)PE38 on days 10 and 11 of cycle 1 and days 2 and 3 of subsequent cycles will also be counted as a dose towards pentostatin prophylaxis
b. Pentostatin Premedicate: -Dexamethasone 12 mg IV, and ondansetron 8 mg IV 60 min prior to each dose; -Dexamethasone 4 mg po daily for 2 days following pentostatin Ondansetron 8 mg po Q 12 h for D 1 thru 12 in Cycle 1 (one evening dose on days when IV is given) and for D1 through 4 for Subsequent cycles (one evening dose on day 1 when IV is given). **Note** PO dexamethasone taken prophylactically for pentostatin on days 10 and 11 of cycle 1 and days 2 and 3 of subsequent cycles will also be counted as a dose towards SS1(dsFv)PE38 prophylaxis.
c. sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN, Albumin, calcium, alkaline phosphatase, SGOT [AST], SGPT [ALT], total bilirubin, total protein.
d. PK sample times for each cycle: Dose 1: Pre, End of Infusion (EOI), 1, 1.5, 2, 4, 8, 12 hours, Dose 2: Pre, EOI, Dose 3: Pre, EOI.
e. Pregnancy test (women of childbearing potential).
f. Anytime during enrollment on study
g. After the last treatment cycle, which may occur at cycle 4 or after SS1(dsFv)PE38 is discontinued for toxicity or neutralization, patients will be evaluated in clinic with PE, Labs and repeat imaging until off study, and then contacted yearly for survival.
h. Off-therapy evaluation.
i. continue until 30 days after the completion of treatment
j. Prior to the 1st dose of each cycle.

k. cell surface markers of immune cells (memory vs. naïve markers, Treg cell markers) and determination of T cell function
l. neutralizing antibodies may be sampled in +/-2 days of these time points
m. vaccination schedule: influenza – lifelong, seasonal starting before HSCT and resuming after 6 months; pneumococcal – at 6, 12 & 18 months post transplant; pneumococcal 23 valent – 24 months post transplant; DTaP, Hib conjugate, IPV - 12, 18, 24 months post transplant; MMR, VSV – 24 months post transplant only if patient is immunocompetent at 24 months
n. Samples will be obtained prior to each SS1(dsFv)PE38 administration

17.4 APPENDIX D: ESTIMATED CREATININE CLEARANCE BY COCKROFT-GAULT FORMULA

A commonly used surrogate marker for actual creatinine clearance is the Cockroft-Gault formula, which employs creatinine measurements and a patient's weight to predict the clearance. The formula, as originally published, is:

$$x = \frac{(140 - age) \times weight}{72 \times creatinine}$$

This formula expects weight (actually mass) to be measured in kilograms and creatinine to be measured in mg/dL, as is standard in the USA. The resulting value is multiplied by a constant of 0.85 if the patient is female. This formula is useful because the calculations are relatively simple and can often be performed without the aid of a calculator.

A modification of this formula, useful for the common units of measure, is:

$$x = \frac{(140 - age) \times weight \times constant}{creatinine}$$

This formula uses metric units (weight in kilograms, creatinine in µmol/L). The constant is 1.23 for men and 1.04 for women.

17.5 APPENDIX E: MEDICATION DIARY FOR CYCLOPHOSPHAMIDE

SUBJECT DIARY

Instructions:

Cyclophosphamide, 200mg, should be taken in the morning, preferably on an empty stomach, at approximately the same time each day. While taking cyclophosphamide, you should drink 2-4 liters of fluid per day to maintain clear color urine and empty your bladder frequently and prior to sleeping.

Please complete the form below to keep track of your doses. For each day, complete the **date and time** for when you took the cyclophosphamide. If you experienced any **symptoms** that you feel might have been related to the drug, write those down as well. Always take this form with you when you attend your visits with the clinic or hospital.

CYCLE 1

DAY	DATE	TIME	SYMPTOMS
1		am	
2		am	
3		am	
4		am	
5		am	
6		am	
7		am	
8		am	
9		am	
10		am	
11		am	
12		am	

Signature: _____ Date: _____

CYCLES 2 THROUGH 4

DAY	DATE	TIME	SYMPTOMS
1		am	
2		am	
3		am	
4		am	

Signature:_____ Date:_____

INSTITUTE: National Cancer Institute

STUDY NUMBER: 11-C-0160 PRINCIPAL INVESTIGATOR: Raffit Hassan, M.D.

STUDY TITLE: A Pilot/Phase II Study of Pentostatin Plus Cyclophosphamide Immune Depletion to Decrease Immunogenicity of SS1P in Patients with Mesothelioma, Lung Cancer or Pancreatic Cancer

Continuing Review Approved by the IRB on 09/12/16
Amendment Approved by the IRB 07/06/17 (M) Date Posted to Web: 07/15/17
Standard – Regimen A – Lot 073I0809

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary**.**

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

Why is this study being done?

The purpose of this study to test the safety and feasibility of giving an experimental cancer antibody, called SS1(dsFv)PE38, or SS1P for short, to people with mesothelioma who have had their immune systems suppressed with a combination of drugs, pentostatin and cyclophosphamide. In addition, this study will determine if the study regimen helps your tumor to shrink.

Malignant mesothelioma is a form of cancer that develops on the protective lining that covers the body's internal organs. It most commonly occurs on the lining of the lungs and chest wall, called pleural mesothelioma, or the lining of the abdomen, called peritoneal mesothelioma.

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Because there is no known effective cure for everyone with mesothelioma, we are searching for new effective ways to treat it.

Mesothelin is a protein that is found in mesothelioma, and other types of cancer. SS1P is an anti-mesothelin antibody which has been combined with PE38, a toxic piece of bacteria called *Pseudomonas.* The SS1P and PE38 have been combined genetically to form the experimental agent SS1(dsFv)PE38 (SS1P) that in laboratory studies has been able to kill mesothelin-expressing cells (like your mesothelioma cells), but not similar cells that do not express mesothelin (like your normal cells). SS1P is experimental drug because it has not been approved by the US Food and Drug Administration (FDA) for treating patients. But the FDA has given us permission to use it in this study. We do not expect that we will seek FDA approval for this agent.

Experiments in people show that frequently the immune system creates cells to fight outside antibodies, called neutralizing antibodies. When this happens, the antibody (such as SS1P) is no longer effective in fighting cancer cells. One way to allow cancer fighting antibodies to work is to 'turn off' or suppress the immune system for a short period of time.

In this study we will give pentostatin, also called Nipent, and cyclophosphamide, also called Cytoxan, to suppress the immune system, followed by the antibody SS1P to see how well it works against your cancer. Pentostatin and cyclophosphamide are both chemotherapy drugs approved by the FDA for use in cancer patients. Thirty-two subjects have already been seen on study and have received one of 2 regimens. 11 subjects received regimen A and 8 subjects received regimen B. Subjects on regimen B received more cyclophosphamide and pentostatin than subjects on regimen A. Regimen A was well tolerated with no subjects developing infections due to the drugs, and most of the 11 regimen A subjects were able to begin a second cycle. Only 2 were able to have more than 2 cycles because antibodies developed. Of the 8 subjects on regimen B, so far 3 have been able to get more than 2 cycles of treatment with 2 more still on their second cycle. However, subjects in regimen B showed more side effects from the study drugs including fatigue, shortness of breath and irregular heart beat. Because of these results, the next 13 subjects with mesothelioma received Regimen A

We will continue to test Regimen A in patients with mesothelioma, but also in patients with lung cancer and pancreatic cancer – which are also known to have mesothelin on the surface; however, those patients (as well as the later patients with mesothelioma) will sign a different consent because a slightly different version of SS1P will be used on them. Your version of drug is lot 073I0809 as indicated in the consent title.

Why are you being asked to take part in this study?

You are being invited to take part in this study because you have either pleural or peritoneal mesothelioma which has not been cured by previous treatments.

How many people will take part in this study?

Up to 75 subjects will be enrolled on this study, including the subjects already seen.

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Description of Research Study

What will happen if you take part in this research study?

Before you begin the study

All research studies have specific criteria for entry to allow for valid interpretation of the study results and safety of participants, known as eligibility criteria. Before you begin this study you will need to have the following exams and tests to make sure you are eligible for this study. The exams and tests are part of regular cancer care and may be done even if you do not join the study. You will probably have these tests done while you are on the NCI Screening Protocol. If you recently had some of the tests, they may not need to be repeated.

- History and physical examination, including vital signs (height, weight, blood pressure, heart rate, temperature, breathing rate)
- Standard blood tests to measure your liver and kidney function, white blood cells, red blood cells and platelets, and blood electrolytes. If you are a woman able to get pregnant, you will also have a pregnancy test done.
- Scans and x-rays to measure your disease, including a CT scan of the chest, abdomen and pelvis, and a FDG-PET scan
- Electrocardiogram (ECG)
- A sample of tissue from any previous surgery or biopsy will be tested at NCI.

In addition, we will test a sample of your blood to make sure you don't already have neutralizing antibodies against SS1P. If you meet the eligibility criteria for this study you will be offered the option of participating and you will be asked to sign this informed consent document before participating in the study

During the study

This study will be done in Cycles:

1. Cycle 1 will be different that the other cycles as you will receive more chemotherapy to get the immune system ready for the SSP1
2. Cycles 2 through 4
3. Follow up regimen

<u>Cycle 1</u>

Cycle 1 will last 30 days and on Cycle 1 you will receive more chemotherapy in an effort to prepare your immune system for the investigational antibody SS1P. Pentostatin will be given to you on Days 1, 5, and 9 of Cycle 1. Pentostatin will be given to you through an IV (intravenous catheter, a small plastic tube that is put into a vein in your arm or neck) over about 30-60

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minutes. Investigators will discuss the option of inserting a central catheter, a catheter that will remain in your vein throughout the time you are on the study.

About 60 minutes before each dose of pentostatin you will be given 2 medicines in your IV to help prevent any side effects:

1) Ondansetron (also called Zofran) to help prevent nausea and vomiting
2) Dexamethasone (a type of steroid) to prevent nausea and vomiting

You will also be asked to take dexamethasone tablet(s) once a day on days 2 – 3, 6 – 7 and 10 – 15. If your study doctor feels that your side effects are severe enough, you may be asked to take dexamethasone twice a day on days 10 – 15. The oral dexamethasone is given to prevent nausea, vomiting and inflammation in the lining of your lungs (pleuritis). During cycle 1 you will take ondansetron tablet(s) twice a day for 12 days, morning and evening, starting on the evening of your pentostatin dose to help prevent nausea and vomiting. You will not take a morning dose of ondansetron on the day of your pentostatin dose.

You will take cyclophosphamide tablets every morning for 12 days during Cycle 1 (Days 1 through 12). You should drink plenty of liquids every day (2-4 liters, or ½ to 1 gallon per day) to keep your urine clear colored. You should also empty your bladder frequently during the day and before you go to sleep to keep the cyclophosphamide from damaging the tissues in your bladder, which can cause inflammation and bleeding (cystitis). We need you to keep a diary of when you take the cyclophosphamide and any symptoms you may have. The nurse will give you a diary form for this purpose. Please bring it with you to clinic when you come at the end of each cycle. If you are unable to take the cyclophosphamide tablets, you may be asked to come to the clinic on these days to receive the medication through an IV.

During Cycle 1 you will be given SS1P the investigational antibody on Days 10, 12 and 14 though an IV over about 30 minutes.

You will be given the following before each dose of SS1P to help prevent side effects:

1) A bag of salt water will be given in your IV for 2-4 hours before and after each dose. Ranitidine tablet(s) (also called Zantac) and hydroxyzine capsule(s) or tablet(s) (also known as Vistaril or Atarax) will be given to you one hour before each dose of SS1P.

<u>Cycles 2 through 4</u>

Upon completing Cycle 1 and after each cycle, you will have a physical exam and standard blood tests, and blood taken for research tests. The schedule of drugs you will receive during the Cycles 2 through 4 is listed in the chart below. Cycles 2 through 4 <u>will last 21</u> days each. At the conclusion of every 2 cycles and at the end of your last cycle you will also have a CT scan of your disease. The following is a summary of what you will receive:

Pentostatin will be given to you in your IV (with the 2 premedications listed above) on Day 1 of each cycle.

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You will take Cyclophosphamide tablets on Days 1 through 4 of each cycle. Again, you may receive this medication through an IV if you are unable to take orally.

SS1P will be given to you in your IV on Days 2, 4, and 6 of each cycle.

You will also be asked to take dexamethasone tablet(s) once a day on days 2 -7. If your study doctor feels that your side effects are severe enough, you may be asked to take dexamethasone twice a day. During cycles 2 through 4 you will take ondansetron tablet(s) twice a day for 4 days, morning and evening, starting on the evening of your pentostatin dose to help prevent nausea and vomiting. You will not take a morning dose of ondansetron on the day of your pentostatin dose.

We will watch you closely during and after the medicines you are given, checking your blood counts twice a week and checking your urine at specified times. Other medicines may be given to you if you experience any side effects. The dose of chemotherapy may be adjusted or doses delayed depending upon your blood tests and your side effects. In addition you may be given antibiotics and throughout this study you will be given other medicines to prevent viral or fungal infections while your immune system is affected. The chemotherapy agents will lower your white cells, red cells and platelets, in addition to lowering the cells in the immune system; therefore we may give you a drug called filgrastim (G-CSF) as a daily shot under the skin, if your white cells get too low.

To test if your immune system is 'neutralizing' the effects of the SS1P, we will test a blood sample taken on Day 24 and 30 of Cycle 1 and Day 16 and 22 of cycles 2 through 4. . These dates may be moved up or back two days depending on your individual needs. If the test shows that your body is making antibodies against the SS1P, the treatment may need to be stopped.

Research blood tests

An important part of this study is testing how your body handles the SS1P, called pharmacokinetic studies or PKs. We will take less than 1/2 teaspoon of blood before and after each dose of SS1P. In addition, after your first dose of each cycle we will take less than 1/2 teaspoon of blood at the following time points: 1, 1 1/2, 2, 4, 8 and 12 hours after the SS1P, for a total of about 24 mL or approximately 5 teaspoons. In addition, blood will be taken to study the effects of this treatment on the immune cells in your blood. We will take approximately 5 ½ teaspoons of blood before you receive the study drug during each cycle, and then during each of your follow up visits (every 3 months). We will also take approximately 1 - 2 teaspoon of blood to study the levels of certain proteins in the blood before you receive study drug during each cycle and before each dose of SS1P. These blood tests are part of the research and are not optional.

When you are finished taking the drugs (treatment) – Follow up Regimen

Once the treatment regimen is stopped, we will ask you to have a series of vaccinations against a number of potential infections. You will complete most of the vaccinations within 2 years of your transplant; however you will be asked to receive lifelong annual vaccinations for the

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seasonal flu. We will also ask you come to the Outpatient Clinic for a physical exam, standard blood tests and scans of your cancer every three (3) months. If your cancer gets worse we will refer you back to your local doctor for further treatment but will contact you every year to see how you are. The scans will include a CT scan of your chest and abdomen, a FDG-PET scan and may include an MRI if indicated.

Study Chart

Day	**Each Cycle** **What to do and what will happen to you**
After signing the consent, before starting Treatment	Get routine blood tests. Provide a history of how you feel and undergo a physical examination by the research team's Health Care Provider. Research blood samples will be taken CT scan of the chest, abdomen and pelvis, FDG-PET and MRI if indicated.
CYCLE 1	**30 Days long**
Days 1, 5 and 9	Routine blood and urine tests Dexamethasone IV, and ondansetron IV 60 minutes before Pentostatin Pentostatin IV over 30-60 minutes Take dexamethasone tablet(s) on days 2 -3, 6-7, and 10 – 15.
Days 1 through 12	Take Cyclophosphamide tablet(s) every morning. Drink 2-4 liters or ½ to 1 gallon of liquid per day to keep urine clear yellow Take Ondansetron tablet(s) every morning and every night, except on days receiving Pentostatin, only take a night dose of Ondansetron tablet(s)
Days 1, 10, 12 and 14	Ranitidine tablet and hydroxyzine one hour before SSP1 SS1P IV over about 30 minutes Research blood samples will be drawn
Days 24 and 30*	Blood test for immune 'neutralizing' assay.
Day 30 (may also be Day 1 of Cycle 2)	Return to the NIH clinic to see your doctor. Provide a history of how you feel and have a physical exam by your Health Care Provider. Blood and urine will be taken for routine cancer care tests and research blood samples. If you are tolerating the treatment, the next cycle will begin.
Cycles 2 through 4	**21 Days Long**
Day 1	Dexamethasone IV, and ondansetron IV 60 minutes before Pentostatin

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Day	Each Cycle What to do and what will happen to you
	Pentostatin IV over 30-60 minutes Take dexamethasone tablet(s) on days 2 – 7.
Days 1 through 4	Take Cyclophosphamide tablet(s) every morning Drink 2-4 liters or ½ to 1 gallon of liquid per day to keep urine clear yellow Take ondansetron tablet(s) every morning and every night, except on the day of your Pentostatin infusion. On that day, only take the night dose of ondansetron tablet(s)
Days 1, 2, 4 and 6	Ranitidine tablet and hydroxyzine one hour before SSP1 SS1P IV over about 30 minutes Research blood samples will be drawn
Days 16 and 22*	Blood test for immune 'neutralizing' assay.
Day 21 of Cycle 2	Return to the NIH clinic to see your doctor. Provide a history of how you feel and have a physical exam by your Health Care Provider. Blood and urine will be taken for routine cancer care tests and research blood samples. CT scan of the chest, abdomen and pelvis, FDG-PET and MRI if indicated. If you are tolerating the treatment and your disease has not gotten worse, cycle 3 will begin.
Cycles 3 - 4	Will be given exactly like Cycle 2
After stopping Treatment	Return to the NIH clinic to see your doctor about every 3 months until your cancer gets worse and you return to your local doctor for other treatment. Provide a history of how you feel and have a physical exam by your Health Care Provider. Blood and urine will be taken for routine cancer care tests and research blood samples drawn. CT scan of the chest, abdomen and pelvis, FDG-PET and MRI if indicated. You will receive a series of vaccinations to help prevent infection.
Follow Up	At least yearly, a member of the research team will contact you or your referring physician to ask about your condition.

* Assays may be taken two days earlier or two days later than scheduled depending on your individual needs

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What does this study involve?

Birth Control

If you are a woman who is breast feeding or pregnant, you may not take part in the study because we don't know how this medicine would affect your baby or your unborn child. If you are a woman who can become pregnant, or are the partner of a woman who can become pregnant, you will need to practice an effective form of birth control before starting study treatment, during study treatment, and for 3 months after you finish study treatment. If you think that you or your partner is pregnant, you should tell your study doctor or nurse at once.

Acceptable effective forms of birth control include:

- abstinence
- intrauterine device (IUD)
- tubal ligation
- vasectomy

Risks or Discomforts of Participation

What side effects or risks can I expect from being in this study?

If you choose to take part in this study, there is a risk that:

- You may lose time at work or home and spend more time in the hospital or doctor's office than usual
- You may be asked sensitive or private questions which you normally do not discuss

The SS1P used in this study may affect how different parts of your body work such as your liver, kidneys, heart, and blood. The study doctor will be testing your blood and will let you know if changes occur that may affect your health.

There is also a risk that you could have side effects from the study drug(s)/study approach.

Here are important points about side effects:

- The study doctors do not know who will or will not have side effects.
- Some side effects may go away soon, some may last a long time, or some may never go away.
- Some side effects may interfere with your ability to have children.
- Some side effects may be serious and may even result in death.

Here are important points about how you and the study doctor can make side effects less of a problem:

- Tell the study doctor if you notice or feel anything different so they can see if you are having a side effect.

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- The study doctor may be able to treat some side effects.
- The study doctor may adjust the study drugs to try to reduce side effects.

The tables below show the most common and the most serious side effects that researchers know about. There might be other side effects that researchers do not yet know about. If important new side effects are found, the study doctor will discuss these with you..

While the combination of pentostatin and cyclophosphamide may reduce the likelihood that you will make neutralizing antibodies to SS1P, it may also be associated with severe or prolonged immune suppression. As part of this study, you will receive additional medications to prevent infections that are known to occur in patients with suppressed immune systems.

Possible Side Effects of Pentostatin (Deoxycoformycin, dCF)

COMMON, SOME MAY BE SERIOUS In 100 people receiving Pentostatin (deoxycoformycin, dCF), more than 20 and up to 100 may have:
• Anemia which may require blood transfusions • Nausea, vomiting • Fever • Tiredness • Bruising, bleeding • Infection, especially when white blood cell count is low • Itching, rash

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Pentostatin (deoxycoformycin, dCF), from 4 to 20 may have:
• Anemia, kidney problems which may cause swelling, or may require dialysis • Blood clot • Diarrhea, loss of appetite • Sores in mouth which may cause difficulty swallowing • Chills • Pain • Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Headache • Cough, stuffy nose • Confusion

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RARE, AND SERIOUS In 100 people receiving Pentostatin (deoxycoformycin, dCF), 3 or fewer may have:
• Kidney damage

Possible Side Effects of Cyclophosphamide

COMMON, SOME MAY BE SERIOUS In 100 people receiving Cyclophosphamide, more than 20 and up to 100 may have:
• Hair loss • Nausea, vomiting, loss of appetite • Sores in mouth • Infection, especially when white blood cell count is low • Absence of menstrual period which may decrease the ability to have children • Blood in urine

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Cyclophosphamide, from 4 to 20 may have:
• Damage to the bone marrow (irreversible) which may cause infection, bleeding, may require transfusions • Loss or absence of sperm which may lead to an inability to father children • Stuffy nose • Fluid around the heart

RARE, AND SERIOUS In 100 people receiving Cyclophosphamide, 3 or fewer may have:
• Severe skin rash with blisters and peeling which can involve mouth and other parts of the body • Damage to the heart or heart failure which may cause shortness of breath, swelling of ankles, cough or tiredness • A new cancer including cancer of bone marrow (leukemia) caused by chemotherapy • Swelling of the body including the brain which may cause dizziness, confusion • Scarring of the lungs

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SS1(dsFv)PE38:

The side effects that may happen as a result of the SS1P include:

POSSIBLE, SOME MAY BE SERIOUS
• Fluid in the body which may cause low blood pressure, shortness of breath, or swelling of ankles • Abnormal heartbeat • Pain • Diarrhea, nausea, vomiting • Chills, tiredness, fever • Swelling of the body • Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Weight gain • Dizziness, headache • Shortness of breath • Itching, rash, hives • Low blood pressure which may cause feeling faint

Side effects of support medications: You will be given numerous medications to treat or prevent certain side effects of this study. These medications also have possible side effects.

Filgrastim: If your blood counts fall very low you will be given filgrastim, also known as G-CSF, to help stimulate your immune cells to re-expand. Bone pain is the most common side effect of the filgrastim, which can usually be controlled with acetaminophen (Tylenol). Rare events of adult respiratory distress syndrome and splenic rupture have been reported with filgrastim.

Bactrim: One of the medicines you may be given to prevent a type of pneumonia is called TMP/SMX double strength. This medicine can cause fever, nausea and vomiting or a skin rash with itching, or it can reduce the number of white blood cells in your blood. This medicine will be continued until after you have finished the immune suppressing drugs and the number of CD4 cells (a type of white blood cell) has been above 200 for three months.

Acyclovir: To prevent herpes infections we will give you a medicine called acyclovir if you can swallow your medications. Acyclovir can cause reversible kidney damage, delirium, tremors, coma, emotional changes, and abnormal Electroencephalogram (EEGs) when taken in higher doses. Stomach upset, headache or nausea, rash or hives; sweating; blood in the urine; and low blood pressure and low platelet count have been reported. Hair loss from prolonged use has also been reported. This medicine will be continued until after you have finished the

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immune suppressing drugs and the number of CD4 cells (a type of white blood cell) has been above 200 for three months.

Dexamethasone: A steroid to prevent allergic reactions which can cause high blood pressure and irregular heartbeats, dry skin, rash or impaired healing of skin wounds, high blood sugar, changes in blood electrolytes such as potassium or sodium, loss of muscle mass, weight gain, mood swings, insomnia, and in rare cases, allergic reaction.

Ranitidine: To help prevent upset stomach, it can cause constipation, diarrheas, headache, nausea or upset stomach. Rare serious side effects include severe allergic reaction with rash, hives, itching, difficulty breathing; confusion, dark urine, depression, fast or slow heartbeat, unusual bruising or bleeding, yellowing of the eyes or skin (indicating liver damage).

Hydroxyzine: It can cause dry mouth. Rare serious side effects include severe allergic reaction with rash, hives, itching, difficulty breathing; involuntary movements.

If your red blood cells or platelets go too low, you may require transfusion with red cells or platelets.

You may experience none of these effects, or you may experience many of these effects. Tell your doctor and nurse about your side effects and they will try and minimize them as much as possible.

Potential Benefits of Participation

The aim of this study is to see if this experimental treatment will cause your tumors to shrink. We do not know if you will receive personal, medical benefit from taking part in this study. These potential benefits could include shrinking of your tumor or lessening of your symptoms, such as pain, that are caused by the cancer. Because there is not much information about the drug's effect on your cancer, we do not know if you will benefit from taking part in this study, although the knowledge gained from this study may help others in the future who have cancer.

Alternative Approaches or Treatments

What other choices do I have if I do not take part in this study?

Instead of being in this study, you have these options**:**

- Getting treatment or care for your cancer without being in a study
- Taking part in another study
- Getting comfort care, also called palliative care. This type of care helps reduce pain, tiredness, appetite problems and other problems caused by the cancer. It does not treat the cancer directly. Instead, it tries to improve how you feel. Comfort care tries to keep you as active and comfortable as possible.

Please talk to your doctor about these and other options.

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Research Subject's Rights

What are the costs of taking part in this study?

If you choose to take part in the study, the following will apply, in keeping with the NIH policy:

- You will receive study treatment at no charge to you. This may include surgery, medicines, laboratory testing, x-rays or scans done at the Clinical Center, National Institutes of Health (NIH), or arranged for you by the research team to be done outside the Clinical Center, NIH if the study related treatment is not available at the NIH.
- There are limited funds available to cover the cost of some tests and procedures performed outside the Clinical Center, NIH. You may have to pay for these costs if they are not covered by your insurance company.
- Medicines that are not part of the study treatment will not be provided or paid for by the Clinical Center, NIH.
- Once you have completed taking part in the study, medical care will no longer be provided by the Clinical Center, NIH.

Will your medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), which are involved in keeping research safe for people.
- National Cancer Institute Institutional Review Board
- Qualified representatives from the pharmaceutical collaborator for the SS1P antibody

A description of this clinical trial will be available on http://www.Clinicaltrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most the Web site will include a summary of the results. You can search this Web site at any time.

Stopping Therapy

Your doctor may decide to stop your therapy for the following reasons:

- if he/she believes that it is in your best interest;
- if your disease comes back during treatment;
- if you have side effects from the treatment that your doctor thinks are too severe;
- if new information shows that another treatment would be better for you.

In this case, you will be informed of the reason therapy is being stopped.

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You can stop taking part in the study at any time. However, if you decide to stop taking part in the study, we would like you to talk to the study doctor and your regular doctor first.

If you decide at any time to withdraw your consent to participate in the trial, we will not collect any additional medical information about you. However, according to FDA guidelines, information collected on you up to that point may still be provided to the Cancer Therapy Evaluation Program (NCI) or designated representatives. If you withdraw your consent and leave the trial, any samples of yours that have been obtained for the study and stored at the NCI can be destroyed upon request. However, any samples and data generated from the samples that have already been distributed to other researchers or placed in the research databases can**not** be recalled and destroyed.

Conflict of Interest

The National Institutes of Health (NIH) reviews NIH staff researchers at least yearly for conflicts of interest. This process is detailed in a Protocol Review Guide. You may ask your research team for a copy of the Protocol Review Guide or for more information. Members of the research team who do not work for NIH are expected to follow these guidelines but they do not need to report their personal finances to the NIH.

Members of the research team working on this study may have up to $15,000 of stock in the companies that make products used in this study. This is allowed under federal rules and is not a conflict of interest.

The National Institutes of Health and the research team for this study have developed a drug being used in this study. This means it is possible that the results of this study could lead to payments to NIH scientists and to the NIH. By law, government scientists are required to receive such payments for their inventions. You will not receive any money from the development of SS1(dsFv)PE38.

Optional Biopsy

We would like to collect a biopsy 3 times during the study. A biopsy is a procedure using a needle to remove a piece of tissue or a sample of cells from your body so that it can be analyzed in a laboratory and is used to diagnose or evaluate the cancer. With your permission, a tumor biopsy will be performed: before you have received study drug; after 2 cycles of treatment; and at the end of the last cycle of treatment or at follow-up. We may use ultrasound or MRI to guide us in collecting the sample as well as CT scans that have already been performed. We will take no more than 4 pieces of tissue from each tumor site.

Your doctor will discuss the risks associated with obtaining tissue samples. Generally during a biopsy, local anesthetic is given and one just feels some minimal pain at this site where the sample is obtained. Rarely, infection or bleeding may occur at the sample site.

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The biopsy to be performed is exclusively for research purposes and will not benefit you. It might help other people in the future. You will be given the opportunity to decide whether you would like to participate at the time of the procedure.

Use of Specimens and Data for Future Research

To advance science, it is helpful for researchers to share information they get from studying human samples. They do this by putting it into one or more scientific databases, where it is stored along with information from other studies. A researcher who wants to study the information must apply to the database and be approved. Researchers use specimens and data stored in scientific databases to advance science and learn about health and disease.

We plan to keep some of your specimens and data that we collect and use them for future research and share them with other researchers. We will not contact you to ask about each of these future uses. These specimens and data will be stripped of identifiers such as name, address or account number, so that they may be used for future research on any topic and shared broadly for research purposes. Your specimens and data will be used for research purposes only and will not benefit you. It is also possible that the stored specimens and data may never be used. Results of research done on your specimens and data will not be available to you or your doctor. It might help people who have cancer and other diseases in the future.

If you do not want your stored specimens and data used for future research, please contact us in writing and let us know that you do not want us to use your specimens and/or data. Then any specimens that have not already been used or shared will be destroyed and your data will not be used for future research. However, it may not be possible to withdraw or delete materials or data once they have been shared with other researchers.

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OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Raffit Hassan, M.D., Building 10, Room 4-5330**,** Telephone: 301-451-8742. You may also call the Clinical Center Patient Representative at (301) 496-2626. If you have any questions about the use of your specimens or data for future research studies, you may also contact the Office of the Clinical Director, Telephone: 301-496-4251.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

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COMPLETE APPROPRIATE ITEM(S) BELOW:	
A. Adult Patient's Consent I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study. _____ _____ Signature of Adult Patient/ Legal Representative Date _____ Print Name	**B. Parent's Permission for Minor Patient.** I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study. (Attach NIH 2514-2, Minor's Assent, if applicable.) _____ _____ Signature of Parent(s)/ Guardian Date _____ Print Name

C. Child's Verbal Assent (If Applicable)
The information in the above consent was described to my child and my child agrees to participate in the study.

_____ _____ _____
Signature of Parent(s)/Guardian Date Print Name

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM SEPTEMBER 12, 2016 THROUGH SEPTEMBER 11, 2017.

Signature of Investigator	Date	Signature of Witness	Date
Print Name		Print Name	

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INSTITUTE: National Cancer Institute

STUDY NUMBER: 11-C-0160 PRINCIPAL INVESTIGATOR: Raffit Hassan, M.D.

STUDY TITLE: A Pilot/Phase II Study of Pentostatin Plus Cyclophosphamide Immune Depletion to Decrease Immunogenicity of SS1P in Patients with Mesothelioma, Lung Cancer or Pancreatic Cancer

Continuing Review Approved by the IRB on 09/12/16
Amendment Approved by the IRB 07/06/17 (M) Date Posted to Web: 07/15/17
Standard – Regimen A – Lot FIL129J01

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary**.**

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

Why is this study being done?

The purpose of this study to test the safety and feasibility of giving an experimental cancer antibody-type therapeutic, called SS1(dsFv)PE38, or SS1P for short, to people with mesothelioma, pancreatic cancer or mesothelin expressing lung cancer who have had their immune systems suppressed with a combination of drugs, pentostatin and cyclophosphamide. In addition, this study will determine if the study regimen helps your tumor to shrink.

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Mesothelin is a protein that is expressed (found) on the surface of some cancer cells like mesothelioma, almost all pancreatic cancer and thirty to fifty percent of lung cancer. It may also be found in other types of cancer. SS1P is an anti-mesothelin antibody which has been fused with PE38, a therapeutic derived from a toxic substance made by a bacteria called *Pseudomonas.* The SS1P and PE38 have been combined genetically to form the experimental agent SS1(dsFv)PE38 (SS1P). In laboratory studies, SS1P has been able to kill mesothelin-expressing cells (like your tumor cells), but does not kill similar cells that do not express mesothelin (like your normal cells). SS1P is an experimental drug because it has not been approved by the US Food and Drug Administration (FDA) for treating patients. The FDA has given us permission to use it in this study. We do not expect that we will seek FDA approval for this agent.

Experiments in people have shown that the immune system creates cells to fight antibody-type therapeutics (like SS1P) and other substances that are not usually part of our body. These immune cells make substances called neutralizing antibodies that bind the foreign therapeutic and remove it from the body. When this happens, the therapeutic (such as SS1P) is no longer effective in fighting cancer cells. One way to allow SS1P to work better is to 'turn off' or suppress the immune system for a short period of time.

In this study we will give pentostatin, also called Nipent, and cyclophosphamide, also called Cytoxan, to suppress the immune system, followed by SS1P to see how well it works against your cancer. Pentostatin and cyclophosphamide are both chemotherapy drugs approved by the FDA for use in cancer patients.

Thirty-two subjects with mesothelioma have already been seen on study and have received one of 2 regimens. Initially 11 subjects received regimen A and 8 subjects received regimen B. Subjects on regimen B received more cyclophosphamide and pentostatin than subjects on regimen A. Regimen A was well tolerated with no subjects developing infections due to the drugs, and most of the 11 regimen A subjects were able to begin a second cycle. Only 2 were able to have more than 2 cycles because antibodies developed. More patients on regimen B were able to have more than 2 cycles; however, subjects in regimen B showed more side effects from the study drugs including fatigue, shortness of breath and irregular heart beat. Because of these results, the next 13 subjects with mesothelioma received Regimen A.

We would now like to investigate whether Regimen A will shrink tumors in patients with other tumor types that are known to express mesothelin; therefore, patients with lung cancer that makes mesothelin (this is determined by testing your tumor specimen) and patients with pancreatic cancer are being added to the study. In addition, although a safe dose of SS1P(dsFv)PE38 was established earlier in the study, we have a new batch of study drug from a different manufacturer than what was tested before. From our experience with (in the lab and in a few patients who received the drug), it was shown that the new batch might be slightly more active than the old batch. As a result, patients had higher blood levels of SS1P than expected from previous studies. Therefore, we will first determine the safe dose for the new batch (called lot FIL129J01). We will then use lot FIL129J01 to investigate how well Regimen A works in all the included tumor types. As we have never used anti-mesothelin antibodies in patients with lung

Revision Copy

or pancreatic cancer before, we do not know for certain what the side effects will be, but we assume that they will be similar to those in patients with mesothelioma.

Why are you being asked to take part in this study?

You are being invited to take part in this study because you have pleural mesothelioma, peritoneal mesothelioma, pancreatic cancer or mesothelin expressing lung cancer that has not been cured by previous treatments.

How many people will take part in this study?

Up to 75 subjects will be enrolled on this study, including the subjects already seen.

Description of Research Study

What will happen if you take part in this research study?

Before you begin the study

All research studies have specific criteria for entry to allow for valid interpretation of the study results and safety of participants, known as eligibility criteria. Before you begin this study you will need to have the following exams and tests to make sure you are eligible for this study. The exams and tests are part of regular cancer care and may be done even if you do not join the study. You will probably have these tests done while you are on the NCI Screening Protocol. If you recently had some of the tests, they may not need to be repeated.

- History and physical examination, including vital signs (height, weight, blood pressure, heart rate, temperature, breathing rate)
- Standard blood tests to measure your liver and kidney function, white blood cells, red blood cells and platelets, and blood electrolytes. If you are a woman able to get pregnant, you will also have a pregnancy test done.
- Scans and x-rays to measure your disease, including a CT scan of the chest, abdomen and pelvis, and a FDG-PET scan
- Electrocardiogram (ECG)
- A sample of tissue from any previous surgery or biopsy will be tested at NCI.

In addition, we will test a sample of your blood to make sure you don't already have neutralizing antibodies against SS1P. If you meet the eligibility criteria for this study you will be offered the option of participating and you will be asked to sign this informed consent document before participating in the study

During the study

If you are among the first 3 to 12 patients assigned to the FIL129J01 batch, you will take part in a dose de-escalation study. Three subjects will be assigned to take SS1P at the starting dose level in combination with pentostatin and cyclophosphamide. If there are no intolerable side

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effects, this will be determined as the safe dose. If only one subject has an intolerable side effect, three more patients will be assigned to the starting dose level. If there are no additional intolerable side effects, the starting dose level will be the safe dose.

If 2 or more out of 3 or 6 subjects has an intolerable side effect, the next 3 to 6 subjects will be assigned to the next lowest dose level in the same manner as described above. If 0 of 3 or 1 of 6 subjects has an intolerable side effect, the next lowest dose level will be determined as the safe dose.

If 2 or more of 3 or 6 subjects has an intolerable side effect, no more patients will be assigned to take the FIL129J01 batch.

However, if a safe dose is established, all subsequent subjects will be assigned to that safe dose level in order to determine how well the drug combination works at shrinking tumors in the various disease types being tested.

This study will be done in Cycles in the dose de-escalation portion as well as after the safe dose is established:

1. Cycle 1 will be different that the other cycles as you will receive more chemotherapy to get the immune system ready for the SS1P
2. Cycles 2 through 4
3. Follow up regimen

<u>Cycle 1</u>

Cycle 1 will last 30 days and on Cycle 1 you will receive more chemotherapy in an effort to prepare your immune system for the investigational therapeutic SS1P. Pentostatin will be given to you on Days 1, 5, and 9 of Cycle 1. Pentostatin will be given to you through an IV (intravenous catheter, a small plastic tube that is put into a vein in your arm or neck) over about 30-60 minutes. Investigators will discuss the option of inserting a central catheter, a catheter that will remain in your vein throughout the time you are on the study.

About 60 minutes before each dose of pentostatin you will be given 2 medicines in your IV to help prevent any side effects:

1) Ondansetron (also called Zofran) to help prevent nausea and vomiting
2) Dexamethasone (a type of steroid) to prevent nausea and vomiting

You will also be asked to take dexamethasone tablet(s) once a day on days 2 – 3, 6 – 7 and 10 – 15. If your study doctor feels that your side effects are severe enough, you may be asked to take dexamethasone twice a day on days 10 – 15. The oral dexamethasone is given to prevent nausea, vomiting and inflammation in the lining of your lungs (pleuritis). During cycle 1 you will take ondansetron tablet(s) twice a day for 12 days, morning and evening, starting on the evening of your pentostatin dose to help prevent nausea and vomiting. You will not take a morning dose of ondansetron on the day of your pentostatin dose.

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You will take cyclophosphamide tablets every morning for 12 days during Cycle 1 (Days 1 through 12). You should drink plenty of liquids every day (2-4 liters, or ½ to 1 gallon per day) to keep your urine clear colored. You should also empty your bladder frequently during the day and before you go to sleep to keep the cyclophosphamide from damaging the tissues in your bladder, which can cause inflammation and bleeding (cystitis). We need you to keep a diary of when you take the cyclophosphamide and any symptoms you may have. The nurse will give you a diary form for this purpose. Please bring it with you to clinic when you come at the end of each cycle. If you are unable to take the cyclophosphamide tablets, you may be asked to come to the clinic on these days to receive the medication through an IV.

During Cycle 1 you will be given SS1P the investigational antibody-type therapeutic on Days 10, 12 and 14 though an IV over about 30 minutes.

You will be given the following before each dose of SS1P to help prevent side effects:

1) A bag of salt water will be given in your IV for 2-4 hours before and after each dose. Ranitidine tablet(s) (also called Zantac) and hydroxyzine capsule(s) or tablet(s) (also known as Vistaril or Atarax) will be given to you one hour before each dose of SS1P.

Cycles 2 through 4

Upon completing Cycle 1 and after each cycle, you will have a physical exam and standard blood tests, and blood taken for research tests. The schedule of drugs you will receive during the Cycles 2 through 4 is listed in the chart below. Cycles 2 through 4 will last 21 days each. At the conclusion of every 2 cycles and at the end of your last cycle you will also have a CT scan of your disease. The following is a summary of what you will receive:

Pentostatin will be given to you in your IV (with the 2 premedications listed above) on Day 1 of each cycle.

You will take Cyclophosphamide tablets on Days 1 through 4 of each cycle. Again, you may receive this medication through an IV if you are unable to take orally.

SS1P will be given to you in your IV on Days 2, 4, and 6 of each cycle.

You will also be asked to take dexamethasone tablet(s) once a day on days 2 -7. If your study doctor feels that your side effects are severe enough, you may be asked to take dexamethasone twice a day. During cycles 2 through 4 you will take ondansetron tablet(s) twice a day for 4 days, morning and evening, starting on the evening of your pentostatin dose to help prevent nausea and vomiting. You will not take a morning dose of ondansetron on the day of your pentostatin dose.

We will watch you closely during and after you get the medicines, checking your blood counts twice a week and checking your urine at specified times. Other medicines may be given to you if you experience any side effects in order to help reduce those side effects. The dose of chemotherapy may be adjusted or doses delayed depending upon your blood tests and your side effects. In addition you will be given antibiotics to take throughout this study in order to prevent

Revision Copy

viral or fungal infections while your immune system is suppressed. Additional antibiotics may be required if you develop fever or infection. The chemotherapy agents will lower your white cells, red cells and platelets, in addition to lowering the cells in the immune system; therefore we may give you a drug called filgrastim (G-CSF) as a daily shot under the skin, if your white cells get too low.

To test if your immune system is 'neutralizing' the effects of the SS1P, we will test a blood sample taken on Day 24 and 30 of Cycle 1 and Day 16 and 22 of cycles 2 through 4. These dates may be moved up or back two days depending on your individual needs. If the test shows that your body is making antibodies against the SS1P, the treatment may need to be stopped.

Research blood tests

An important part of this study is testing how your body handles the SS1P, called pharmacokinetic studies or PKs. We will take less than 1/2 teaspoon of blood before and after each dose of SS1P. In addition, after your first dose of each cycle we will take less than 1/2 teaspoon of blood at the following time points: 1, 1 1/2, 2, 4, 8 and 12 hours after the SS1P, for a total of about 24 mL or approximately 5 teaspoons. In addition, blood will be taken to study the effects of this treatment on the immune cells in your blood. We will take approximately 5 ½ teaspoons of blood before you receive the study drug during each cycle, and then during each of your follow up visits (every 3 months). We will also take approximately 1 - 2 teaspoon of blood to study the levels of certain proteins in the blood before you receive study drug during each cycle and before each dose of SS1P. These blood tests are part of the research and are not optional.

When you are finished taking the drugs (treatment) – Follow up Regimen

Once the treatment regimen is stopped, we will ask you to have a series of vaccinations against a number of potential infections. You will complete most of the vaccinations within 2 years of your participation on the study; however you will be asked to receive lifelong annual vaccinations for the seasonal flu. We will also ask you come to the Outpatient Clinic for a physical exam, standard blood tests and scans of your cancer every three (3) months. If your cancer gets worse we will refer you back to your local doctor for further treatment but will contact you every year to see how you are. The scans will include a CT scan of your chest and abdomen, a FDG-PET scan and may include an MRI if indicated.

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Study Chart

Day	Each Cycle What to do and what will happen to you
After signing the consent, before starting Treatment	Get routine blood tests. Provide a history of how you feel and undergo a physical examination by the research team's Health Care Provider. Research blood samples will be taken CT scan of the chest, abdomen and pelvis, FDG-PET and MRI if indicated.
CYCLE 1	**30 Days long**
Days 1, 5 and 9	Routine blood and urine tests Dexamethasone IV, and ondansetron IV 60 minutes before Pentostatin Pentostatin IV over 30-60 minutes Take dexamethasone tablet(s) on days 2 -3, 6-7, and 10 – 15.
Days 1 through 12	Take Cyclophosphamide tablet(s) every morning. Drink 2-4 liters or ½ to 1 gallon of liquid per day to keep urine clear yellow Take Ondansetron tablet(s) every morning and every night, except on days receiving Pentostatin, only take a night dose of Ondansetron tablet(s)
Days 1, 10, 12 and 14	Ranitidine tablet and hydroxyzine one hour before SSP1 SS1P IV over about 30 minutes Research blood samples will be drawn
Days 24 and 30*	Blood test for immune 'neutralizing' assay.
Day 30 (may also be Day 1 of Cycle 2)	Return to the NIH clinic to see your doctor. Provide a history of how you feel and have a physical exam by your Health Care Provider. Blood and urine will be taken for routine cancer care tests and research blood samples. If you are tolerating the treatment, the next cycle will begin.
Cycles 2 through 4	**21 Days Long**
Day 1	Dexamethasone IV, and ondansetron IV 60 minutes before Pentostatin Pentostatin IV over 30-60 minutes Take dexamethasone tablet(s) on days 2 – 7.

Revision Copy

Day	Each Cycle What to do and what will happen to you
Days 1 through 4	Take Cyclophosphamide tablet(s) every morning Drink 2-4 liters or ½ to 1 gallon of liquid per day to keep urine clear yellow Take ondansetron tablet(s) every morning and every night, except on the day of your Pentostatin infusion. On that day, only take the night dose of ondansetron tablet(s)
Days 1, 2, 4 and 6	Ranitidine tablet and hydroxyzine one hour before SSP1 SS1P IV over about 30 minutes Research blood samples will be drawn
Days 16 and 22*	Blood test for immune 'neutralizing' assay.
Day 21 of Cycle 2	Return to the NIH clinic to see your doctor. Provide a history of how you feel and have a physical exam by your Health Care Provider. Blood and urine will be taken for routine cancer care tests and research blood samples. CT scan of the chest, abdomen and pelvis, FDG-PET and MRI if indicated. If you are tolerating the treatment and your disease has not gotten worse, cycle 3 will begin.
Cycles 3 - 4	Will be given exactly like Cycle 2
After stopping Treatment	Return to the NIH clinic to see your doctor about every 3 months until your cancer gets worse and you return to your local doctor for other treatment. Provide a history of how you feel and have a physical exam by your Health Care Provider. Blood and urine will be taken for routine cancer care tests and research blood samples drawn. CT scan of the chest, abdomen and pelvis, FDG-PET and MRI if indicated. You will receive a series of vaccinations to help prevent infection.
Follow Up	At least yearly, a member of the research team will contact you or your referring physician to ask about your condition.

* Assays may be taken two days earlier or two days later than scheduled depending on your individual needs

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What does this study involve?

Birth Control

If you are a woman who is breast feeding or pregnant, you may not take part in the study because we don't know how this medicine would affect your baby or your unborn child. If you are a woman who can become pregnant, or are the partner of a woman who can become pregnant, you will need to practice an effective form of birth control before starting study treatment, during study treatment, and for 3 months after you finish study treatment. If you think that you or your partner is pregnant, you should tell your study doctor or nurse at once.

Acceptable effective forms of birth control include:

- abstinence
- intrauterine device (IUD)
- tubal ligation
- vasectomy

Risks or Discomforts of Participation

What side effects or risks can I expect from being in this study?

If you choose to take part in this study, there is a risk that:

- You may lose time at work or home and spend more time in the hospital or doctor's office than usual
- You may be asked sensitive or private questions which you normally do not discuss

The SS1P used in this study may affect how different parts of your body work such as your liver, kidneys, heart, and blood. The study doctor will be testing your blood and will let you know if changes occur that may affect your health.

There is also a risk that you could have side effects from the study drug(s)/study approach.

Here are important points about side effects:

- The study doctors do not know who will or will not have side effects.
- Some side effects may go away soon, some may last a long time, or some may never go away.
- Some side effects may interfere with your ability to have children.
- Some side effects may be serious and may even result in death.

Here are important points about how you and the study doctor can make side effects less of a problem:

- Tell the study doctor if you notice or feel anything different so they can see if you are having a side effect.
- The study doctor may be able to treat some side effects.

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- The study doctor may adjust the study drugs to try to reduce side effects.

The tables below show the most common and the most serious side effects that researchers know about. There might be other side effects that researchers do not yet know about. If important new side effects are found, the study doctor will discuss these with you..

While the combination of pentostatin and cyclophosphamide may reduce the likelihood that you will make neutralizing antibodies to SS1P, it may also be associated with severe or prolonged immune suppression. As part of this study, you will receive additional medications to prevent infections that are known to occur in patients with suppressed immune systems.

Possible Side Effects of Pentostatin (Deoxycoformycin, dCF)

COMMON, SOME MAY BE SERIOUS In 100 people receiving Pentostatin (deoxycoformycin, dCF), more than 20 and up to 100 may have:
• Anemia which may require blood transfusions • Nausea, vomiting • Fever • Tiredness • Bruising, bleeding • Infection, especially when white blood cell count is low • Itching, rash

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Pentostatin (deoxycoformycin, dCF), from 4 to 20 may have:
• Anemia, kidney problems which may cause swelling, or may require dialysis • Blood clot • Diarrhea, loss of appetite • Sores in mouth which may cause difficulty swallowing • Chills • Pain • Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Headache • Cough, stuffy nose • Confusion

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RARE, AND SERIOUS In 100 people receiving Pentostatin (deoxycoformycin, dCF), 3 or fewer may have:
• Kidney damage

Possible Side Effects of Cyclophosphamide

COMMON, SOME MAY BE SERIOUS In 100 people receiving Cyclophosphamide, more than 20 and up to 100 may have:
• Hair loss • Nausea, vomiting, loss of appetite • Sores in mouth • Infection, especially when white blood cell count is low • Absence of menstrual period which may decrease the ability to have children • Blood in urine

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Cyclophosphamide, from 4 to 20 may have:
• Damage to the bone marrow (irreversible) which may cause infection, bleeding, may require transfusions • Loss or absence of sperm which may lead to an inability to father children • Stuffy nose • Fluid around the heart

RARE, AND SERIOUS In 100 people receiving Cyclophosphamide, 3 or fewer may have:
• Severe skin rash with blisters and peeling which can involve mouth and other parts of the body • Damage to the heart or heart failure which may cause shortness of breath, swelling of ankles, cough or tiredness • A new cancer including cancer of bone marrow (leukemia) caused by chemotherapy • Swelling of the body including the brain which may cause dizziness, confusion • Scarring of the lungs

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SS1(dsFv)PE38:

The side effects that may happen as a result of the SS1P include:

POSSIBLE, SOME MAY BE SERIOUS
• Fluid in the body which may cause low blood pressure, shortness of breath, or swelling of ankles • Abnormal heartbeat • Pain • Diarrhea, nausea, vomiting • Chills, tiredness, fever • Swelling of the body • Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Weight gain • Dizziness, headache • Shortness of breath • Itching, rash, hives • Low blood pressure which may cause feeling faint

Side effects of support medications: You will be given numerous medications to treat or prevent certain side effects of this study. These medications also have possible side effects.

Filgrastim: If your blood counts fall very low you will be given filgrastim, also known as G-CSF, to help stimulate your immune cells to re-expand. Bone pain is the most common side effect of the filgrastim, which can usually be controlled with acetaminophen (Tylenol). Rare events of adult respiratory distress syndrome and splenic rupture have been reported with filgrastim.

Bactrim: One of the medicines you may be given to prevent a type of pneumonia is called TMP/SMX double strength. This medicine can cause fever, nausea and vomiting or a skin rash with itching, or it can reduce the number of white blood cells in your blood. This medicine will be continued until after you have finished the immune suppressing drugs and the number of CD4 cells (a type of white blood cell) has been above 200 for three months.

Acyclovir: To prevent herpes infections we will give you a medicine called acyclovir if you can swallow your medications. Acyclovir can cause reversible kidney damage, delirium, tremors, coma, emotional changes, and abnormal Electroencephalogram (EEGs) when taken in higher doses. Stomach upset, headache or nausea, rash or hives; sweating; blood in the urine; and low blood pressure and low platelet count have been reported. Hair loss from prolonged use has also been reported. This medicine will be continued until after you have finished the

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immune suppressing drugs and the number of CD4 cells (a type of white blood cell) has been above 200 for three months.

Dexamethasone: A steroid to prevent allergic reactions which can cause high blood pressure and irregular heartbeats, dry skin, rash or impaired healing of skin wounds, high blood sugar, changes in blood electrolytes such as potassium or sodium, loss of muscle mass, weight gain, mood swings, insomnia, and in rare cases, allergic reaction.

Ranitidine: To help prevent upset stomach, it can cause constipation, diarrheas, headache, nausea or upset stomach. Rare serious side effects include severe allergic reaction with rash, hives, itching, difficulty breathing; confusion, dark urine, depression, fast or slow heartbeat, unusual bruising or bleeding, yellowing of the eyes or skin (indicating liver damage).

Hydroxyzine: It can cause dry mouth. Rare serious side effects include severe allergic reaction with rash, hives, itching, difficulty breathing; involuntary movements.

If your red blood cells or platelets go too low, you may require transfusion with red cells or platelets.

You may experience none of these effects, or you may experience many of these effects. Tell your doctor and nurse about your side effects and they will try and minimize them as much as possible.

Potential Benefits of Participation

The aim of this study is to see if this experimental treatment will cause your tumors to shrink. We do not know if you will receive personal, medical benefit from taking part in this study. These potential benefits could include shrinking of your tumor or lessening of your symptoms, such as pain, that are caused by the cancer. Because there is not much information about the drug's effect on your cancer, we do not know if you will benefit from taking part in this study, although the knowledge gained from this study may help others in the future who have cancer.

Alternative Approaches or Treatments

What other choices do I have if I do not take part in this study?

Instead of being in this study, you have these options**:**

- Getting treatment or care for your cancer without being in a study
- Taking part in another study
- Getting comfort care, also called palliative care. This type of care helps reduce pain, tiredness, poor appetite and other problems caused by the cancer. It does not treat the cancer directly. Instead, it tries to improve how you feel. Comfort care tries to keep you as active and comfortable as possible.

Please talk to your doctor about these and other options.

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Research Subject's Rights

What are the costs of taking part in this study?

If you choose to take part in the study, the following will apply, in keeping with the NIH policy:

- You will receive study treatment at no charge to you. This may include surgery, medicines, laboratory testing, x-rays or scans done at the Clinical Center, National Institutes of Health (NIH), or arranged for you by the research team to be done outside the Clinical Center, NIH if the study related treatment is not available at the NIH.
- There are limited funds available to cover the cost of some tests and procedures performed outside the Clinical Center, NIH. You may have to pay for these costs if they are not covered by your insurance company.
- Medicines that are not part of the study treatment will not be provided or paid for by the Clinical Center, NIH.
- Once you have completed taking part in the study, medical care will no longer be provided by the Clinical Center, NIH.

Will your medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), which are involved in keeping research safe for people.
- National Cancer Institute Institutional Review Board
- Qualified representatives from the pharmaceutical collaborator for the SS1P antibody

A description of this clinical trial will be available on http://www.Clinicaltrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most the Web site will include a summary of the results. You can search this Web site at any time.

Stopping Therapy

Your doctor may decide to stop your therapy for the following reasons:
- if he/she believes that it is in your best interest;
- if your disease comes back during treatment;
- if you have side effects from the treatment that your doctor thinks are too severe;
- if new information shows that another treatment would be better for you.

In this case, you will be informed of the reason therapy is being stopped.

You can stop taking part in the study at any time. However, if you decide to stop taking part in the study, we would like you to talk to the study doctor and your regular doctor first.

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If you decide at any time to withdraw your consent to participate in the trial, we will not collect any additional medical information about you. However, according to FDA guidelines, information collected on you up to that point may still be provided to the Cancer Therapy Evaluation Program (NCI) or designated representatives. If you withdraw your consent and leave the trial, any samples of yours that have been obtained for the study and stored at the NCI can be destroyed upon request. However, any samples and data generated from the samples that have already been distributed to other researchers or placed in the research databases can**not** be recalled and destroyed.

Conflict of Interest

The National Institutes of Health (NIH) reviews NIH staff researchers at least yearly for conflicts of interest. This process is detailed in a Protocol Review Guide. You may ask your research team for a copy of the Protocol Review Guide or for more information. Members of the research team who do not work for NIH are expected to follow these guidelines but they do not need to report their personal finances to the NIH.

Members of the research team working on this study may have up to $15,000 of stock in the companies that make products used in this study. This is allowed under federal rules and is not a conflict of interest.

The National Institutes of Health and the research team for this study have developed a drug being used in this study. This means it is possible that the results of this study could lead to payments to NIH scientists and to the NIH. By law, government scientists are required to receive such payments for their inventions. You will not receive any money from the development of SS1(dsFv)PE38.

Optional Biopsy

We would like to collect a biopsy 3 times during the study. A biopsy is a procedure using a needle to remove a piece of tissue or a sample of cells from your body so that it can be analyzed in a laboratory and is used to diagnose or evaluate the cancer. With your permission, a tumor biopsy will be performed: before you have received study drug (mandatory in patients with lung cancer); after 2 cycles of treatment; and at the end of the last cycle of treatment or at follow-up. We may use ultrasound or MRI to guide us in collecting the sample as well as CT scans that have already been performed. We will take no more than 4 pieces of tissue from each tumor site.

Your doctor will discuss the risks associated with obtaining tissue samples. Generally during a biopsy, local anesthetic is given and one just feels some minimal pain at this site where the sample is obtained. Rarely, infection or bleeding may occur at the sample site.

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The biopsy to be performed is exclusively for research purposes and will not benefit you. It might help other people in the future. You will be given the opportunity to decide whether you would like to participate at the time of the procedure.

Use of Specimens and Data for Future Research

To advance science, it is helpful for researchers to share information they get from studying human samples. They do this by putting it into one or more scientific databases, where it is stored along with information from other studies. A researcher who wants to study the information must apply to the database and be approved. Researchers use specimens and data stored in scientific databases to advance science and learn about health and disease.
We plan to keep some of your specimens and data that we collect and use them for future research and share them with other researchers. We will not contact you to ask about each of these future uses. These specimens and data will be stripped of identifiers such as name, address or account number, so that they may be used for future research on any topic and shared broadly for research purposes. Your specimens and data will be used for research purposes only and will not benefit you. It is also possible that the stored specimens and data may never be used. Results of research done on your specimens and data will not be available to you or your doctor. It might help people who have cancer and other diseases in the future.

If you do not want your stored specimens and data used for future research, please contact us in writing and let us know that you do not want us to use your specimens and/or data. Then any specimens that have not already been used or shared will be destroyed and your data will not be used for future research. However, it may not be possible to withdraw or delete materials or data once they have been shared with other researchers.

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OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Raffit Hassan, M.D., Building 10, Room 4-5330**,** Telephone: 301-451-8742. You may also call the Clinical Center Patient Representative at (301) 496-2626. If you have any questions about the use of your specimens or data for future research studies, you may also contact the Office of the Clinical Director, Telephone: 301-496-4251.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

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COMPLETE APPROPRIATE ITEM(S) BELOW:	
A. Adult Patient's Consent I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study. _____ _____ Signature of Adult Patient/ Legal Representative Date _____ Print Name	**B. Parent's Permission for Minor Patient.** I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study. (Attach NIH 2514-2, Minor's Assent, if applicable.) _____ _____ Signature of Parent(s)/ Guardian Date _____ Print Name

C. Child's Verbal Assent (If Applicable)
The information in the above consent was described to my child and my child agrees to participate in the study.

_____ _____ _____
Signature of Parent(s)/Guardian Date Print Name

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM SEPTEMBER 12, 2016 THROUGH SEPTEMBER 11, 2017.

_____ _____ _____ _____
Signature of Investigator Date Signature of Witness Date

_____ _____
Print Name Print Name

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