Supplemental Material

- Protocol (Approved 21JAN2015)
- Protocol Amendment A(16) (Approved 27APR2020)
- CAR T Manufacturing Information

A Phase I Clinical Trial of Malignant Pleural Disease Treated with Autologous T Cells Genetically Engineered to Target the Cancer-Cell Surface Antigen Mesothelin

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Title: A phase I clinical trial of malignant pleural disease (MPD) treated with autologous T cells genetically engineered to target the cancer-cell surface antigen mesothelin

Objectives: The primary objective of this trial is to assess the safety, dose requirement, and targeting efficiency of intrapleurally administered genetically directed autologous human T cells targeted to mesothelin—a cell surface cancer antigen widely expressed in mesothelioma, lung cancer, and breast cancer cells—using a chimeric antigen receptor (CAR).

Secondary objectives include assessment of the biological and antitumor effects of T cell treatment, measuring serum soluble mesothelin related peptide (SMRP) levels, as well as assessment of T cell targeting, accumulation, persistence, and antitumor immune response, using molecular biology techniques on samples from peripheral blood, pleural fluid and tumor biopsies. A recommended dose and a schedule for future investigation will also be determined. Any evidence of antitumor activity will be noted. If unacceptable toxicity occurs that is probably or likely related to anti-mesothelin CAR T cells, assess the capacity for AP1903, a dimerizing agent, to mediate clearance of the genetically engineered cells and resolve toxicity.

Methodology:

Design: This is an open-label, dose-escalating, nonrandomized, single-center, phase I study of mesothelin-targeted T cells administered intrapleurally as a single infusion in patients with a diagnosis (histologically or cytologically documented) of MPD from mesothelioma, lung cancer, or breast cancer. The total number of patients studied will depend on the number of dose levels tested, up to a maximum dose of 3×10^6 mesothelin-targeted T cells/kg or until the maximum tolerated dose (MTD) is reached. For this study, we anticipate infusing a minimum of 4 and a maximum of 24 evaluable patients.

Population: The study population will comprise patients who have been diagnosed with MPD from mesothelioma, lung cancer, or breast cancer. We anticipate that 2 different cohorts of patients with MPD will be eligible for this trial: (a) patients with mesothelioma with a free or partially free pleural space and (b) patients with MPD from lung or breast cancer that failed standard chemotherapy management (patients who received at least one chemotherapeutic regimen and are documented to have progressing tumor) with a free or partially free pleural space deemed to be clinically stable.

Safety evaluations: The safety of iCasp9M28z+ T cells will be assessed by evaluation of the type, frequency, and severity of adverse events (AEs); changes in clinical laboratory test findings (hematologic and chemistry); and physical examination. All AEs and laboratory toxicities will be graded using version 4 of the CTCAE.

At baseline (i.e., within 14 days before the start of treatment), daily during the first 3 days after treatment, and at day 60 ± 5, laboratory testing will consist of complete blood count

(CBC), SMA-12 blood chemistries, and routine urinalysis. Following T-cell administration, C-reactive protein titers will be performed as clinically indicated.

Vital signs (i.e., blood pressure, temperature, and pulse rate) will be charted immediately before treatment; Q15 min during infusion, at 30, and 60 minutes after iCasp9M28z T cell treatment; every 8 hours thereafter for 24 hours; at the clinical follow-up visit (weekly for 8 weeks after treatment); and at day 60 ± 5. An electrocardiogram (ECG) will be performed at baseline.

As an added safety measure, the vector includes a suicide switch comprising a caspase dimerization domain (ICD9) that can be activated by a small molecule and induce death of the genetically engineered cells if they were to induce untoward toxicity.The following medication will be available for use in case of a severe toxicity related to this agent: AP1903, at 0.4 mg/kg, given intravenously as a single-dose over 2 hours.If toxicity persists, multiple doses of AP1903, up to 6 doses every other day, may be administered.

Assessment of tumor T cell infiltration: Patients can undergo video-assisted thoracic surgery (VATS) or open thoracotomy when clinically indicated (e.g., to obtain pleural biopsies or to perform pleurodesis or surgical resection). Pleural biopsies will be performed to assess tumor T cell infiltration—specifically iCasp9M28z T cell infiltration—in tumor and surrounding tissues. Flow cytometric analysis of fresh tissue will be performed to assess the phenotype of the infiltrating iCasp9M28z T cells. The clinical indications for a surgical procedure at the time of pleural biopsy are the following: (a) drainage of unresolved/loculated pleural effusion, with or without pleurodesis; (b) surgical resection of pleural disease by pleurectomy; and (c) exploration for intended surgical resection (aborted because of unresectability).

Immunophenotyping and cytokine analysis (immune response): Phenotyping of peripheral blood iCasp9M28z T cells, along with analysis of lymphocyte subsets (e.g., CD4 and CD8) and cytokine profile (serum IFN-γ and TNF-α), will be performed on blood at baseline (within 14 days before the start of treatment) and on days 2 and/or 3 posttreatment.

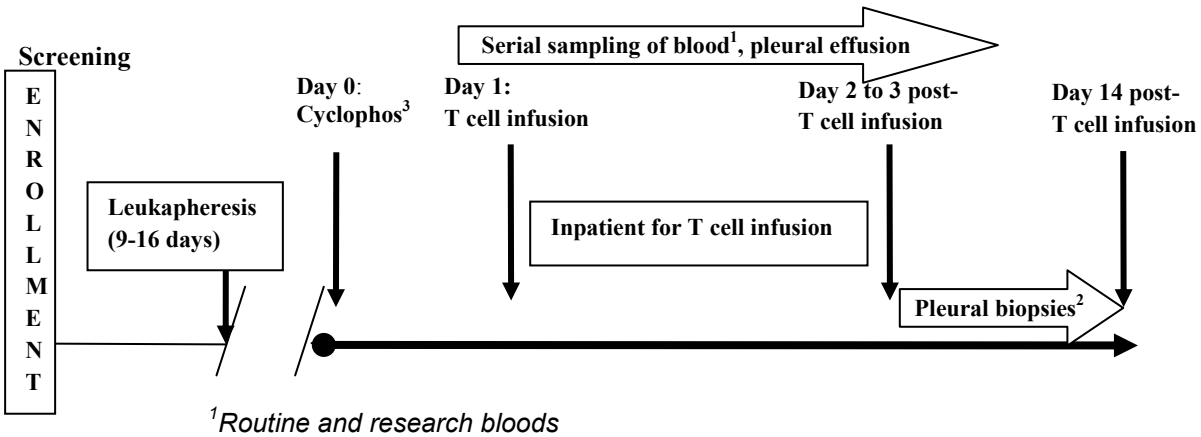
Number of patients: A precise sample size cannot be defined, as it is dependent on the observed toxicity. Cohorts of 3 patients will be treated at each dose level, up to a maximum of 3×10^6 iCasp9M28z T cells/kg or until the MTD has been reached. It is expected that a minimum of 4 and a maximum of 24 evaluable patients will be accrued for this study.

Study design: This study will be conducted under the umbrella of the Cellular Therapeutics arm of the MSKCC Center for Cell Engineering. Patients with malignant pleural effusion and MPD will undergo insertion of an intrapleural catheter by either the thoracic surgery service or the interventional radiology / pulmonary service, as clinically indicated. The clinical indications for insertion of a pleural catheter are (a) patient with symptomatic pleural effusion; (b) patient with multiloculated pleural effusion; or (c) patient undergoing pleural biopsy by either VATS or thoracotomy, with a history of recurrent pleural effusion. All patients with a functional pleural catheter are eligible for the study, as long as there are no clinical concerns of infection. After obtaining a screening consent, patients tumor, pleural fluid and/or blood will be tested for presence of tumor marker – mesothelin. In patients with mesothelin-positive

tumors, a single blood volume leukapheresis for harvesting of PBMCs will be performed, after obtaining informed consent for the study. As the transduced T cells will be frozen, the timing of leukapheresis is not defined and can vary from patient to patient. All obtainable pleural fluid will be drained from the chest by thoracentesis or through a pleural catheter and will be preserved for analysis (cytokines, tumor cells, and SMRP). Subsequently, a single dose of mesothelin-targeted T cells will be instilled via the catheter into the pleural space. The catheter will then be flushed and will be capped for at least 12 hours, to maximize T cell delivery to the intrapleural tumor. Patients will be monitored in the hospital and discharged home after 72 hours, with the pleural catheter capped. Patients will be monitored closely as outpatients for the next 2 months. Patients will be followed weekly for the first 8 weeks after the treatment. The pleural catheter will be removed as clinically indicated (i.e., when it is no longer needed for the clinical management of malignant pleural effusion). In Cohorts 2 to 4, patients will be admitted, hydrated intravenously, premedicated with acetaminophen and diphenhydramine, and administered cyclophosphamide at 1.5 g/m^2 (day 0) 1 day before administration of mesothelin-targeted T cells.

Treatment plan: Patients will be admitted to the M-12 floor under Thoracic Surgery Inpatient Service at MSKCC for T cell infusion, observation, and laboratory blood tests, including immune and molecular monitoring. The patients will remain inpatients for 3 nights (including admission on day 0 for cyclophosphamide administration), unless undue toxicity or unexpected side effects occur; maximum expected inpatient stay is 3 nights. Patients will receive the T cell infusion at MKSCC under the supervision of the principal investigator. The dosing scheme is detailed below.

Schema:



[1]*Routine and research bloods*
[2]*If patient undergoes a surgical procedure, pleural biopsy specimens will be harvested*
[3]*No cyclophosphamide in cohort 1*

In cases of study procedures occurring on weekends/holidays or in cases of other logistical issues, an allowance of +/-3 days is considered to be included for all time points stated in this protocol, if not stated otherwise. A medical procedure or test conducted before signed consent is obtained that is the standard of care or that would have been conducted regardless of whether the patient considered participation in this clinical trial may be used in place of a screening procedure (e.g., CT scan).

CAR T-cell oversight committee: Decisions to escalate to the next level or, when appropriate, to deescalate to a lower level will be recommended to the institutional CAR T-cell Oversight Committee by the principal investigator (PI). The CAR T-cell Oversight Committee will make the final decision of whether to proceed to the next dose level. In addition, the PI will coordinate regular meetings/communication with CAR T-cell Oversight Committee to

a. present the data for each cohort of patients and study progress
b. discuss and obtain recommendations prior to escalating to the next dose level or if appropriate deescalating the dose level
c. report any significant toxicities associated with the CAR T-cell intervention.
d. present any proposed significant changes in the protocol.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary endpoint: The primary endpoint of this study is to evaluate the safety of adoptive transfer of genetically modified, autologous, mesothelin-targeted T cells into the pleural cavity of patients with MPD, with and without administration of cyclophosphamide—as well as to establish the MTD for this treatment.

Secondary endpoints:

1) Changes in the absolute value of the biomarker SMRP in serum before and after adoptive transfer of T cells
2) The pattern of change in SMRP levels from before to after adoptive transfer of T cells
3) The persistence of adoptively transferred T cells in peripheral blood, assessed by quantitative molecular techniques
4) The persistence of adoptively transferred T cells in tumor tissue from patients undergoing a pleural-access surgical procedure anytime after receiving T cells
5) The cytokine profile of serum and pleural effusion before and after adoptive transfer of T cells
6) The capacity for AP1903, a dimerizing agent, to mediate clearance of the genetically engineered mesothelin-targeted T cells and resolve toxicity.

3.0 BACKGROUND AND RATIONALE

MPD: The pleural cavity is a common site for cancer, both primary (mesothelioma) and metastatic (lung, breast, and other solid cancers).[1] The incidence of MPD is estimated to be >150,000 patients/year in the U.S. alone.[2] Pleural mesothelioma is a regionally aggressive primary malignancy of the pleura with a median survival of 9 to 17 months, even after aggressive combined-modality therapy.[3] At least 25% of patients with lung adenocarcinoma (LAC), a common form of lung cancer,

develop MPD during the course of their illness.[1,4] The pleura is the most common site of metastatic breast cancer recurrence[5] and the site of the first and only manifestation of recurrence in 40% of patients.[6]

Mesothelioma: Mesothelioma is expected to cause >450,000 deaths worldwide during the next 20 years.[78] U.S. Army personnel exposed to asbestos in construction projects, ships, brakes, clutches, and military gas masks before the 1970s are at risk for developing mesothelioma.[8,9] With combined chemoradiotherapy and surgical resection, survival is prolonged to 9 to 12 months (compared with no treatment), with a higher rate of pleural and mediastinal lymph node recurrence.[10,11] In the largest series to date (945 patients), which was reported by our group, even among the patients carefully screened and selected for resection, one-third were unresectable because of locally advanced tumor at the time of operation.[3] Only 8% of patients had distant metastases.[3] *Its localized nature, potential accessibility, and relative lack of metastases make mesothelioma a suitable candidate for regional targeted therapies.* As available treatment regimens have poor outcomes, patients with untreated mesothelioma can be considered eligible for this protocol.

Tumor microenvironment and its effect on immunotherapy: Patients with mesothelioma with high levels of CD8+ tumor-infiltrating lymphocytes (TILs) have better survival than those with low levels of TILs (3-year survival: 83% vs 28%).[13,14] High levels of CD8+ TILs also correlated with higher levels of TUNEL-positive apoptotic tumor cells.[14] High levels of CD8+ TILs remained an independent prognostic factor for delayed recurrence and better survival[14] in multivariate analysis. We have convincing data (unpublished) that increased infiltration of the tumor by T lymphocytes conveys a survival advantage. Interestingly, a significant correlation was demonstrated between levels of CD8+ TILs and induction chemotherapy with cisplatin/pemetrexed,[14] the only regimen shown to have a significant survival benefit in randomized clinical trials of mesothelioma.[12,13] *Thus, the rationale exists for pursuing combination chemoimmunostimulatory strategies that will increase the number and efficacy of TILs in the treatment of mesothelioma.* Published immunotherapy studies have shown that IFN-γ, IL-2, IL-12, and GM-CSF have a direct cytotoxic effect on mesothelioma cells, which further strengthens our immune intervention approach.[14-1922] Our group has reviewed all of the published studies on intrapleural immunotherapies for mesothelioma, which demonstrate efficacy without occurrence of a limiting toxicity.[23]

Publications from our laboratory and those of others have documented the prognostic role of the tumor immune microenvironment in lung[20,21] and breast[22-25] cancers. Specifically, tumor-targeted cytotoxic immune cell responses have been shown to be beneficial.

3.1 Immunologic approaches

3.1.1. Adoptive T cell therapy with engineered T lymphocytes

Cell engineering can be used to redirect T cells toward tumor antigens, to enhance T cell function, and to potentially resolve many previously observed shortcomings afflicting adoptively transferred cytotoxic T lymphocytes (CTLs).[26] An impetus for performing genetic T cell modification is the prospect of enhancing T cell survival and expansion, generating memory lymphocytes, and offsetting T cell death, anergy, and immune suppression.

CARs: Tumor-specific T cells can be generated by transferring genes that encode CARs.[26-31] CARs consist of a tumor antigen–binding domain that is fused to an intracellular signaling domain capable of activating T cells. The design of CARs must therefore reconcile antigen recognition with signal transduction, two functions that are physiologically borne by two separate complexes, the T cell

receptor (TCR) heterodimer and the CD3 complex. The CAR's extracellular binding domain is usually derived from a murine or human monoclonal antibody or from receptors or their ligands. *Antigen recognition is therefore not MHC-restricted,[32,33] as is the case for physiologic TCR-mediated antigen recognition.* Ligand binding by the chimeric receptor triggers phosphorylation of immunoreceptor tyrosine-based activation motifs in the cytoplasmic region; this activates a signaling cascade that is required for cytolysis induction, cytokine secretion, and proliferation. Since the requirements for MHC restriction in the interaction of effector cells with target cells are bypassed, the binding of tumor cells to CTLs grafted with CARs is *not affected by human leukocyte antigen (HLA) downregulation or by defects in the antigen-presentation machinery*.

Requirements of T cells for expansion and survival: To proliferate in response to antigen, T cells must receive two signals. One is provided by TCR recognition of antigenic peptide/MHC complexes displayed on the surface of antigen-presenting cells (APCs)[30]; the other is provided by a T cell costimulatory receptor, such as the CD28 molecule. Whereas the cytolytic activity of T cells does not require concomitant costimulation, recent studies, including some of our own, have established that the provision of costimulatory signals is critical for the antitumor activity of adoptively transferred T cells.[29,34-36] *A first step toward providing costimulation to T cells that recognize CD28-negative tumor cells was achieved by creating CD28-derived CAR (Adusumilli et al. Science Translational Medicine, in press, anticipated publication Nov 2014).*

3.1.2. Our approach to the treatment of MPD

The primary goal of this research proposal is to promote T cell infiltration of the tumor by administering genetically engineered human primary T lymphocytes that have been redirected to target the MPD tumor antigen mesothelin, and to further enhance the efficacy of such targeted T cells by regional delivery. *In recent years, there have been several published reports of long-term remission of metastatic melanoma,[37-39] a solid tumor, after treatment with adoptive cells. These findings provide strong support to investigate this approach for other regionally accessible solid tumors, such as MPD.* The occurrence of some remarkable, albeit infrequent, complete responses in patients with metastatic disease underscores the potential of targeted T cells, as well as the need to increase the potency of these therapies through improvements in host conditioning and CAR design. Our mesothelin CAR is derived from a human V_L and V_H cDNA[44] obviating the risk of human antimouse antibody immunogenicity, which is the case for CARs derived from mouse humanized antibodies. Although T cells may access disease sites, their survival, function, and persistence are influenced by the tumor microenvironment. Thus, an essential aspect of our approach is to not merely generate tumor-targeted T cells for adoptive therapy, but to enhance T cell function through the design of improved antigen receptors and through intervention in the host microenvironment by intrapleural delivery.

An ongoing phase I clinical trial of intrapleural delivery of vaccinia virus has demonstrated the feasibility of intrapleural therapies delivered via pleural catheters. Furthermore, tissue biopsy specimens and pleural effusion obtained from the pleural cavity will provide us with the scientific rationale to study T cell persistence and phenotypic changes and will advance our ability to understand and improve T cell therapy to benefit patients with MPD.[40]

3.2 Mesothelin

3.2.1. Rationale for targeting mesothelin

Mesothelin is an immunogenic cell surface antigen[41,42] that is expressed at high levels in MPD and mesothelioma.[42-47] In normal tissues, it is expressed only in the pleura, pericardium, and peritoneum, at low levels.[42,48] Mesothelin is involved in cell proliferation,[53] adhesion,[49,50] cell signaling,[54] and metastases.[56] It has uniform and strong expression in 80% of patients with mesothelioma.[42,46,47,51,52] Serum SMRP secreted by mesothelin-expressing MPD tumors can be measured both in humans[46,47,53-58] and in mice and has been shown to correlate with therapy response and prognosis. Our laboratory has shown—both in preclinical mouse models and in patients—that overexpression of mesothelin in mesothelioma cells promotes invasion and is associated with secretion of MMP-9. In patients with LAC, overexpression of mesothelin is associated with reduced recurrence-free and overall survival and is an independent factor of poor prognosis. In patients with triple-negative breast cancer, overexpression of mesothelin is associated with reduced disease-free and overall survival, as well as increased incidence of distant metastases.
Our laboratory investigation of primary lung and breast cancer tumors revealed that 60% of patients with LAC and 16% of patients with breast cancer (including 36% of patients with triple-negative breast cancer) have tumors that overexpress mesothelin.

Antimesothelin recombinant immunotoxin SS1P and MorAb-009 have shown *in vivo* specificity and significant antitumor activity,[42,59,60] with no off-target toxicities noted. In a trial of pancreatic cancer vaccine, patients with a survival advantage had strong and consistent CD8+ T cell responses to mesothelin epitopes, which was associated with a vaccine-induced delayed-type hypersensitivity response.[61] Specific T cell epitopes derived from mesothelin have been shown to activate human T cells to efficiently lyse human tumors expressing mesothelin.[62] *Thus, there is strong evidence that adoptive immunotherapy with a mesothelin receptor will be tumor specific.*

3.2.2. Mesothelin CAR T cells

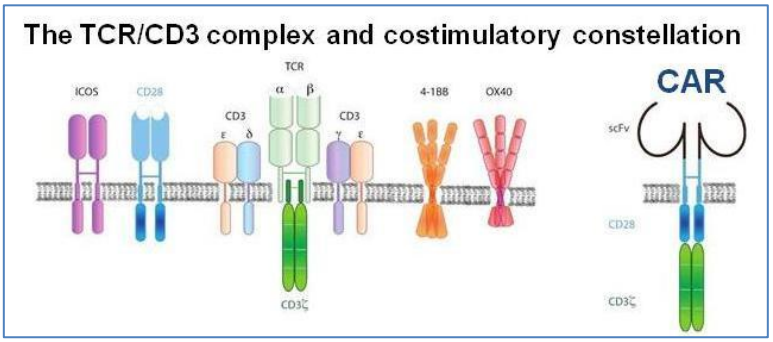
CAR-mediated mesothelin antigen recognition offers distinct advantages—for instance, receptor specificity is easily generated, as the *human Fab* we are using as part of the mesothelin CAR is highly specific and has high affinity. As these receptors can transduce both CD4+ and CD8+ T cells (*Adusumilli et al. Science Translational Medicine, Nov 5;6(261)ra151)*, transduction of a patient's T cells with CARs could generate helper and CTL responses, possibly resulting in a sustained antitumor response. CAR-modified CD4+ T cells exhibit direct cytolytic activity against tumor cells, indicating that genetically targeted CD4+ T cells serve as both helper cells and effector cells. Finally, soluble tumor antigens (CEA, HER-2, and Lewis Y) that are known to decrease the efficacy of monoclonal antibody therapies have been shown to have no effect on CAR T cells.[63-65] Given the toxicities associated with chemotherapy, alternative therapies are viewed favorably. Several recent clinical trials have suggested that immune-based therapies with vaccines, either alone or in combination with cytokines, might provide a more targeted approach for treating patients with MPD, with fewer resulting AEs.

3.2.3 Targeted elimination of MPD by genetically directed human lymphocytes

The genetic engineering of T cells is a novel strategy designed to accelerate the generation of tumor-specific T cells and remedy the biological limitations that constrain the antitumor functions of normal T cells.[26-28] Unlike the physiologic TCR, CARs encompass immunoglobulin-variable regions or receptor ligands as antigen-recognition elements, thus permitting T cells to recognize cell-surface tumor antigens in the absence of HLA expression.[30] T cell activation is mediated by the cytoplasmic domain of the CAR, which is typically derived from the CD3ζ chain or the FcRIγ chain (Figure 1). Our group has shown that ζ chain–based CARs can induce strong activation capable of sustaining T cell

proliferation and permitting secondary antigenic restimulation *in vitro*, provided that antigen is presented in the context of CD28-mediated costimulation.[26,27,29,31,34-36] In an effort to determine whether human T cells expand in this manner, whether they can mediate tumor eradication *in vivo*, and whether further *in vivo* costimulation is needed to sustain their function, tumor models using SCID-bg/bg and NOD-SCID mice were developed. These models showed that mesothelin-targeted T cells can effectively eliminate MPD. Our orthotopic mouse model of mesothelioma was extensively characterized to resemble human mesothelioma. T cells were transduced with iCasp9M28z, a CAR that targets human mesothelin. The M28z receptor encompasses the ζ chain of the CD3 complex as its activation domain and specifically redirects *in vitro* cytolysis against mesothelin-positive tumor cells. The tumor models used included orthotopic and subcutaneous MPD. Tumor eradication was directly proportional to the *in vivo* effector-to-tumor cell ratio and mesothelin expression. The administration of iCasp9M28z-transduced T cells induced objective responses in all mice and cured a substantial portion of them (*Adusumilli et al. Science Translational Medicine, Nov 5;6(261)ra151*). The data strongly support the feasibility of targeting MPD with autologous T lymphocytes directed against mesothelin by a transduced ζ chain–based receptor. MSKCC investigators have previously shown that similar technology and approaches can be used to treat other diseases, including hematologic malignancies ("A Phase I Trial for the Treatment of Purine Analog-Refractory Chronic Lymphocytic Leukemia Using Autologous T Cells Genetically Targeted to the B Cell Specific Antigen CD19," MSKCC IRB protocol 06-138, J. Park, PI; "A phase I trial of Precursor B Cell Acute Lymphoblastic Leukemia (B-ALL) Treated with Autologous T cellsGenetically Targeted to the B Cell Specific Antigen CD19", MSKCC IRB protocol 09-114, J.Park, PI).

A.



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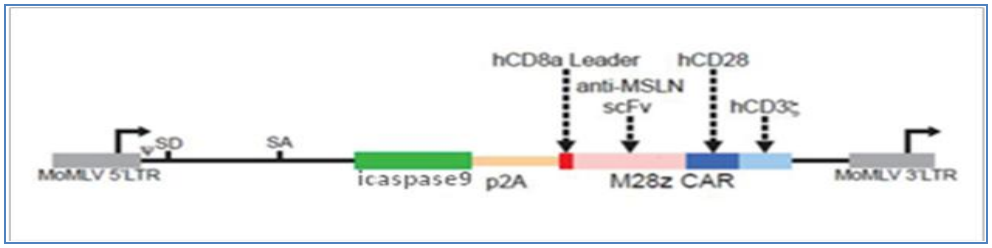


Figure 1. Schematic representation of (A) the physiological TCR and the scFV-ζ chain chimeric antigen receptor (CAR) structure, and (B) iCasM28z CAR

3.2.4 Rationale for using genetically redirected, adoptively transferred T cells

The advent of effective methods for gene transfer in T cells has provided a new means for creating tumor-specific T cells. In principle, genetic reprogramming could be used to improve T cell survival, augment T cell expansion, generate memory lymphocytes, and offset T cell death, anergy, and

immune suppression. Such genetic alterations are distinct from redirecting antigen specificity and may eventually prove to be critical for sustaining immunity to the tumor. CARs that comprise both CD28 and CD3 cytoplasmic domains have been shown, by our group[26-28,31,66,67] and others,[68-70] to better support T cell stimulation by target cells that present antigen in the absence of activating costimulatory ligands. The M28z receptor has thus been selected for clinical investigation. Because of the potential risk of initiating any immune response against normal tissues that express very low levels of mesothelin, the ICaspase-9 gene will be cotransferred with the cDNA encoding the M28z receptor, by use of the M28z gamma-retroviral vector. Constitutive expression of ICaspase-9 has been extensively investigated by our group and others and has been shown to render T cells sensitive to AP1903, providing a means to eliminate T cells if required. Furthermore, expression of ICaspase-9 enables elimination of >90% of T cells, as demonstrated by our group's preclinical studies and by other groups' clinical studies.[77,97] Intravenously administered AP1903 eliminated intrapleural intratumoral iCasp9M28z T cells within 1 hour.

3.2.5 Rationale for combining T cell therapy with cyclophosphamide

The rationale for combining genetically modified T cell therapy with cyclophosphamide chemotherapy in Cohorts 2 to 4 is two-fold. First, lymphodepleting chemotherapy may enhance the ability of adoptively transferred tumor-specific T cells to proliferate *in vivo* through homeostatic proliferation. Second, pretreatment with cyclophosphamide may transiently reduce the numbers of patient CD4+CD25+ regulatory T cells, which would otherwise suppress the function of adoptively transferred tumor-specific T cells. Ongoing clinical trials of adoptive T cell therapy have established the benefit of cyclophosphamide as a preparatory lymphodepleting agent. The infused T cells comprise both CD4+ and CD8+ T cells, both of which will be targeted to mesothelin. In studies in patients with melanoma, both subsets have been shown to possess antitumor activity. The T cell doses proposed for this study are within the range of T cell doses administered in other comparable studies. *In this study, only 1 dose of cyclophosphamide will be administered in Cohorts 2 to 4 (as a lymphodepleting agent) before infusion of T cells. Multiple doses are not administered, as cyclophosphamide is not the standard of care for MPD and as only 1 dose of T cells is administered*.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a phase I dose-escalation study to assess the safety and tolerability of administering increasing doses of engineered autologous T cells that have been targeted to mesothelin, without (Cohort 1) and following (Cohorts 2-4) pretreatment with cyclophosphamide. Patients diagnosed with malignant pleural mesothelioma, lung or breast cancer who have evidence of pleural disease are eligible for initial screening. Patients will be potentially be eligible to progress to the treatment phase of this trial if laboratory testing confirms that their tumors express the target protein mesothelin.

4.2 Intervention

To conduct a clinical trial of adoptive therapy with genetically modified T cells in patients with MPD, we have established an *ex vivo* transduction and expansion protocol that is capable of generating clinical-grade iCasp9M28z T cells for treatment. Autologous peripheral blood lymphocytes will first be isolated by leukapheresis. CD4+ and CD8+ T cells will then be activated, to allow for the efficient retroviral transduction of the iCasp9M28z CAR, using a highly efficient gamma-retroviral T cell

transduction methodology. Cells will then be expanded for a short period (9 to 16 days), as required by the dose-escalation scheme.

These T cells will be engineered using a “second-generation” CAR (with tandem CD28 and CD3zeta signaling domains). The transduction and expansion protocol will be conducted using a closed system developed at MSKCC that has been previously approved for ongoing clinical trials of chronic and acute lymphocytic leukemia (CLL and ALL) (“A Phase I Trial for the Treatment of Purine Analog-Refractory Chronic Lymphocytic Leukemia Using Autologous T Cells Genetically Targeted to the B Cell Specific Antigen CD19,” MSKCC IRB protocol 06-138, J. Park, PI; “A phase I trial of Precursor B Cell Acute Lymphoblastic Leukemia (B-ALL) Treated with Autologous T cellsGenetically Targeted to the B Cell Specific Antigen CD19”, MSKCC IRB protocol 09-114, J.Park, PI).

The proposed trial will test 3 levels of T cell doses. The dose-escalation scheme is described in detail below (section 9.1.4). In Cohorts 2-4, on the basis of our experience in an ongoing study of CLL, pretreatment with lymphodepleting cyclophosphamide will be administered at 1.5 g/m^2 1 day before infusion of T cells.

4.3 TREATMENT OF on-target, off-tumor toxicity –

Patients will be monitored throughout the trial for potential on-target, off-tumor reactions in response to the genetically engineered T cells including signs of inflammation within vital organs. If such reaction is suspected, consideration will be given to administer corticosteroid therapy as clinically indicated. If unacceptable toxicity occurs that is probably or likely related to anti-mesothelin CAR T cells, AP1903, a dimerizing agent, may be administered to mediate clearance of the genetically engineered cells and resolve toxicity. AP1903 will be considered for the following: • Any grade 4 toxicity that is felt to be probably or likely related to the mesothelin-CAR T cells will trigger administration of AP1903 if in the judgment of the Principal Investigator the syndrome cannot be adequately treated with routine supportive care including corticosteroids, and/or clinically available agents for neutralizing inflammatory cytokines (anti-TNF, IL1R antagonists, anti-IL6R mAbs). In the event that the PI is unavailable this judgement will be made by one of the Co-Principal Investigators, or a member of the Cell Therapy team. Persistent Grade 3 toxicity may also trigger administration of AP1903 if, in the judgment of the Principal Investigator, the risk of long-term morbidity is high. Any other Grade 3 or 4 toxicity may trigger administration of AP1903 if, in the judgment of the Principal Investigator, the risk for short-term or long-term moribidy is high and the toxicity is not responsive to standard supportive care, including administration of corticosteroids. AP1903 will be administered as a 2-hour infusion at a dose of 0.4 mg/kg. Where feasible, mesothelin-CAR persistence studies will be obtained from the start of the infusion, within 3 hours of the start of the infusion, and at 24 hours after infusion of AP1903.

The decision for use of AP1903 will be made by the study PI. In case study PI is not available, study Co-PI or CTC attending will make the judgment. In case of disagreement between the treating team and the PI regarding the use of AP1903, CAR Oversight committee should be approached for recommendation.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Cyclophosphamide

- Nitrogen mustard derivative alkylating agent; converted to activated alkylating metabolites by hepatic microsomal enzymes. It interferes with DNA replication and RNA transcription.
- Common indications: Hodgkin's disease, lymphoma, multiple myeloma, CLL, acute lymphocytic leukemia, acute myeloblastic leukemia, mycosis fungoides, neuroblastoma, retinoblastoma, mesothelioma, ovarian carcinoma, and breast carcinoma.
- Supplied as lyophilized powder: 200-mg, 500-mg, and 2000-mg vials.

The drug is reconstituted with sterile water to result in a concentration of 20 mg/mL. It is stable for 24 hours at room temperature or for 6 days refrigerated. No significant toxicity has been noted at daily doses of 1.5 g/m^2.[78-82] Baseline renal and hepatic function will be assessed before administration of cyclophosphamide.

5.2 Mesothelin-targeted T cells

Patients with therapy-refractory MPD will be treated by intrapleural infusion of autologous T cells genetically modified *ex vivo* to express the iCasp9M28z chimeric antigen receptor. This section of the protocol will describe the iCasp9M28z retroviral vector; the protocol for isolation, transduction, and expansion of iCasp9M28z T cells; and the analysis performed on the transduced T cells to measure gene expression, function, purity, and microbial sterility before infusion.

5.2.1 The vector

The iCasp9M28z retroviral vector is based on SFG, a splicing vector in which transgene expression is under the control of the Mo-MuLV long terminal repeat (LTR). The vector encodes a CAR consisting of a mesothelin-specific human scFv[44] fused to the transmembrane and cytoplasmic signaling domain of CD28, fused to the cytoplasmic signaling domain of the CD3ζ chain. It is linked to the ICaspase-9 gene through a P2A site derived from porcine teschovirus-1. Expression of both ICaspase-9 and M28z is driven by the retroviral LTR. The M28z vector will be transfected into the 293GP-R30 packaging cell line (RD114 envelope),[71] and cell-free supernatant will be used to infect the 293GP-GLV9 packaging cell line (GaLV pseudotyped).[71] The titration of individual 293GP-GLV9 clones is performed by infecting T cells with serial dilutions of the vector stocks and subsequently determining (by real-time PCR [RT-PCR] using sequences specific for the SFG vector) the average vector copy number in the genomic DNA of T cells. A high-titer 293GP-GLV9-iCasp9M28z packaging cell clone has been selected. The absence of replication-competent retroviruses (RCRs) has been ascertained using the S+L- assay, after amplification of 293 cells, in accordance with Federal Drug Administration (FDA) guidelines. A master cell bank (MCB) of the resulting 293GP-GLV9-iCasp9M28z clone and clinical lots of the retroviral vector M28z supernatant derived from the MCB were produced in the Cell Therapy and Cell Engineering Facility at MSKCC (CTCEF), in accordance with FDA and NIH recommendations and guidelines (see Appendix 3).

5.2.2 Generation and isolation of iCasp9M28z patient T cells for adoptive therapy

Please refer to the IND application for further details regarding the iCasp9M28z$^+$ retroviral vector and the generation/safety testing of a clinical grade packaging cell line used in this protocol.

5.2.3 Quality control for release of transduced cells

Before infusion, the transduced CD3+ T cells will be quality tested for number, purity, viability, and sterility. Please refer to the IND application for details regarding analyses performed on iCasp9M28z$^+$ T cells to measure gene expression, function, purity, and sterility prior to infusion.

5.2.4 Turnover of cell preparations

Depending on scheduling, patients will likely undergo infusion of their autologous transduced T cells 9 to 16 days after leukapheresis. T cells will be frozen and stored after leukapheresis. After transduction of thawed T cells, the transduced cells will either be immediately infused into the patient or frozen for future infusion.

5.2.5 AP1903 DIMERIZING AGENT

Availability: Supplied by Bellicum Pharmaceuticals.
Preparation: Formulated at 5 mg/ml in 25% Solutol HS15 (a non-ionic stabilizer).
Description: AP1903 is a lipid-permeable molecule with homodimerizing activity. Dimerizer drug AP1903 homodimerizes an analogue of human protein FKBP12 (Fv) which contains a single acid substitution (Phe36Val) so that AP1903 binds to wild-type FKBP12 with 1000-fold lower affinity. Upon binding of AP1903 to the engineered FKBP12, caspase 9 activation ensues leading to endogenous caspase 3 activation and cellular apoptosis, beginning within 30 minutes and peaking at 3 hours.
Chemical Formula*:* C78H98N4O20
Stability: Stable for at least 24 months when stored at 2-8°C.
Administration: Premedicate with Tylenol and Benadryl using standard dosing 15-30 minutes prior to AP1903. AP1903 should be warmed to room temperature prior to dilution. AP1903 is incompatible with material containing plasticizer or DEHP and materials sterilized with ethylene oxide. Diluted to 200 ml in normal saline and administered at a dose of 0.4 mg/kg over 2 hours.
Pharmacology: T1/2 3.6 hours in mice
Toxicity: none expected, the no observed effect level in dogs was 1000mg/kg, which is much beyond the prescribed 0.4 mg/kg dose. Urticaria and flushing observed in one patient, which did not occur with subsequent AP1903 administration administered after premedication. In the same trial, one patient experienced a cytokine release reaction after receiving AP1903 following dendritic cell infusions. Please refer to the Investigative Brochre on AP1903 provided by the Bellicum Pharamceuticals. In the event that the AP1903 is used, we will report the use to Bellicum Pharmaceuticals along with any relevant clinical data regarding use of the agent.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

Patients deemed eligible for the study are patients with MPD. Patients with malignant pleural effusion and MPD will undergo insertion of an intrapleural catheter as clinically indicated. All patients with a functional pleural catheter are eligible for the study, as long as there are no clinical concerns of infection. After obtaining a screening consent, patients tumor and/or blood will be tested for presence of tumor marker – mesothelin. In patients with mesothelin-positive tumors, a single blood volume leukapheresis for harvesting of PBMCs will be performed, after obtaining informed consent for the study. All obtainable pleural fluid will be drained from the chest through a pleural catheter and will be preserved for analysis. Subsequently, a single dose of mesothelin-targeted T cells will be instilled via the catheter into the pleural space. If a patient requires a thoracic surgical procedure (ex. VATS) for any clinical indication after instillation of CAR T cells, pleural biopsies will be obtained. Patients with PleurX catheters for management of MPD for whom VATS is not considered are eligible for this study.

6.1 Subject Inclusion Criteria

1. Patients with MPD aged ≥18 years
2. Karnofsky performance status ≥70%
3. Patients with malignant pleural disease (MPD), pathologically confirmed at MSKCC, and defined as one of the following:
    a. Malignant pleural mesothelioma – previously treated with at least one prior treatment regimen.
    b. Non-small cell lung cancer metastatic to the pleura—previously treated with at least one prior treatment regimen (chemotherapy or targeted agent) and documented progression of disease. Patients with disease outside of the pleura will be discussed among study PI and Co-PIs prior to considered eligible for the study. Disease outside of the pleura must not require any immediate therapy.
    c. Breast cancer metastatic to the pleura— previously treated with at least one prior treatment regimen (chemotherapy or targeted agent) and documented progression of disease. Patients with disease outside of the pleura will be discussed among study PI and Co-PIs prior to considered eligible for the study. Disease outside of the pleura must not require any immediate therapy.
4. Expression of mesothelin must be confirmed by meeting one of the following criteria.
    a. Mesothelin expression (>10% of the tumor expressing mesothelin) by immunohistochemical (IHC) analysis
    b. Elevated serum SMRP levels (>0.4 nM/L).
5. Patients must have a free flowing pleural effusion requiring management by placement of a pleural catheter. Patients with a functional pleural catheter already in place are eligible for the study, as long as there are no clinical concerns of infection.
6. Chemotherapy, targeted therapy (such as a tyrosine kinase inhibitor) or radiotherapy must have been completed at least 28 days prior to administration of T-cells. Continuation of hormonal therapy (ie for breast cancer) is acceptable. Prior immunotherapy with checkpoint blockade (i.e. PD1 inhibitor, PDL1 inhibitor or CTL4-antagonist or similar agent) must have been completed more than 6 months prior to the T cell infusion.
7. Any major thoracic (thoracotomy with lung or esophageal resection) or abdominal (laparotomy with organ resection) operation must have occurred at least 28 days before study enrollment. Patients who have undergone diagnostic VATS or laparoscopy can be included in the study.
8. All acute toxic effects of any previous radiotherapy, chemotherapy, or surgical procedures must have resolved to grade I or lower according to CTCAE (version 4.0).
9. Lab requirements (hematology)
    - White blood cell (WBC) count ≥3000 cells/mm^3
    - Absolute neutrophil count ≥1500 neutrophils/mm^3
    - Platelet count ≥100,000 platelets/mm^3
10. Lab requirements (serum chemistry)
    - Bilirubin ≤1.5x upper limit of normal (ULN)
    - Serum alanine aminotransferase/serum aspartate aminotransferase (ALT/AST) ≤2.5x ULN
    - Serum creatinine ≤1.5x ULN or Cr > 1.5x ULN, but calculated clearances of >60
11. Negative screen for human immunodeficiency virus (HIV), hepatitis B virus (HBV) antigen, and hepatitis C virus (HCV). If testing was performed during the previous 3 months, there is no need to repeat testing, as long as documentation of results is

provided to the study site. Subjects must receive counseling and sign a separate informed consent form for HIV testing.

12. Subjects and their partners with reproductive potential must agree to use an effective form of contraception during the period of drug administration and for 4 weeks after completion of the last administration of the study drug. An effective form of contraception is defined as oral contraceptives plus 1 form of barrier or double-barrier method contraception (condom with spermicide or condom with diaphragm).
13. Subjects must be able to understand the potential risks and benefits of the study and must be able to read and provide written, informed consent for the study

6.2 Subject Exclusion Criteria

1. Any prior history of brain metastases
2. Non-small cell lung cancer metastatic to the pleura that extends outside of the pleura requiring immediate therapy
3. Breast cancer metastatic to the pleura that extends outside of the pleura requiring immediate therapy
4. Prior history of seizure disorder
5. Patients currently receiveing treatment for concurrent active malignancy
6. Autoimmune or antibody-mediated disease, including but not limited to systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, Crohn's disease, and temporal arteritis (Patients with a history of hypothyroidism will not be excluded)
7. Clinically significant cardiac disease (New York Heart Association class III/IV) or severe debilitating pulmonary disease
8. Pregnant or lactating women
9. An infection requiring antibiotic treatment within 7 days before the start of treatment (day 0)
10. A requirement for daily systemic corticosteroids for any reason or a requirement for other immunosuppressive or immunomodulatory agents. Topical, nasal, and inhaled steroids are permitted.
11. Administration of live, attenuated vaccine within 8 weeks before the start of treatment (day 0) and throughout the study
12. Any other medical condition that, in the opinion of the PI, may interfere with a subject's participation in or compliance with the study
13. Participation in a therapeutic research study or receipt of an investigational drug within 30 days before the screening visit

7.0 RECRUITMENT PLAN

This study will be conducted at MSKCC. A minimum of 4 and a maximum of 24 patients will be enrolled in this study. All patients will be recruited through by the Thoracic Surgical or Thoracic Medical Oncology Service of MSKCC. Only patients aged 18 years or older who have therapy-refractory disease will be eligible for this trial.

Table 2: Race/Ethnicity

Sex	White, not of Hispanic origin	Black, not of Hispanic origin	Hispanic	Asian or Pacific Islander	Unknown	Total

Male/ Female	20	2	0	2	0	24

Potential research subjects will be identified by a member of the patient's treatment team, the PI, or the research team at MSKCC. If the PI is a member of the treatment team, he or she will screen his or her patients' medical records for suitable research study participants and discuss the study and the patient's potential for enrolling in the research study with the patient. Potential subjects contacted by their treating physician will be referred to the PI/research staff to record appropriate contact information, so that these patients can be approached about enrolling in the study.

The PI/research staff may also screen the medical records of patients they do not have a treatment relationship with, for the limited purpose of identifying patients who are eligible.

During the initial conversation between the PI/research staff and the patient, the patient may be asked to provide certain health information that is necessary for the recruitment and enrollment process. The PI/research staff may also review portions of the patient's medical records at MSKCC to further assess eligibility. They will use the information provided by the patient and/or the medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study or declines to participate, the research staff will destroy all information collected on the patient during the initial conversation and review of medical records, except for any information that must be maintained for screening log purposes.

Subjects will be required to sign a statement of informed consent that meets the requirements of the Code of Federal Regulations ([CFR] Federal Register Vol. 46, No. 17, Jan. 27, 1981, Title 21, Part 50) and the Institutional Review Board (IRB) of MSKCC. The medical record will include a statement that written, informed consent was obtained, along with the date that written consent was obtained—thereby documenting that written, informed consent was obtained before the subject was enrolled in the study.

8.0 PRETREATMENT EVALUATION

The study calendar for this protocol can be found in section 10. Documented cycle delays that occur outside of the allowable window of variance (+/-3 days, unless otherwise noted) because of holidays, weekends, weather, or other unforeseen circumstances will constitute a protocol deviation and will be reported to IRB.

There are 2 phases to the study: the Screening Phase and the Intervention Phase. Only patients identified as eligible from the Screening Phase may enroll in the Intervention Phase.

Screening Phase: After signing Informed Consent 1 (Screening Informed Consent), the patient's mesothelin tumor expression will be determined as previously described. In order to be eligible for this protocol, the patient's carcinoma must express the mesothelin protein detectable by IHC analysis of banked (paraffin embedded) or fresh biopsied tumor and/or elevated serum mesothelin levels.

After signing Informed Consent 1, if a patient's tumor is found to express mesothelin, patients will be offered Informed Consent 2 (Treatment Informed Consent) to enroll in the Treatment Phase of the study. Patients will then undergo leukapheresis for the collection of PBMC. Subsequently, the leukapheresis product will be used to generate the iCasp9M28z^{+} genetically-modified T cells.

Intervention Phase: Patients must sign Informed Consent 2 (Intervention Informed Consent) before receiving treatment on the study.

To be performed before study treatment is scheduled
- Leukapheresis
- Successful generation of T cells

To be performed within 4 weeks before the start of treatment:
- A complete history and physical examination
- Concomitant medication inquiry
- ECG

To be performed within 2 weeks before the start of treatment:
- Hematology blood tests: CBC, differential count, and platelet count
- Serum chemistry: electrolytes (Na, K, Cl, $HC0_2$), BUN, glucose, creatinine, bilirubin, AST, ALT, calcium, phosphorus, uric acid, total protein, albumin, LDH, PT/PTT, and serum SMRP
- Negative screen for HIV, HBV antigen, and HCV. If testing was performed during the previous 3 months, there is no need to repeat testing, as long as documentation of results is provided to the study site.
- Research blood tests (~30 cc)
  -Lymphocyte subsets (CD4 and CD8) and cytokine profile (serum IFN-γ and TNF-α)
  -RCR test
- Urinalysis and culture & sensitivity (C&S; urinalysis includes specific gravity, pH, ketones, sugar, protein, bilirubin, blood, WBC, and microscopic examination of sediment)

9.0 TREATMENT/INTERVENTION PLAN

9.1.1 Production of genetically modified T cells

Following enrollment, leukapheresis product will be obtained in the blood donor facility at MSKCC and cryopreserved in the CTCEF. Before protocol treatment, leukapheresis product will be thawed, and T cell isolation, transduction, and expansion of iCasp928z T cells will be performed in the MSKCC Cell Therapy and Cell Engineering Facility. It is estimated that it will take approximately 9 to 16 days to generate T cells for treatment.

9.1.2 Pretreatment

In Cohorts 2 to 4, patients will receive cyclophosphamide intravenously (at 1.5 g/m^2) as inpatients, 1 day before admission for T cell infusion. The next day, patients will be admitted to the MSKCC Thoracic Surgery Inpatient Service (if not already inpatients) for intravenous hydration, clinical monitoring, and blood work for immune monitoring. Standard MSKCC antiemetic therapy will be administered prior to chemotherapy to prevent nausea/vomiting. Administration of corticosteroids will be avoided as steroids may impede the efficacy of CAR T cells.

9.1.3 Infusion of iCasp9M28z genetically modified T cells

On the first day of hospitalization for T cell infusion, patients will be treated with genetically modified T cells. Thirty to 60 minutes before T cell infusion, patients will be given 650 mg of acetaminophen orally and 50 mg of diphenhydramine orally or intravenously, to prevent infusion-related reactions. The genetically modified T cells will be infused for 1 to 2 hours thereafter through the indwelling

pleural catheter. The T cells will be administered slowly, during a period of 1 hour. A physician will be available during the infusion.

9.1.4 Treatment scheme

A minimum of 4 patients and a maximum of 24 patients will be treated with 3 escalating T cell doses. Since only limited published data are available regarding the safety of genetically modified T cells in patients with cancer, the proposed treatment doses in this study are based on safety data from previously published studies that used cloned autologous CD4+ or CD8+ T cells in patients with melanoma. The safety of the lymphodepleting cyclophosphamide dose proposed in this study (1.5 g/m^2) is based on our previous experiences at MSKCC with high-dose cyclophosphamide in the treatment of patients with melanoma and CLL. *Ongoing trials at MSKCC have delivered higher initial doses of genetically modified T cells via IV infusion (e.g., CLL trial: 3×10^6 CAR+ T cells/kg; ALL trial: 3×10^6 to 1×10^6 CAR+ T cells/kg). To our knowledge, this proposed trial is the first at MSKCC and other centers to deliver modified T cell intrapleurally. A conservative starting dose of 3×10^5 was chosen to ensure patient safety.*

Table 3

Cohort	Dose **	Number of doses	Total volume of each injection
-1*	1×10^5	1	Final volume of preparation will be approximately 50 mL, to be administered as a bolus.
1	3×10^5	1	
2	3×10^5+Cyclophosphamide	1	
3	1×10^6+Cyclophosphamide	1	
4	3×10^6+Cyclophosphamide	1	

*Necessary only if toxicity is encountered at the initial dose level.

**Mesothelin-targeted T cells/kg; intermediate dose levels may be evaluated, if indicated.

At least 3 patients will be treated starting at dose level 1 with an accrual of 1 patient per month. All patients treated in this step will be observed a minimum of 4 weeks before the initiation of dose level 2 occurs. However, patients can be prescreened to be ready for the next enrollment.

Dose-escalation scheme

The dose-escalation scheme is as follows.

- If none of the initial 3 patients at this dose level experiences a dose limiting toxicity (DLT), the next T cell dose will be administered to the next cohort of 3 patients.

- If 1 of the initial 3 patients at a dose level experiences a DLT, then up to 3 additional patients will be treated at the same dose level. If then one in 6 of the patients at the same dose level experiences a DLT, the next dose level will be investigated. Thus, dose escalation will only proceed with 0 of three, or 1 of six, patients experiencing a DLT per dose level.

- In the event that 2 of 3 patients, or 2 of 6 patients in the first cohort (dose level 1) experiences DLT, then the next cohort of patients will be treated at a lower dose level (dose level -1) with 1×10^5 T cells/kg. Three patients will be enrolled at this dose level, if 0 or 1 patients experiences a DLT, a further 3 patients will be enrolled at this dose level. If overall 0 or 1 patient experiences a DLT at this dose level, this dose will be established as the MTD.

CAR T-cell oversight committee: Decisions to escalate to the next level or, when appropriate, to deescalate to a lower level will be recommended to the institutional CAR T-cell Oversight Committee by the principal investigator (PI). The CAR T-cell Oversight Committee will make the final decision of

whether to proceed to the next dose level. In addition, the PI will coordinate regular meetings/communication with CAR T-cell Oversight Committee to

a. present the data for each cohort of patients and study progress
b. discuss and obtain recommendations prior to escalating to the next dose level or if appropriate deescalating the dose level
c. report any significant toxicities associated with the CAR T-cell intervention.
d. present any proposed significant changes in the protocol.

9.2 DLTs

DLT is defined as any of the following occurring within 30 days from the infusion of the iCasp9M28z^{+} T cells.

- Grade 4 leukopenia (WBC < 1000/ml) lasting 30 days or more from the time of infusion (in patients with a pre-treatment WBC of > 1000/ml).
- New grade 3 or 4 non-hematologic toxicities lasting 14 days or more, which is probably or definitively attributed to T cell infusion, not attributed to any chemotherapy received or persistent disease.
- Grade 4 neurotoxicity (encephalopathy), severe cytokine release syndrome (CRS) as defined in the section below not improving after 48 hours of systemic steroids.
- Grade 3 EEG-confirmed seizures or Grade 4 multiple seizures that are not responsive to the neurological treatment by the Neurology team.
- Any grade 5 toxicity including death, attributable (definitely, probably, or possibly) to T cell infusion.

9.3 Stopping Rules for Delayed Toxicity:

Dose escalation will proceed based on DLT experienced within the treatment and observation periods as described. Delayed toxicities (any of the toxicities specified above seen after 30 days) which are likely or definitely related to treatment with genetically-modified T cells will be collected and evaluated by the investigators and reported to the MSKCC IRB, Investigational Drug Committee, and the FDA. Accrual might be held pending analysis of adverse events and will be restarted only after approval of the IRB and FDA.

9.4 Management of Infusion-Related Reactions:

- If a grade 1 infusion-related reaction occurs, T cell infusion may be continued at the same dose and rate of administration.
- Fever, chills, and rigors may be treated with acetaminophen po, diphenhydramine IV or po (or alternative antihistamine), meperidine IV, and hydrocortisone IV or methylprednisolone IV as clinically indicated. Nausea and vomiting may be treated using previously established MSKCC guidelines.
- If a grade 2 infusion-related reaction occurs, treatment may be continued with a reduction in the infusion rate of T cells. Symptoms may be treated as above, as clinically indicated.
- If a grade 3 infusion-related reaction occurs, the infusion of T cells will be interrupted. Symptomatic treatment, as outlined above, will be administered as clinically necessary,

and the infusion can be resumed at a reduced rate after resolution of symptoms. If grade 3 reactions recur after resumption of the infusion, T cell infusion will be discontinued.

- If a grade 4 infusion-related toxicity recurs, T cell infusion will be discontinued. Symptoms will be treated using the guidelines above, as well as established MSKCC adult hypersensitivity guidelines, and no further T cells will be administered.

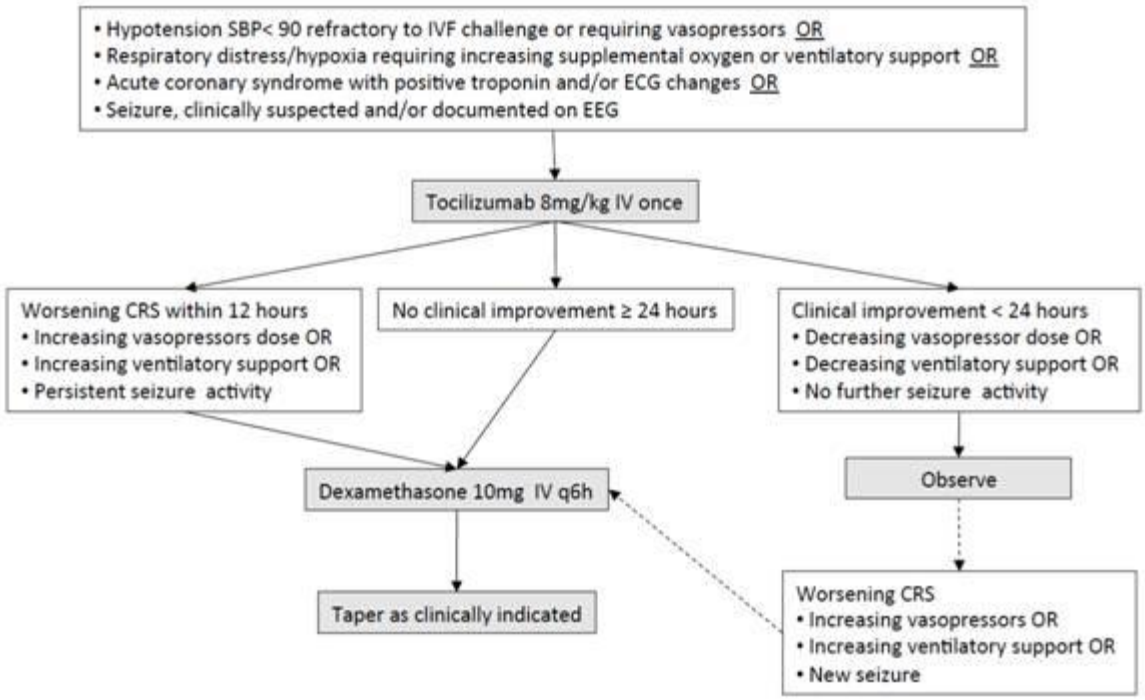
9.5 Management of Cytokine Release Syndrome (CRS): Following infusion of iCasp9M28z^{+} T cells, patients may develop severe CRS. Severe CRS is defined by the presence of one of the following clinical and laboratory parameters, not expected in the post-operative period if the patient underwent a surgical procedure.:

- Hypotension: SBP<90 refractory to IV fluids or requiring at least one vasopressors
- Respiratory distress/hypoxia requiring increasing supplemental oxygen (not related to post-operative oxygen requirement) or ventilator support
- Acute coronary syndrome (ACS) with positive troponin and/or EKG changes concerning for ACS
- Seizure, clinically suspected and/or documented on EEG

In order to ensure adequate monitoring and treatment of any sCRS or other serious toxicity in patients receiving modified T cells, patients will be admitted to Memorial Hospital as inpatients prior to their first infusion of modified T cells, and will remain hospitalized until at least 2 days after the second infusion. The first cohort of patients to be treated, and the first patient treated in each subsequent cohort, will be admitted to the ICU; subsequent patients may be admitted to regular inpatient units (subject to the clinical judgment of the treating physician).

In an effort to reduce complications related to these CRS toxicities, the following management algorithm will be used.

Severe Cytokine Release Syndrome (CRS) Management Algorithm



If the patient is not responding to the above measures, or worsening despite the treatment proposed, PI will make the judgment to infuse AP1903 as described in section 4.3.

9.6 Management of Neurological Toxicities: Patients with severe CRS may develop neurological complications including confusion, delirium, expressive aphasia, obtundation, myoclonus and seizure-like activity. Some of the previously treated patients required ICU stay due to these side effects, and few patients required intubation. All of these side effects were observed in patients with morphologic ALL at the time of T cell infusion. The following specific measures will be taken to minimize toxicity in treated patients.

- Upon admission, all patients baseline neurological examination will be documented in the history&physical examination prior to T cell infusion.
- All patients will receive levetiracetam (Keppra®)for seizure prophylaxis prior to initial T cell infusion. In cases of allergic reactions to levetiracetam, alternative anti-seizure medications will be used under the guidance of the Neurology team.
- After T cell infusion, if patient develops neurological signs, a Neurology consult will be requested. These patients will be followed by the Neurology team, at least 3 times a week (or as clinically indicated) for the first 2 weeks or until neurological symptoms resolve. If neurological toxicities including seizure are observed, these symptoms will be managed as per the institutional neurology guidelines

9.7 Management of 'On-target, Off-tumor' Toxicities: patients may develop 'on-target, off-tumor' toxicity due to very low levels of mesothelin expression in the normal pleura, pericardium or peritoneum. Patients will be monitored for evidence of pleuritis, pericarditis or peritonitis. 'On-target, Off-tumor' toxicity will be defined as –

* Symptoms of persistent pleuritis that may worsen with deep inspiration, pain not relived by routine pain management and is unrelated to post-operative incisional or chest pain

* Symptoms of pericarditis diagnosed by sharp, persistent, retrosternal chest pain that may become worse with deep inspiration and is associated with new EKG changes and/or new pericardial effusion diagnosed by echocardiogram.
* Symptoms of peritonitis diagnosed by diffuse abdominal pain that may worsen following deep inspiration or cough with rebound tenderness on examination, that may be associated with previously unknown ascites.

Patients with suspected 'on-target, off-tumor' toxicity will be closely monitored as clinically indicated. If patient continues to have above criteria, AP1903 will be administered as indicated in section 4.3.

9.8 Supportive Treatment:

- Patients will receive antibiotics, red blood and platelet cell transfusions according to MSKCC standard care guidelines.
- Patients may receive subcutaneous filgrastim or pegfilgrastim at the discretion of the treating physician following conditioning chemotherapy and infusion of modified T cells.
- The patient may be discharged from the hospital, at the discretion of the treating physician if the patient is clinically stable and there is no evidence of significant tumor lysis, transfusion reactions, or unforeseen adverse reactions after T cell infusion.
  The ICU staff will be made aware in of all patients treated with T cells on this protocol.
- In the event of symptoms related to an adverse reaction to infused T cells including fever, hypotension, renal failure, or hypoxia, the ICU staff will immediately be consulted to provide close monitoring and assessment for possible transfer to the ICU for closer observation.
- If there is laboratory evidence of acute renal failure (increased creatinine of 1.5x over pre-T cell infusions levels), the MSKCC renal service will be consulted

If the patient develops fevers, chills or other evidence of infection on outpatient followup, she will be admitted to the inpatient service for further evaluation and IV antibiotics as clinically indicated, ID service will be consulted.

Management of tumor lysis syndrome per institutional guidelines (may include allopurinol).

10.0 EVALUATION DURING TREATMENT/INTERVENTION

STUDY FLOW CHART

Procedure		**Visit**						
	Screening	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**3 days after discharge**	**Weeks 1-8**	**Monthly F/U for 6 months**
Medical history[1]	X*					X	X	X
Physical examination	X*	X				X	X	X
RCR test[8]	X+						X	
T cell polyclonality testing[9]								X
Hematology[2]	X+	X	X	X	X		X	X
Serum chemistry[3]	X+		X	X	X			
Serum C-Reactive Protein[10]			X	X	X		X	X
Serum SMRP	X+						X	X

HIV testing, serologic testing for HBV and HCV[6]	X[8]							
Immunophenotyping and cytokine profile	X=			X	X			
Urinalysis[4] and C&S	X+							
ECG	X							
Administration of cyclophosphamide [7]		X						
Administer T cells[5]			X					
Concomitant medication inquiry	X*	X						X
AE inquiry		X	X	X		X	X	
Leukapheresis	X							
Twice weekly communication [11]							X	

*Within 4 weeks of study treatment (day 0)

+Within 2 weeks of study treatment (day 0)

[1] Record complete medical history
[2] CBC, differential count, and platelet count
[3] Electrolytes (Na, K, Cl, $HC0_2$), BUN, glucose, creatinine, bilirubin, AST, ALT, calcium, phosphorus, uric acid, total protein, albumin, PT/PTT, and LDH
[4]Urinalysis includes specific gravity, pH, ketones, sugar, protein, bilirubin, blood, WBC, and microscopic examination of sediment
[5]Treatment with antimesothelin T cells at escalating doses ($3x10^5$, $1x10^6$, $3x10^6$ T cells/kg), administered intrapleurally. Dose escalation will occur on the basis of toxicity/safety profiles. Cells may be infused within 2 to 4 weeks after the leukapheresis procedure.
[6]If testing was performed within the previous 3 months, there is no need to repeat testing, as long as documentation of results is provided to the study site.
[7]Administration of cyclophosphamide at 1.5 g/m^2 intravenously 1 day before infusion of T cells
[8]RCR samples will be obtained at screening, 3, 6, 12 weeks (+/-5 days) and yearly thereafter (see 10.4)
[9]T cell polyclonality analysis will be assessed at day 60 (+/-5 days) after therapy (see 10.5)
[10] Following T-cell administration, C-reactive protein titers will be performed as clinically indicated
[11]Twice-weekly communications will be performed by the PI, co-PIs, CTC MDs or CTC research nurse.

Specimen handling: collecting blood samples

A. Venous blood samples (7 mL/tube) for biochemical and metabolic markers will be collected in a red speckled tube, stored at room temperature until processing, and forwarded to the Hematology/Clinical Chemistry laboratories. PBMCs will be collected in cell preparation tubes and kept on ice until distribution to either Dr. Riviere's or Dr. Adusumilli's laboratory. Samples will then be stored as indicated in Section 5.0.

B. Labeling blood/pathologic samples
Submitted patient documents will have a unique identifier consisting of a study number derived from the site identification and the order in which the patient was enrolled in the study, followed by the patient's initials and the cycle of enrollment (e.g., MSKCC-7-JD-C1). This identifier will be used for the purposes of the study only and will be distinct from the patient identification (medical records) number used. Each sample will be accompanied by a sample log. Pathologic specimens will be stored at -80^0C in Dr. Adusumilli's freezer in the Schwartz building.

C. Collection of blood and blood products carries low risks for the patient. Minimal discomfort and bruising at the site of needle entry are common. No central line for collection of peripheral blood is needed; leukapheresis is performed once and has the

same risks of discomfort and bruising at the site of needle entry as does collection of peripheral blood.

Details of specialized tests are provided below.

10.1 T Cell infusion evaluation

Temperature, pulse, respirations, and blood pressure will be measured and recorded in the patient's chart. Patients will be evaluated clinically by vital signs approximately 30-90 minutes pre-infusion of T cells, approximately every 15 minutes during infusions and then approximately 30 minutes x4 post infusion of T cells. All infusions of modified T cells, whether IV or IP, will be administered by MDs or RNs/ APPs under MD supervision. Chemistry profiles and CBCs will be obtained daily for at least 2 days (days 2 and 3). Following T-cell administration, C-reactive protein titers will be performed as clinically indicated. For the first cohort, patients will be treated in the Intensive Care Unit, and they will remain inpatients for 1 night, unless undue toxicity or unexpected AEs occur; maximum expected inpatient stay is 2 nights. Treatment will be administered under the supervision of Dr. Adusumilli or Dr. O'Cearbhaill or Dr. Krug. Patients will remain as an inpatient for at least 2 days after any T cell infusion. They will be seen daily during the inpatient stay by an attending from the Cellular Therapeutics Center or an attending from the Medical Oncology service. A Cellular Therapeutics Center attending will also be contactable via the CTC pager.
The research blood work will be stored at room temperature. Either the RSA or study investigator will bring research samples to the laboratory of Dr Adusumilli as needed, and the samples will be processed in Dr Adusumilli's laboratory. A Research Study Assistant (RSA) will be assigned to the study to facilitate collection and delivery of the research specimens.

10.2 Follow-up evaluation

Patients will be seen as an outpatient within 3 days of discharge. Patients will have at least twice weekly communication with study team for toxicity assessment in addition to being seen in clinic weekly for at least 8 weeks following therapy. Thereafter, follow-up visits will be at least monthly for 6 months. Thereafter, the frequency of further follow-up will be determined by the treating physician, as clinically indicated. At each follow-up visit, patients will undergo a complete history and physical examination. If the pleural catheter is still in place, pleural fluid samples will be obtained for analysis. CBCs will be obtained and AE assessments will be performed at each clinic visit.

10.3 Assessment of genetically modified T cell survival

To assess and quantify the survival of genetically modified T cells after infusion, peripheral blood analysis will be performed 1 to 3 days post infusion, at week 4, monthly for the first 6 months and subsequently as needed until the CAR T cells become undetectable by FACS analysis (using PE-conjugated rabbit polyclonal antihuman IgG antibody specific to the iCasp9M28z CAR) and by quantitative RT-PCR analysis (using primers and probe targeting retroviral-specific sequences) in Dr. Adusumilli's laboratory or in Cell Therapy and Cell Engineering Facility. Biopsies will be performed for selected patients on the basis of availability and access to the involved tumor site, as well as the willingness of the patient to undergo the procedure.

10.4 RCR testing

At the time of T cell infusion, the retroviral vector copy number of the infused T cells will be assessed by RT-PCR. Samples will undergo RCR testing at the time of infusion. Peripheral blood samples (~7 cc/collection) will undergo RCR testing at 0 days (prior to infusion), 3 months, 6 months, 12 months after treatment and archived yearly thereafter.

10.5 Pathologic sample archiving

Pathologic samples (both frozen and paraffin-embedded) obtained 4 weeks or later after any clinically indicated surgical procedure will be archived. Specimens will be analyzed for mesothelin-expressing tumor, CD3 infiltration, and necrosis as needed.

10.6 Long-term follow-up plan

In compliance with CBER/Biological Response Modifiers Advisory Committee recommendations for gene-transfer research protocols, long-term follow-up of enrolled patients will include:

1) A minimum of 1 physical examination per year, with documentation of significant-interval medical history, to be performed at MSKCC for 15 years after completion of treatment.

2) All information obtained during follow-up will be compiled by the research study assistant assigned to this study and reviewed by the PI. The PI will provide an annual summary of long-term follow-up results, which will be provided to the FDA in conjunction with each investigational new drug (IND) annual report. In the unlikely event that a plan change becomes necessary, this information will also be provided.

10.7 Contingencies for patients lost to follow-up

As the long-term follow-up extends to 15 years, it is likely that some patients will be lost to follow-up. To minimize this risk, enrolled patients will be requested to provide forwarding information if they move out of the area. Furthermore, we will make every effort to obtain contact information of close relatives of patients at the time of enrollment. The research study assistant will be responsible for tracking and locating patients enrolled in this study during the follow-up period.

10.8 Contingencies for loss of the PI

In the event that the PI leaves the institution, the role of the PI and the responsibilities for further patient follow-up associated with this title will be transferred to a co-PI or other member of the MSKCC Thoracic Surgery Service, at the discretion of the service chief and with approval of the institution's IRB.

10.9 Contingencies for death of a patient during study

In the event that a patient dies during the study period, the patient's family will be requested to have an autopsy performed, regardless of the cause of death. If an autopsy is permitted by the family, samples of blood, all tumor sites, brain, liver, kidney, lung, and gonads, as well as marrow and spleen, will be obtained. These tissues will be analyzed histochemically for evidence of residual tumor cells and for evidence of residual iCasp9M28z T cells. In addition, samples will be obtained for PCR-amplified analysis of tissue fractions to detect for evidence of residual iCasp9M28z+ T cells. DNA from the tissue samples will be obtained to assess vector sequences detectable in the tissues and to define sites of insertion of the vectors, if possible. In addition, attempts will be made to expand iCasp9M28z T cells from the bone marrow, blood, and spleen cell populations. The expanded cell populations will be further analyzed for appropriate vector sequence and insertion site.

11.0 TOXICITIES/SIDE EFFECTS

Adverse Event: An adverse event is any noxious, pathologic, or unintended change in anatomical, physiologic, or metabolic functions, as indicated by physical signs, symptoms, and/or laboratory changes occurring in any phase of the clinical trial, whether associated with drug or placebo and whether or not considered drug related. All of the following are to be considered adverse events:

- An exacerbation of a pre-existing condition
- An intercurrent illness
- Any drug interaction
- Any event related to a concomitant medication
- Development of an abnormal laboratory value or a significant change from baseline in a laboratory value within the range of normal, considered by the investigator to be clinically important
- An unexpected significant worsening of the cancer under treatment. Anticipated day-to-day fluctuations in the activity of the cancer or the anticipated progression of the cancer (other than death) should not be considered an adverse event.

Serious Adverse Event: A serious adverse event is one that is fatal or life-threatening (see below), is temporarily or permanently disabling, requires inpatient hospitalization (initial or prolonged), or is associated with a congenital anomaly, a new cancer or a drug overdose (either accidental or intentional). In addition, any event suggesting a significant hazard, contraindication, side effect or precaution should also be considered serious.

Life-threatening: means an immediate risk of death from the reaction as it occurred. Life- threatening does not include a reaction that, had it occurred in a more serious form, might have caused death. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening even though drug-induced hepatitis can be fatal

Toxicity will be graded on a scale of I to V, as described in version 4 of the CTCAE (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

11.1 Autoimmunity

Autoimmune reactions mediated by the infused autologous T cells are a theoretical possibility if anergic autoimmune T cells are stimulated by activation through the M28z receptor or if normal mesothelin-positive tissues (pleura, pericardium, and peritoneum) are targeted. Patients who develop evidence of either hematologic or unforeseen autoimmune reactions during therapy will be treated with standard autoimmune regimens, including corticosteroids and/or AP1903 if elimination of transduced T cells is indicated. As a secondary alternative, AP1903 may be administered to specifically delete the infused T cells.

11.2 Insertional oncogenesis

In a previous study in a pediatric population with X-linked SCID, wherein patient bone marrow stem cells were retrovirally transduced with the common γc chain gene, 5 cases of subsequent T cell acute lymphoblastic leukemia thought to be related to the insertion of the retroviral vector at or near a cellular protooncogene were reported. In at least 4 cases, the insertion of the retroviral vector was at or near the LMO-2 protooncogene. Unfortunately, 1 of the first 3 patients diagnosed with T cell leukemia has subsequently died of complications related to GvHD associated with allogeneic bone marrow transplantation therapy for this leukemia. The results from this trial illustrate the significant risk of insertional oncogenesis related to retroviral gene therapy in stem cells. However, several significant differences exist between the X-linked SCID trial and our proposed adoptive T cell trial. First, whereas the X-linked SCID trial involved gene transfer into undifferentiated pluripotent stem cells, our trial involves modification of fully differentiated T cells. Second, whereas stem cells

undergo a high number of recombination events during maturation after retroviral transduction (potentially creating secondary mutations that could enhance the potential of generating a leukemic clone), mature T cells no longer undergo further differentiation, and recombination thereby minimizes the risk of further oncogenic mutations. Viewed from another perspective, patients enrolled in the X-linked SCID trial had favorable long-term survival. The subsequent T cell leukemias developed >3 years after treatment. In our patient population, prolonging survival to >3 years may represent a significant benefit from therapy, thereby mitigating the potential risk associated with this treatment. Third, it is worth noting that, to date, of the >300 SCID-beige mice treated with retrovirally modified human T cells in our preclinical studies—with >100 surviving for >1 year—none has had any evidence of clonal human T cell expansion or human T cell leukemia. Fourth, patients treated with T cells genetically modified to express LNGF-R, HSV-tk, neomycin, adenosine deaminase, or an anti–HIV-1 *tat* ribozyme have not developed any evidence of T cell clonal expansion after up to 10 years of follow-up.[62,66-68] Nevertheless, we plan to monitor for insertional oncogenesis in treated patients. Specifically, we will closely monitor T cell number and, when appropriate, assess clonal T cell expansion by use of PCR, FACS, and/or immunoscope analysis, as described above. Finally, in over 100 patients treated with CAR therapy including more than 50 patients at MSKCC, none has to date shown evidence of insertional oncogenesis. Furthermore, our CAR has iCasp9 suicide gene that can be turned on when needed.

11.3.1 Generalized inflammatory response generated by infused genetically modified T cells

The potential toxicities include transfusion reactions at the time of T cell infusion. Although all previously published data regarding the infusion of *ex vivo* expanded T cells have reported it to be safe, the infusion of T cells may nevertheless elicit a generalized inflammatory response. There is a theoretical possibility that the patient will develop fever, chills, arthralgias and myalgias, or diarrhea. Another possibility includes anaphylaxis or immediate hypersensitivity reaction characterized by facial swelling, itching, or difficulty breathing. For this reason, we have chosen an initial T cell dose (3×10^5 T cells/kg) that is significantly lower than those cited in the published literature, thereby minimizing the risk of unanticipated inflammatory reactions. To avoid the possibility of reactions, patients will be premedicated with 650 mg of acetaminophen orally and 50 mg of diphenhydramine orally or intravenously. Furthermore, if any untoward cytokine storm occurs, >90% of the infused T cells can be eliminated by intravenous injection of 1 dose of Ap1903.

11.3.2 Description of reported DLTs after CAR treatment

A thorough description of expected DLTs, based on previous data on use of CARs, can be found in the following publications.

1) Adverse events following infusion of T cells for adoptive immunotherapy: a 10-year experience. *Cytotherapy.* **2010**;12:743-749.

   Summary: The authors reviewed infusion-related AEs following administration of *ex vivo* expanded T cell products (antigen-specific CTLs, allo-depleted T cells, and genetically modified T cells) in IND studies. From 1998 to 2008, 381 T cell products were administered to 180 recipients, enrolled in 18 studies, receiving T cells targeting malignancies or posttransplant viral infections. There were no grade III or IV infusion reactions during initial monitoring or the 24-hour follow-up. Twenty-four mild (grade I to II) AEs occurred in 21 infusions, either during or immediately after infusion (up to 6 hours); the most common AEs were nausea and vomiting (10/24; 41.6%), probably because of the dimethyl sulfoxide cryoprotectant, and hypotension (20.8%), attributable to premedication with diphenhydramine. Twenty-two additional nonsevere events were reported within 24 hours of infusion; most common among these were culture-negative fever, chills, and nausea. An increased risk of AEs was associated with age (incidence rate ratio [IRR], 0.98; 95%

confidence interval [CI], 0.96 to 1.00; $P < 0.05$), while the risk of immediate infusion-related events was higher in patients reporting allergies (IRR, 2.72; 95% CI, 1.00 to 7.40; $P < 0.05$). Sex, disease type, and T cell source (allogeneic or autologous) had no effect on the frequency of AEs. The authors conclude that infusion of these T cell products was safe in the outpatient setting and was associated with no severe reactions, so monitoring for 1 hour after infusion is probably sufficient. As many of the AEs were attributable to premedication with diphenhydramine, a lower dose (0.25 mg/kg) should be selected.

2) Case report of a serious adverse event following the administration of T cells transduced with a CAR recognizing ERBB2. *Mol Ther.* **2010**;18(4):843-851.

   Summary: An optimized CAR vector containing CD28, 4-1BB, and CD3ζ signaling moieties was assembled in a γ-retroviral vector and used to transduce autologous peripheral blood lymphocytes from a patient with colon cancer that was metastatic to the lungs and liver and refractory to multiple standard treatments. The gene transfer efficiency into autologous T cells was 79% CAR+ in CD3+ cells, and these cells demonstrated high specific reactivity in *in vitro* coculture assays. Following completion of nonmyeloablative conditioning, the patient received 1×10^{10} cells intravenously. Within 15 minutes after infusion, the patient experienced respiratory distress and displayed a dramatic pulmonary infiltrate on chest X-ray. She was intubated, and despite intensive medical intervention, the patient died 5 days after treatment. Serum samples obtained after cell infusion showed marked increases in IFN-γ, GM-CSF, TNF-α, IL-6, and IL-10, consistent with a cytokine storm. The authors speculated that the large number of administered cells localized to the lung immediately after infusion and were triggered to release cytokine by the recognition of low levels of ERBB2 on lung epithelial cells.

3) CARs on track in the clinic. *Mol Ther.* **2011**;19(3):432-438.

   Summary: A patient death related to treatment with anti-CD19 CAR occurred shortly after the patient received cyclophosphamide for lymphodepletion and infusion of CAR-transduced cells. Although the precise etiology of this patient's death remains uncertain, it was consistent with an inflammatory cytokine cascade after administration of cyclophosphamide, which worsened after infusion of T cells, to give a clinical picture of acute sepsis, renal failure and resultant shock, and adult respiratory distress syndrome. Importantly, this patient's death did not appear to be directly caused by the cellular product, and this trial has been reopened.

4) Toxicity management of CAR T cell therapy at MSK: Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med 2014 Feb 19:6(224).

   Summary: The Center for Cell Engineering group reported on 16 patients with relapsed or refractory B cell acute lymphoblastic leukemia (B-ALL) treated with autologous T cells expressing the 19-28z chimeric antigen receptor (CAR) specific to the CD19 antigen. The overall complete response rate was 88%, which allowed to transition most of these patients to a standard-of-care allogeneic hematopoietic stem cell transplant (allo-SCT). Through systematic analysis of clinical data and serum cytokine levels over the first 21 days after T cell infusion, our group has defined diagnostic criteria for a severe cytokine release syndrome (sCRS), with the goal of better identifying the subset of patients who will likely require therapeutic intervention with corticosteroids or interleukin-6 receptor blockade to curb the sCRS. Additionally, we found that serum C-reactive protein, a readily available laboratory study, can serve as a reliable indicator for the severity of the CRS. This data provides a

strong rationale in monitoring CAR T-cell therapy patients and expected toxicity management.

The use of 19-28z gene-modified T cells in adults with CLL

We have infused 19-28z gene-modified T cells in eight adults with CLL. The first three adults were treated with 19-28z gene-modified T cells (3 x 10^7 T cells/kg) and no DLTs were noted. All three patients have died from complications from progressive CLL. The fourth patient was pre-treated with cyclophosphamide (1.5 gm/m^2), which was followed by a single dose of gene-modified T cells (3 x 10^7 T cells/kg). Unfortunately, this patient died approximately two days after receiving the T cells. The protocol was placed on hold to allow a comprehensive evaluation of this death. In addition, an autopsy was granted and performed. After a thorough review of all clinical data by institutional and federal regulators the leading suspicion for cause of death is sepsis. However, we cannot rule out that death was related to infusion with the gene-modified T cells so we have updated the consents to include the above information. In addition, we have modified the protocol to 1) split the dose and 2) reduce the total dose of T cells (1 x 10^7 T cells/kg). Since these changes, six other patients have been treated with cyclophosphamide and split-dose, reduced dose T cells without any significant complications to date.

Nonetheless, it remains possible that these anti-mesothelin targeted chimeric antigen receptor T cells used here could result in serious cytokine release syndrome, or in off-tumor, on-target effects on mesothelin expressing tissues. In addition, two patients had neurological events after receiving CAR T cells. To monitor cytokine release syndrome and possible neurological events, a treatment algorithm is developed as outlined in section 9.5. On-target, Off-tumor toxicity is a major reason for the incorporation of an inducible suicide gene as described below which would be used to eradicate the genetically engineered cells if substantial, unexpected autoimmune toxicity was observed. If Grade 4 toxicity is observed, or if Grade 3 toxicity is observed and believed to be causing substantial risk to the patient, then the AP1903 drug will be used as a suicide switch to deplete the genetically engineered cells. The proof-of-principle of this approach has been used in a graft-versus-host disease clinical setting and substantial preclinical data demonstrates that this agent is capable of rapid, efficient eradication of >99% of transduced T cells. Given the steep dose response curves with chimeric antigen receptors, we are confident that such two log depletion would be sufficient to prevent substantial clinical toxicity.

11.3.3 INCORPORATION OF CASPACIDE CELL THERAPY SAFETY SWITCH AS A SAFETY MEASURE - Given the theoretical risk of toxicity in this trial due to on-target, off-tumor effects on mesothelin+ non-malignant tissues, we have incorporated a suicide switch into the genetic vector that would allow rapid killing of the engineered cells in the event of unacceptable toxicity. The transgene iCasp9 consists of the sequence of the human FK506-binding protein (FKBP12; GenBank number, AH002818) with an F36V mutation, connected through a Ser-Gly-Gly-Gly-Ser linker to the gene encoding human caspase 9 (CASP9; GenBank number, NM001229), which has had its endogenous caspase activation and recruitment domain deleted. FKBP12-F36V binds with high affinity to an otherwise bioinert small-molecule dimerizing agent, AP1903. In the presence of AP1903, the iCasp9 promolecule dimerizes and activates the intrinsic apoptotic pathway, leading to cell death. The safety and efficacy of the transgene was initially tested *in vitro* and in small-animal models, and subsequently shown to mediate efficient apoptosis and clearance of GVHD inducing T cells in humans following allogeneic stem cell transplantation. Indeed, a single dose of the drug (0.4 mg/kg as a 2 hour infusion), given to four patients who developed GVHD, led to elimination of more than 90% of the genetically engineered T cells within 30 minutes of administration, followed by a subsequent reduction of 0.5-1.0 log during the subsequent 24 hours. GVHD resolved and did not recur. The dimerizing inducing agent is clinically inert and lacks biologic activity other than inducing

apoptosis of genetically engineered cells. Experience in humans comprises the study by DiStasi noted above, a follow on study of an additional 10 patients, 3 of whom similarly responded rapidly and completely with resolution of GvHD after administration of AP1903, as well as a pharmacokinetic analysis of AP1903 in 28 healthy male volunteers. Doses of 0.01-1.0 mg/kg resulted in plasma concentrations ranging from 10-1275 ng/ml with a short T1/2 (plasma levels of 18% of maximum 30 minutes after infusion and 7% of maximum 2 hours after infusion)74. The drug was well tolerated without significant toxicity. In a third trial, 18 men with progressive prostate cancer were enrolled in a dose escalation trial of dendritic cells that had been engineered to express prostate specific membrane antigen (PSMA) and iCASP9, with a total of 6 cycles/patient. 24 hours after each dendritic cell injection, the men received AP1903 to induced death of the injected dendritic cells. One patient exhibited a cytokine reaction following AP!903, presumably related to the dendritic cell lysis and a second patient experienced urticarial and flushing after AP1903. Subsequently, all patients received Benadryl and Tylenol premedication and significant toxicity was not observed (ASCO, Abstract #4670, 2011). In all trials, the dose of AP1903 has been 0.4 mg/kg infused IV over two hours. In this trial, patients would receive AP1903 in the event of unacceptable toxicity.

11.4 Risk of infection
There is a small risk of cotransmission of microbial pathogens during the infusion of cultured T cells. For this reason, cultures of infused preparations will be obtained before infusion, to exclude transmission of viral, bacterial, and fungal pathogens.

11.5 Risk of the generation of RCRs
To minimize the risk of RCRs, all vector stocks will be tested in accordance with FDA and RAC guidelines before use in T cell transduction. Furthermore, patient T cells will be tested for RCRs at the time of treatment, and peripheral blood T cells from patients will be tested for RCRs at 3, 6, 12 months and yearly after for 15 years , samples will be archived if the RCR testing are negative during the first year.

11.6 Risk of insertional mutagenesis in genetically modified T cells
The risk that random retroviral insertion may result in adverse mutations in modified T cells is likely very low. Furthermore, this remote risk is markedly less significant than the risks of hormone-refractory MPD, for which this treatment is designed. To date, no deleterious mutational events have been reported in patients treated with retrovirally modified T cells. The inclusion of the ICaspase-9 “suicide gene,” which confers sensitivity to the prodrug Ap1903, provides a potential safeguard as a treatment option if a T cell malignancy were to arise.

11.7 Retroviral transmission to offspring
This is not applicable in this patient population. Although this AE has not been observed in other trials that involved transduction of retrovirally modified T cells, barrier methods of contraception will be strongly recommended to all patients enrolled in the study.

11.8 Cyclophosphamide toxicity
Patients will be closely monitored for the following toxicities and will be treated with appropriate medical management in these settings.

- Nausea, vomiting (patients will receive antiemetics before and during treatment, to prevent this side effect), diarrhea

- Acute water retention attributable to inappropriate antidiuretic hormone release (hydration will be closely monitored and acute water retention treated with diuretics; hydration will be maintained to ensure adequate urine output)
- Cardiomyopathy (pretreatment cardiac function will be assessed to define eligibility); patients will be monitored during treatment to ensure against cardiac dysfunction; as only 1 dose of cyclophosphamide will be administered, the likelihood of cyclophosphamide-induced cardiomyopathy is minor
- Hemorrhagic cystitis (patients with a history of treatment with cyclophosphamide or any other chemotherapy will be excluded)
- Fatigue, loss of fertility, nail changes, mouth sores, secondary malignancies

Common Toxicities Associated with Cyclophosphamide

Hematologic	leukopenia, thrombocytopenia, anemia
Gastrointestinal	anorexia, nausea and vomiting (common with doses > 600mg/m^2), diarrhea
Hepatic	hepatitis, elevations in SGOT and SGPT
Renal	hemorrhagic cystitis
Cardiac	cardiotoxicity with high doses (> 120 mg/kg)
Respiratory	high doses may cause interstitial pulmonary fibrosis
Dermatologic	alopecia, facial flushing
Miscellaneous	nasal stuffiness, syndrome of inappropriate anti-diuretic hormone (SIADH) induced hyponatremia, fever after high-dose therapy

Fever in the setting of neutropenia will be managed in the Thoracic Surgery Inpatient Service at MSKCC with broad-spectrum intravenous antibiotic treatment. Patients will remain hospitalized until neutropenia and fevers have resolved. Patients with radiographic evidence of infection at the time of admission will be discharged, at the treating physician's discretion, once neutrophil reconstitution and radiographic evidence of resolving infections are established.

11.9 Risks of leukapheresis

To obtain sufficient quantities of T lymphocytes for the production of T cells for use in this trial, patients will undergo a leukapheresis procedure. The required volume, generally 250 to 270 mL, is obtained during a 3-hour leukapheresis procedure. The risks of leukapheresis can involve occasional vasovagal responses to venipuncture and minimal hemodynamic alterations associated with single-unit phlebotomies. To protect against these risks, leukapheresis will be conducted in the Blood Bank Donor Room, with full medical and nursing supervision and support systems to prevent and/or address AEs.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Exploratory efficacy assessments

Exploratory immune monitoring assays: processing and testing

Assessment of the presence and quantity of immune responses to mesothelin will be performed by Dr. Adusumilli's laboratory, in collaboration with Dr. Jedd Wolchok at the Ludwig Institute for Cancer Research's Immune Monitoring Facility and Dr. Isabelle Riviere at the Cell Therapy and Cell Engineering Facility.

Flow cytometric assay
This assay detects antibodies that recognize native cell-surface mesothelin on 3T3 cells that have been transfected with mesothelin. Controls for this assay consist of nontransfected 3T3 cells or mesothelin-negative cells: 1×10^6 cells from either cell line are washed in phosphate buffered saline (PBS) containing 0.25% (w/v) bovine serum albumin (staining buffer). Serum is then added (1:100 dilution in PBS) and incubated on ice for 30 minutes. After washing in staining buffer, the cells are incubated with antihuman IgG–fluorescein isothiocyanate (Caltag) for 30 minutes on ice. Cells are washed again in staining buffer and analyzed by flow cytometry using a FACSCalibur instrument (Becton-Dickinson). A positive response is defined as an increase in mean fluorescence intensity that is 5 standard deviations above the mean value for the preimmune serum (assayed in triplicate).

Cellular immunity (mesothelin-specific T cell assay)
All pre- and postimmunization PBMC samples will be analyzed by a mesothelin-specific IFN-γ enzyme-linked immunosorbent spot assay (Immune Monitoring Facility) and by cytotoxic activity (Cell Therapy and Cell Engineering Facility).

Persistence of genetically modified T cells
The quantification of persisting, genetically modified T cells in peripheral blood, and any other cellular specimens will be performed by quantitative PCR (Dr. I. Riviere, Cell Therapy and Cell Engineering Facility).

12.1 Bioeffect summaries
The following measure will be summarized by dose level:

Changes in the biomarker SMRP, assessed at screening and at 60 days (+/-5 days).

13.0 CRITERIA FOR REMOVAL FROM STUDY

Withholding of CAR T-cell administration
If a subject experiences a DLT, as defined in Section 9.0, or if a diagnosis of encephalitis is made, no further study medication will be administered to that subject. Other reasons for the termination of treatment include:

- Initiation of alternative therapy for MPD
- Subject withdraws consent to continue in the trial
- Subject develops an AE or intercurrent illness (regardless of a DLT) that precludes further treatment
- The investigator withdraws the subject, in the subject's best interests
- Subject is lost to follow-up (defined as the inability to contact the subject on 3 separate occasions during a period of 2 months)
- Administrative reasons (e.g., the subject is transferred to hospice care or the subject is unwilling to continue or comply)

Emergency situations

To date, life-threatening events have been rare in a similar MSKCC trial treating patients with CLL ("A Phase I Trial for the Treatment of Purine Analog-Refractory Chronic Lymphocytic Leukemia Using Autologous T Cells Genetically Targeted to the B Cell Specific Antigen CD19," MSKCC IRB protocol 06-138, J. Park, PI). The potential problems could include headache, fever, and chills, which may not be directly relevant to the clinical trial, to cytokine release syndrome which occurs close to infusion while the patient is in-house. Patients will be asked to contact our office in the event of any untoward or unusual symptoms. Patients will be instructed at the time of initiation of the trial that, if they experience any untoward event(s) and are not able to contact our office, they are to proceed to the nearest emergency room for triage and possible treatment. Dr. Adusumilli or his designee will be the contact person should this occur.

14.0 BIOSTATISTICS

14.1 Dose-escalation algorithm

It is anticipated that 3 dose levels will be evaluated in this treatment protocol, as summarized below. Patients will be treated in sequential groups of 3 to 6 patients per T cell dose. The projected trial size for this study is a minimum of 4 and a maximum of 24 patients. The trial will proceed using the dose escalation scheme described in section 9.1.4.

Table 4*

Cohort	Dose **	Number of doses	Total volume of each injection
-1*	1×10^5	1	Final volume of preparation will be 50 mL, to be administered as a bolus.
1	3×10^5	1	
2	3×10^5+Cyclophosphamide	1	
3	1×10^6+Cyclophosphamide	1	
4	3×10^6+Cyclophosphamide	1	

*Necessary only if toxicity is encountered at the initial dose level.
**Mesothelin-targeted T cells/kg; intermediate dose levels may be evaluated, if indicated.

Escalation to the next dose level is probable if the risk of DLT is low and if the likelihood of escalation decreases as the risk of DLT increases, as shown below.

True risk of toxicity	0.10	0.20	0.30	0.40	0.50	0.60
Probability of escalation	0.91	0.71	0.49	0.31	0.17	0.08

14.2 Secondary objectives

The secondary aims of the study (listed as 1 through 5 above) will be addressed by descriptive exploratory statistical analyses, since the sample size is not known in advance and there are no formal hypotheses being tested. These analyses may include descriptions of time patterns for continuous variables measured repeatedly, both at the individual level and aggregated by dose level.

Changes in serum levels of the biomarker SMRP will be assessed at screening and at 60 days (+/-5 days) after treatment.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Approved: 01/21/15

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent by following procedures defined in section entitled Informed Consent Procedures.

During the registration process, individuals undergoing registration will be required to complete a protocol-specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at MSKCC. PPR is available Monday through Friday, from 8:30 a.m. to 5:30 p.m., at (646) 735-8000. The PPR fax numbers are (646) 735-0008 and (646) 735-0003. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

15.2 Randomization

Not applicable.

16.0 DATA MANAGEMENT ISSUES

A research study assistant will be assigned to the study. The responsibilities of the research study assistant include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol study team.

The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

16.1 Data entry

Data collected during this study will be entered into a secure electronic database: the MSKCC Clinical Research Database (CRDB). The CRDB system is compliant with regulations as required by 21 CFR 11. Data entered into the CRDB are stored indefinitely.

16.1.1 Source documents

Study personnel will record clinical data in each patient's source documents (i.e., the patient's medical record). Source documentation will be made available to support the electronic database. Source documentation is stored in the patient's electronic medical record indefinitely. The electronic medical record is the MSKCC gold standard for clinical care. This includes but is not limited to electronic signatures, optical images of patient records, consent forms, and electronic data provided from MSKCC source clinical systems. The compliance requirements for all of the above are stated in "*Part 11, Electronic Records, Electronics Signatures-Scope and Application*."

16.1.2 Record retention

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator will maintain all source documents, study-related documents, and the electronic data.

16.1 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period, and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team at a minimum of 2 times per year, and more frequently if indicated.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) plans at MSKCC were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials," which can be found at http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC DMS plans can be found on the MSKCC intranet at *http://mskweb5.mskcc.org/intranet/_assets/_tables/content/359709/DSMPlans07.pdf.*

There are several different mechanisms by which clinical trials are monitored for data, safety, and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research quality assurance) and departmental procedures for quality control, plus there are 2 institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees—the Data and Safety Monitoring Committee for Phase I and II clinical trials and the Data and Safety Monitoring Board for Phase III clinical trials—report to the center's research council and IRB.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group) will be addressed, and the monitoring procedures will be established at the time of protocol activation.

The mesothelin CAR T-cell infusion-related reactions, adverse events occurred and the management will be reported to the CAR T-cell oversight committee. Any protocol changes or deviations will be immediately reported to the CAR T-cell oversight committee.

Decisions to escalate to the next level or, when appropriate, to deescalate to a lower level will be recommended to the institutional CAR T-cell Oversight Committee by the principal investigator (PI). The CAR T-cell Oversight Committee will make the final decision of whether to proceed to the next dose level. In addition, the PI will coordinate regular meetings/communication with CAR T-cell Oversight Committee to

a. present the data for each cohort of patients and study progress
b. discuss and obtain recommendations prior to escalating to the next dose level or if appropriate deescalating the dose level
c. report any significant toxicities associated with the CAR T-cell intervention.
d. present any proposed significant changes in the protocol.

The CAR T-cell Oversight Committee will not replace the IRB. Any AEs, protocol deviations, etc., still need to be reported to OCR/IRB as well as the CAR T-cell Oversight Committee

17.0 PROTECTION OF HUMAN SUBJECTS

Inclusion of Children in Research: This protocol/project does not include children because the MPD is not seen in children. This statement is based on exclusion 4b of the NIH Policy and Guidelines on the Inclusion of Children as Participants in Research Involving Human Subjects.

Risks and possible toxicities/side effects: Discussed in Section 11.

Potential benefits: There is a potential benefit to society as a whole, in that a successful phase I study could lead to a larger phase II trial which would improve our understanding of the treatment of these diseases and potentially extend survival for patients with MPD. Participation in this study may not provide any benefit to the individual participant, and may or may not provide information which will ultimately benefit others.

Adverse event reporting: Discussed in section 17.2.

Alternative treatment options may include standard chemotherapy off-study, participation in a different clinical trial, or best supportive care/ palliative care.

Financial Costs/Burdens: Patients will be responsible for the costs related to treatment and complications of treatment. Costs to the patient (third party insurer) will include cost of supportive medications and their administration, cost of T cell administration, hospitalizations (including intensive care unit), routine blood and urine tests, diagnostic studies, office visits, EKG, imaging studies, physician services, adult day hospital and outpatient costs. Patients will not be charged the cost of analysis for the research correlates. Costs of generating genetically-modified T cells will not be billable to research participants.

Voluntary Nature of the Study: Participation in this study is voluntary. Whether or not a patient chooses to participate in this study will not affect the availability of standard, supportive, or other investigational treatment at MSKCC.

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the PI and approved by the IRB and Privacy Board.

17.2 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/Privacy Board as soon as possible, but no later than 5 calendar days. The IRB/Privacy Board requires a CRDB SAE report be submitted electronically to the SAE Office at sae@mskcc.org. The report should contain the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the AE occurred
- The AE
- Relationship of the AE to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following:
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication of whether the subject remains in the study
    - If an amendment will need to be made to the protocol and/or consent form

The PI's signature and the date it was signed are required on the completed report.

SAE requiring AP1903 administration will be reported to Bellicum Pharmaceuticals.

For IND/IDE protocols:
The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. The report will be forwarded to the FDA by the SAE Office staff through the IND Office. The report will also be forwarded to the NIH/OBA via the Genetic Modification Clinical Research Information System (GeMCRIS), a web-accessible database sponsored by the NIH and FDA. Confirmation of this report will be forwarded to the SAE Office staff by the PI or co-PIs.

17.2.1 SAE Reporting to the FDA

The report will be forwarded to the FDA by the Institutional SAE Manager through the IND Office no later than 15 days after receipt of the report. For Grade 4 or fatal events, the report will be forwarded to the FDA no later than 7 calendar days.

The report must include the FDA assigned BB-IDE, BB-IND or IND number and name.

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are performed, consenting professionals will explain full details of the protocol and study procedures, as well as the risks involved, to participants before their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB-/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the

Code of Federal Regulations and the IRB/Privacy Board of this center. The consent form will include the following:

1. The nature and objectives, potential risks, and benefits of the intended study
2. The length of study and the likely follow-up required
3. Alternatives to the proposed study (this will include available standard and investigational therapies; in addition, patients will be offered an option of supportive care for therapeutic studies)
4. The name of the investigator(s) responsible for the protocol
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time

Before any protocol-specific procedures can be performed, consenting professionals will fully explain the aspects of patient privacy concerning research-specific information. In addition to signing the IRB informed consent form, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 APPENDICES

APPENDIX I: GUIDELINES FOR THE GENE TRANSFER AND MANUFACTURING

APPENDIX I

GUIDELINES FOR THE GENE TRANSFER AND THE MANUFACTURING PROCESS

Cell Therapy and Cell Engineering Facility

The viral vector production and T lymphocyte transduction involved in this phase I clinical protocol will be performed in the Cell Therapy and Cell Engineering Facility. This facility operates in compliance with NIH and CBER/FDA recommendations and guidelines.

From the point of view of tissue culture, the general conditions of operation are in compliance with "Points to consider in the collection, processing, and testing of *ex-vivo* activated mononuclear leukocytes for administration to humans" (CBER, August 1989). These include standard collection procedures, obligatory bovine serum screening for viral and mycoplasma contamination, and testing of porcine trypsin for porcine parvovirus. Any mitogen, cytokine, growth factor, or chemical used in cell activation and/or culture must be cross-referenced with CBER files. Donors are screened in accordance with the requirements for blood donors (21 CFR 640.3). Use of monoclonal antibodies for cell separation must be performed as described in "Points to consider in the manufacture and testing of monoclonal antibody products for human use" (CBER, 1997).

Approved: 01/21/15

The genetic modification of human cells introduces an additional set of guidelines regarding manufacturing process, quality control, and preclinical testing. The development procedures, preclinical safety, efficacy testing, and manufacturing controls are described in the documents titled "Points to consider in human somatic cell therapy and gene therapy" (CBER, 1998), "Points to consider in the characterization of all cell lines used to produce biologicals" (CBER, 1993), and "Supplemental guidance on testing for RCR in retroviral vector based gene therapy products and during follow-up of patients in clinical trials using retroviral vectors" (FDA guidance, October 2000). The conditions applying to the use of viral vectors to infect target cells are encompassed in the guidelines applying to the generation and characterization of the viral producer cells. The aim of these guidelines is to ensure the generation of a final product that is uniform, consistent, and free of adventitious infectious agents (21 CFR 200, 21 CFR 600, and 21 CFR 610.18). Usage of recombinant retroviral vectors falls within the BL2 category of biohazard safety levels.

Guidance regarding the CTCEF and its personnel is based on:

21 CFR 210: Current Good Manufacturing Practice in Manufacturing, Processing, Packing or Holding of Drugs
21 CFR 211: Current Good Manufacturing Practice for Finished Pharmaceuticals
21 CFR 606: Current Good Manufacturing Practice for Blood and Blood Components
21 CFR 1271 Current Good Tissue Practice for Manufacturers of Human Cellular and Tissue-Based Products

Access to the CTCEF is restricted to authorized personnel. The CTCEF is dedicated solely to the production of clinical-grade vector stocks and to the transduction of patient cells. All the equipment is dedicated to the manufacturing process for the generation of the vector stocks or for the transduction process. Validated decontamination procedures are completed before the manufacture of the clinical material can commence and between processing of individual lots of cells.

The cell culture and testing processes will be continuously supervised by qualified and trained personnel. Protocols for cell culture procedures, formulation techniques, cleaning, and environmental monitoring are in place, including detailed standard operating procedures. Lot records will be maintained, and these will identify all steps in the preparation of each lot of cells, the lot number of every component, and all test results.

A Phase I/II Clinical Trial of Malignant Pleural Disease Treated with Autologous T Cells Genetically Engineered to Target the Cancer-Cell Surface Antigen Mesothelin

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

OneMSK Sites	
Manhattan	Consent and Follow-Up
Basking Ridge	Consent and Follow-Up
Bergen	Consent and Follow-Up
Commack	Consent and Follow-Up
Monmouth	Consent and Follow-Up
Nassau	Consent and Follow-Up
Westchester	Consent and Follow-Up

Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, New York 10065

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Title: A Phase I/II Clinical Trial of Malignant Pleural Disease (MPD) Treated with Autologous T cells Genetically Engineered to Target the Cancer-Cell Surface Antigen Mesothelin

Part I: Phase I Dose-Escalation

Primary Objectives

- To assess the safety, dose requirement, and targeting efficiency of intrapleurally administered genetically directed autologous human T cells targeted to mesothelin—a cell surface cancer antigen widely expressed in mesothelioma, lung cancer, and breast cancer cells—using a chimeric antigen receptor (CAR)
- To determine the recommended phase II dose of mesothelin-targeted CAR T cells in combination with pembrolizumab in patients with malignant pleural mesothelioma

Secondary Objectives

- To assess the biological and antitumor effects of T cell treatment
- To measure serum soluble mesothelin related peptide (SMRP) levels
- To assess T cell targeting, accumulation, persistence, and antitumor immune response, using molecular biology techniques on samples from peripheral blood, pleural fluid and tumor biopsies.
- To assess the capacity for AP1903, a dimerizing agent, to mediate clearance of the genetically engineered cells and resolve toxicity if unacceptable toxicity occurs that is probably or likely related to anti-mesothelin CAR T cells

Part II: Phase II Study of Mesothelin targeted CAR T cells in Combination with Pembrolizumab in patients with mesothelioma

Primary Objectives

- To determine the clinical benefit rate as defined by the proportion of patients with a best response of complete response (CR), partial response (PR), and stable disease (SD) at 12 weeks following the first dose of pembrolizumab as measured by mRECIST criteria.

Secondary Objectives

- To assess the safety of combination CAR T cell and pembrolizumab therapy
- To determine response rate by RECIST 1.1 and iRECIST criteria
- To determine the best response and the duration of best response
- To determine progression-free survival (PFS) and overall survival (OS) of patients at 6, 12, 18, and 24 months
- To explore changes in patient's BMI and serum SMRP

Methodology:

Design: This is an open-label, dose-escalating, nonrandomized, single-center, phase I/II study of mesothelin-targeted T cells administered intrapleurally as aninfusion in patients with a diagnosis (histologically or cytologically documented) of MPD from mesothelioma, lung cancer, or breast cancer. Patients receiving pembrolizumab in cohort 9 only includes patients with a diagnosis of mesothelioma. The total number of patients studied will depend on the number of dose levels tested, up to a maximum dose of $6x10^7$ mesothelin-targeted T cells/kg or until the maximum tolerated dose (MTD) is reached. In Phase I of this study, we anticipate infusing a minimum of 4 and a maximum of

54 evaluable patients. The total number of enrolled patients in Phase II of this study depends on the number of observed responses and ranges from 13-21 evaluable patients, including 6 patients treated at the final dose in Phase I.

Following establishment of safety in initial cohorts, patients in cohort 9 with malignant pleural mesothelioma (MPM) will proceed to receive pembrolizumab 4 weeks following CAR T cell infusion, provided that patients do not have adverse events greater than grade 3 related to the CAR T cell infusion. Checkpoint blockade agents, pembrolizumab and nivolumab, are included in the National Comprehensive Cancer Network (NCCN) guidelines as second line therapy for MPM.[102] Patients will continue to be monitored for CAR T cell related toxicities.

Population: The study population will comprise patients who have been diagnosed with MPD from mesothelioma, lung cancer, or breast cancer. We anticipate that 2 different cohorts of patients with MPD will be eligible for this trial: (a) patients with mesothelioma with a free or partially free pleural space and (b) patients with MPD from lung or breast cancer that failed standard chemotherapy management (patients who received at least one chemotherapeutic regimen and are documented to have progressing tumor) with a free or partially free pleural space, deemed to be clinically stable. Patients in cohort 9 and in the Phase II part of the study will only include patients diagnosed with MPM.

Safety evaluations: The safety of iCasp9M28z+ T cells will be assessed by evaluation of the type, frequency, and severity of adverse events (AEs); changes in clinical laboratory test findings (hematologic and chemistry); and physical examination. All AEs and laboratory toxicities will be graded using version 4 of the CTCAE.

A complete blood count (CBC) and SMA-12 blood chemistries will be tested at baseline, daily during the first 2 days after treatment, and as clinically indicated at the post treatment protocol follow up visits.

Routine urinalysis will be performed at baseline, and as clinically indicated or at PI discretion at all time points after treatment.

Serum troponin will be tested at baseline and daily during the first 2 days following CAR T cell treatment. Additional troponin tests will be ordered as clinically indicated.

C-reactive protein titers will be performed at baseline and as clinically indicated or at PI discretion following CAR T cell administration.

Vital signs (i.e., blood pressure, temperature, and pulse rate) will be charted approximately 15 minutes (+/10 minutes) prior to CAR T infusion, 15 minutes during infusion, 30, 60 minutes (+/10 minutes) after treatment is completed. Following this, vital signs will be assessed as clinically indicated.

An electrocardiogram (ECG) will be performed at baseline, within 4 hours after CAR T cell infusion, daily during the first 2 days following CAR T cell infusion. Additional ECGs will be ordered as clinically indicated.

An echocardiogram will be performed at baseline (prior to leukapheresis). Additional echocardiograms will be ordered as clinically needed.

As an added safety measure, the vector includes a suicide switch comprising a caspase dimerization domain (ICD9) that can be activated by a small molecule and induce death of the genetically engineered cells if they were to induce untoward toxicity. The following medication will be

available for use in case of a severe toxicity related to this agent: AP1903, as per the pharmacy guidelines of the manufacturer.

The safety measures that are standard of care for pembrolizumab in MPM patients will be followed.

Assessment of tumor T cell infiltration: Patients can undergo video-assisted thoracic surgery (VATS) or open thoracotomy when clinically indicated (e.g., to obtain pleural biopsies or to perform pleurodesis or surgical resection). If the patient goes to the operating room, pleural biopsies will be performed to assess tumor T cell infiltration—specifically iCasp9M28z T cell infiltration—in tumor and surrounding tissues. Flow cytometric analysis of fresh tissue will be performed to assess the phenotype of the infiltrating iCasp9M28z T cells. Possible clinical indications for a surgical procedure at the time of pleural biopsy are the following: (a) drainage of unresolved/loculated pleural effusion, with or without pleurodesis; (b) surgical resection of pleural disease by pleurectomy; and (c) exploration for intended surgical resection (aborted because of unresectability).

Immunophenotyping and cytokine analysis (immune response): Presence of peripheral blood iCasp9M28z T cells, along with analysis of lymphocyte subsets (e.g., CD4 and CD8) and cytokine profile (e.g., serum IFN-γ and TNF-α), will be performed on blood at baseline and at least once during hospital admission. Additional blood specimens will be obtained following these time points as indicated by PI.

Number of patients: A precise sample size cannot be defined, as it is dependent on the observed toxicity. Cohorts of 3 patients will be treated at each dose level, up to a maximum of 6×10^7 iCasp9M28z T cells/kg or until the MTD has been reached. In Phase I of this study, we anticipate infusing a minimum of 4 and a maximum of 53 evaluable patients. The total number of enrolled patients in Phase II of this study depends on the number of observed responses and ranges from 13-21 evaluable patients, including 6 patients treated in the final dose in Phase I.

Study design: This study will be conducted under the umbrella of the Cellular Therapeutics arm of the MSKCC Center for Cell Engineering. Patients with malignant pleural effusion and/or MPD will undergo insertion of an intrapleural catheter by either the Thoracic Surgery service or the Interventional Radiology (IR)/Pulmonary service, as clinically indicated. The possible clinical indications for insertion of a pleural catheter are (a) patients with symptomatic pleural effusion; (b) patients with multiloculated pleural effusion; or (c) patients undergoing pleural biopsy by either VATS or thoracotomy, with a history of recurrent pleural effusion. All patients with a functional pleural catheter are eligible for the study, as long as there are no clinical concerns of infection. In patients with documented pleural disease, but no significant effusion present for insertion of a pleural catheter, CAR T cells will be administered by intervention radiology-guided intrapleural injection. The patients' radiographic images will be evaluated by an Interventional Radiologist and the feasibility of injecting the CAR T cells directly into the chest cavity will be assessed. After obtaining a screening consent, patients' tumor, pleural fluid and/or blood will be tested for presence of tumor marker – mesothelin. Note: Patients with epithelioid or biphasic mesothelioma are not required to sign the Screening Informed Consent as these patients' tumors express mesothelin[98]. In patients with mesothelin-positive tumors, a leukapheresis for harvesting of PBMCs will be performed. When the patient is ready for treatment, informed consent for the treatment phase of the study will be obtained. As the transduced T cells will be frozen, the timing of leukapheresis is not defined and can vary from patient to patient. For patients with pleural effusion, all obtainable pleural fluid will be drained from the chest by thoracentesis or through a pleural catheter and will be preserved for analysis (i.e. cytokines, tumor cells, and mesothelin testing).

In Cohorts 2 to 9, patients will be hydrated intravenously, premedicated with acetaminophen and diphenhydramine, and administered cyclophosphamide at 1.5 g/m^2 2- 7 days (Day (-7) – (-2)) before

administration of mesothelin-targeted T cells. Subsequently, a single dose of mesothelin-targeted T cells will be instilled via the catheter or interventional radiology guidance into the pleural space. If instilled via a pleural catheter, following treatment with CAR T cells, the catheter will be flushed and capped for at least 8 to 12 hours, to maximize T cell delivery to the intrapleural tumor.

If instilled via interventional radiology, MSK interventional radiologists have developed techniques to safely perform image-guided biopsy of lesions close to the mediastinum or crucial vascular structures[101]. These methods of creating an artificial pneumothorax during lung ablation develop a working space in the thorax sufficient to displace the target lung lesion from adjacent vulnerable mediastinal or chest wall structures, permitting development of safe "windows" for ablation. These pneumothoraces, induced by the introduction of a needle with a spring-loaded, blunt-tipped obturator into the pleural space, followed by injection of room air, will be used for this study. All eligible patients will be reviewed by the PI and an IR physician together to assess for a) presence of a small loculation, which is a common finding in patients with pleural disease, and b) feasibility and safety of administering the required amount of fluid containing CAR T cells into the loculation. In the absence of such a loculation, both the PI and the IR physician will review patients' clinical and treatment history and review the latest imaging (CT or PET/CT) to determine if a small loculated pneumothorax can be created safely to administer the reagent containing CAR T cells. The IR physician's decision will be final for these patients.

Patients will be monitored in the hospital and ideally discharged home after 72 hours. If the patient has a pleural catheter at discharge, the pleural catheter will be capped. Patients' pleural fluid can be drained at any time as clinically indicated. Patients will be monitored closely as outpatients for the next 2 months. Patients will be followed weekly for the first 4 weeks after the treatment and patients with pleural catheters will have them removed as clinically indicated (i.e. when it is no longer needed for the clinical management of malignant pleural effusion).

For patients who have obtained clinical benefit from the initial T cell therapy and did not experience any non-hematologic grade 4 toxicities, additional modified T cells may be re infused later at the treating physicians' discretion. For patients who were treated and were removed from study, duplicate enrollment is permitted if it is determined the patients could receive benefit. If the patients meet all eligibility criteria, they may receive treatment in a higher dose level cohort. Patients who are re-treated with CAR T cell therapy will not be considered new accruals. Outcomes of re-treated patients will be analyzed separately.

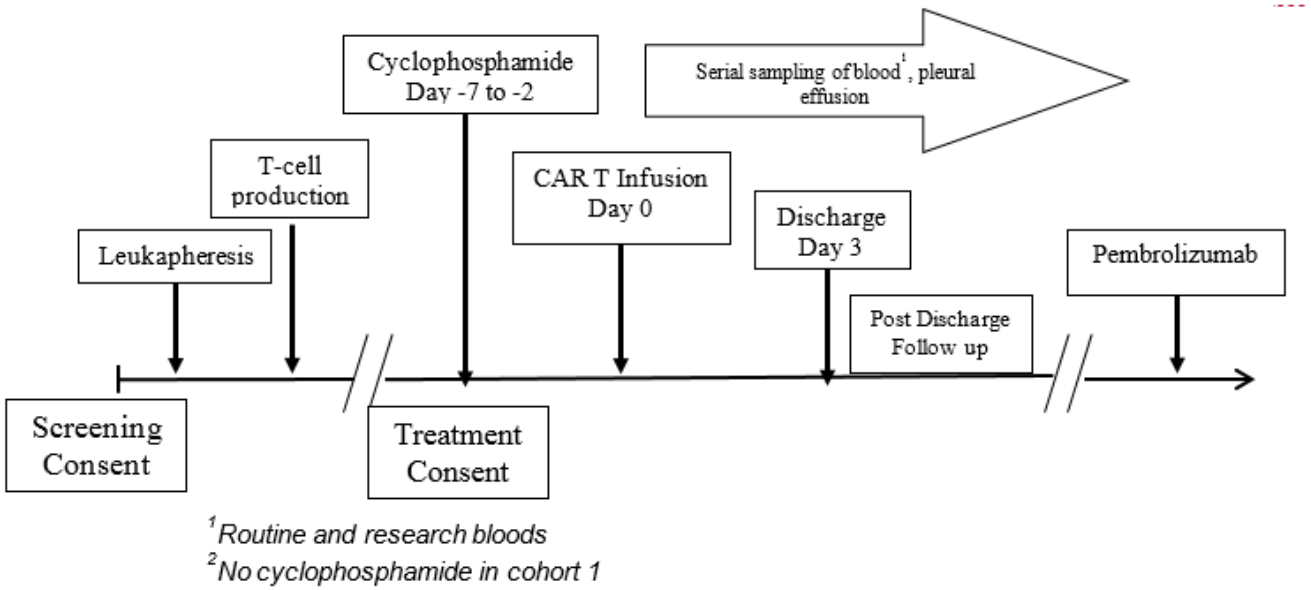
Patients may receive palliative radiotherapy for symptom management prior to or following the CAR T cell infusion. If a patient receives palliative radiotherapy, the study PI, treating Radiation Oncologist, and treating Medical Oncologist will decide whether to proceed with the infusion. Palliative radiotherapy must be completed at least 2 days prior to the administration of cyclophosphamide.

Patients in cohort 9 and in the Phase II portion of the study will begin treatment with pembrolizumab 4 weeks (+3/-1 week window) after completing CAR T cell administration. Patients will receive 3 doses of pembrolizumab given on a recurring schedule followed by reassessmentPatients responding or deriving clinical benefit, without unacceptable toxicity, will be followed for up to 6 months after the first dose of pembrolizumab and may continue pembrolizumab. Patients who cannot receive all 6 doses of pembrolizumab at MSK will be followed and medical records including images will be obtained from the treating physician.

Treatment plan: Patients will be admitted under the BMT, CTC, or Leukemia Inpatient Service at MSKCC for T cell infusion, observation, and laboratory blood tests, including immune and molecular

monitoring. The first patient in each new cohort will have an ICU consult prior to treatment with the modified T cells. Patients receiving the treatment via interventional radiology will be treated in the IR suites and admitted back to the BMT, CTC, or Leukemia service following administration of cells. The patients will remain inpatients for a minimum of 2 nights following T cell infusion, unless undue toxicity or unexpected side effects occur; maximum expected inpatient stay is 3 nights. Patients will receive the CAR T cell infusion at MSKCC. A physician will be available during the time of the infusion. The patient plan is summarized below.

Patient Schema:



[1] *Routine and research bloods*
[2] *No cyclophosphamide in cohort 1*

In cases of study procedures occurring on weekends/holidays or in cases of other logistical issues, an allowance of +/-3 days is considered to be included for all time points stated in this protocol, if not stated otherwise. These cases will not be considered a protocol violation. A medical procedure or test conducted before signed consent is obtained that is the standard of care or that would have been conducted regardless of whether the patient considered participation in this clinical trial may be used in place of a screening procedure (e.g., CT scan). If a biospecimen sample (e.g. pleural fluid, peripheral blood) necessary for any research assay is unable to be obtained, this will not constitute a protocol violation. Samples will be attempted to be obtained within a +/- 3 day window.

CAR T cell Oversight Committee: Decisions to escalate to the next level or, when appropriate, to deescalate to a lower level will be recommended to the institutional CAR T cell Oversight Committee by the principal investigator (PI). The CAR T cell Oversight Committee will make the final decision of whether to proceed to the next dose level. In addition, the PI will coordinate regular meetings/communication with CAR T cell Oversight Committee to

a. present the data for each cohort of patients and study progress
b. discuss and obtain recommendations prior to escalating to the next dose level or if appropriate, deescalating the dose level
c. report any significant toxicities associated with the CAR T cell intervention.
d. present any proposed significant changes in the protocol.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Part I: Phase I Dose-Escalation

Primary Objectives

- To assess the safety, dose requirement, and targeting efficiency of intrapleurally administered genetically directed autologous human T cells targeted to mesothelin—a cell surface cancer antigen widely expressed in mesothelioma, lung cancer, and breast cancer cells—using a chimeric antigen receptor (CAR)
- To determine the recommended phase II dose of mesothelin-targeted CAR T cells in combination with pembrolizumab in patients with malignant pleural mesothelioma

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- To assess the biological and antitumor effects of T cell treatment
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- To assess the capacity for AP1903, a dimerizing agent, to mediate clearance of the genetically engineered cells and resolve toxicity if unacceptable toxicity occurs that is probably or likely related to anti-mesothelin CAR T cells

Part II: Phase II Study of Mesothelin targeted CAR T cells in Combination with Pembrolizumab in patients with mesothelioma

Primary Objectives

- To determine the clinical benefit rate as defined by the proportion of patients with a best response of complete response (CR), partial response (PR), and stable disease (SD) at 12 weeks following the first dose of pembrolizumab as measured by mRECIST criteria.

Secondary Objectives

- To assess the safety of combination CAR T cell and pembrolizumab therapy
- To determine response rate by RECIST 1.1 and iRECIST criteria
- To determine the best response and the duration of best response
- To determine progression-free survival (PFS) and overall survival (OS) of patients at 6, 12, 18, and 24 months
- To explore changes in patient's BMI and serum SMRP

3.0 BACKGROUND AND RATIONALE

MPD: The pleural cavity is a common site for cancer, both primary (mesothelioma) and metastatic (lung, breast, and other solid cancers).[1] The incidence of MPD is estimated to be >150,000 patients/year in the U.S. alone.[2] Pleural mesothelioma is a regionally aggressive primary malignancy of the pleura with a median survival of 9 to 17 months, even after aggressive combined-modality therapy.[3] At least 25% of patients with lung adenocarcinoma (LAC), a common form of lung cancer, develop MPD during the course of their illness.[1,4] The pleura is the most common site of metastatic breast cancer recurrence[5] and the site of the first and only manifestation of recurrence in 40% of patients.[6]

Mesothelioma: Mesothelioma is expected to cause >450,000 deaths worldwide during the next 20 years.[7,8] U.S. Army personnel exposed to asbestos in construction projects, ships, brakes, clutches, and military gas masks before the 1970s are at risk for developing mesothelioma.[8,9] With combined chemoradiotherapy and surgical resection, survival is prolonged to 9 to 12 months (compared with no treatment), with a higher rate of pleural and mediastinal lymph node recurrence.[10,11] In the largest

series to date (945 patients), which was reported by our group, even among patients carefully screened and selected for resection, one-third were unresectable because of locally advanced tumor at the time of operation.[3] Only 8% of patients had distant metastases.[3] *Its localized nature, potential accessibility, and relative lack of metastases make mesothelioma a suitable candidate for regional targeted therapies.* As available treatment regimens have poor outcomes, patients with untreated mesothelioma can be considered eligible for this protocol.

Tumor microenvironment and its effect on immunotherapy: Patients with mesothelioma with high levels of CD8+ tumor-infiltrating lymphocytes (TILs) have better survival than those with low levels of TILs (3-year survival: 83% vs 28%).[13,14] High levels of CD8+ TILs also correlated with higher levels of TUNEL-positive apoptotic tumor cells.[14] High levels of CD8+ TILs remained an independent prognostic factor for delayed recurrence and better survival[14] in multivariate analysis. We have convincing data (unpublished) that increased infiltration of the tumor by T lymphocytes conveys a survival advantage. Interestingly, a significant correlation was demonstrated between levels of CD8+ TILs and induction chemotherapy with cisplatin/pemetrexed,[14] the only regimen shown to have a significant survival benefit in randomized clinical trials of mesothelioma.[12,13] *Thus, the rationale exists for pursuing combination chemoimmunostimulatory strategies that will increase the number and efficacy of TILs in the treatment of mesothelioma.* Published immunotherapy studies have shown that IFN-γ, IL-2, IL-12, and GM-CSF have a direct cytotoxic effect on mesothelioma cells, which further strengthens our immune intervention approach.[14-1922] Our group has reviewed all of the published studies on intrapleural immunotherapies for mesothelioma, which demonstrate efficacy without occurrence of a limiting toxicity.[23]

Publications from our laboratory and those of others have documented the prognostic role of the tumor immune microenvironment in lung[20,21] and breast[22-25] cancers. Specifically, tumor-targeted cytotoxic immune cell responses have been shown to be beneficial.

3.1 Immunologic Approaches

3.1.1. Adoptive T cell therapy with engineered T lymphocytes

Cell engineering can be used to redirect T cells toward tumor antigens, to enhance T cell function, and to potentially resolve many previously observed shortcomings afflicting adoptively transferred cytotoxic T lymphocytes (CTLs).[26] An impetus for performing genetic T cell modification is the prospect of enhancing T cell survival and expansion, generating memory lymphocytes, and offsetting T cell death, anergy, and immune suppression.

CARs: Tumor-specific T cells can be generated by transferring genes that encode CARs.[26-31] CARs consist of a tumor antigen–binding domain that is fused to an intracellular signaling domain capable of activating T cells. The design of CARs must therefore reconcile antigen recognition with signal transduction, two functions that are physiologically borne by two separate complexes, the T cell receptor (TCR) heterodimer and the CD3 complex. The CAR's extracellular binding domain is usually derived from a murine or human monoclonal antibody or from receptors or their ligands. *Antigen recognition is therefore not MHC-restricted,[32,33] as is the case for physiologic TCR-mediated antigen recognition.* Ligand binding by the chimeric receptor triggers phosphorylation of immunoreceptor tyrosine-based activation motifs in the cytoplasmic region; this activates a signaling cascade that is required for cytolysis induction, cytokine secretion, and proliferation. Since the requirements for MHC restriction in the interaction of effector cells with target cells are bypassed, the binding of tumor cells to CTLs grafted with CARs is *not affected by human leukocyte antigen (HLA) downregulation or by defects in the antigen-presentation machinery*.

Requirements of T cells for expansion and survival: To proliferate in response to antigen, T cells must receive two signals. One is provided by TCR recognition of antigenic peptide/MHC complexes displayed on the surface of antigen-presenting cells (APCs)[30]; the other is provided by a T cell costimulatory receptor, such as the CD28 molecule. Whereas the cytolytic activity of T cells does not require concomitant costimulation, recent studies, including some of our own, have established that the provision of costimulatory signals is critical for the antitumor activity of adoptively transferred T cells.[29,34-36] *A first step toward providing costimulation to T cells that recognize CD28-negative tumor cells was achieved by creating CD28-derived CAR (Adusumilli et al. Science Translational Medicine* 2014 Nov 5;6(261):261ra151)

3.1.2. Our approach to the treatment of MPD

The primary goal of this research proposal is to promote T cell infiltration of the tumor by administering genetically engineered human primary T lymphocytes that have been redirected to target the MPD tumor antigen mesothelin, and to further enhance the efficacy of such targeted T cells by regional delivery. *In recent years, there have been several published reports of long-term remission of metastatic melanoma,[37-39] a solid tumor, after treatment with adoptive cells. These findings provide strong support to investigate this approach for other regionally accessible solid tumors, such as MPD.* The occurrence of some remarkable, albeit infrequent, complete responses in patients with metastatic disease underscores the potential of targeted T cells, as well as the need to increase the potency of these therapies through improvements in host conditioning and CAR design.

Our mesothelin CAR is derived from a human V_L and V_H cDNA[44] obviating the risk of human antimouse antibody immunogenicity, which is the case for CARs derived from mouse humanized antibodies. Although T cells may access disease sites, their survival, function, and persistence are influenced by the tumor microenvironment. Thus, an essential aspect of our approach is to not merely generate tumor-targeted T cells for adoptive therapy, but to enhance T cell function through the design of improved antigen receptors and through intervention in the host microenvironment by intrapleural delivery.

A phase I clinical trial of intrapleural delivery of vaccinia virus demonstrated the feasibility of intrapleural therapies delivered via pleural catheters. Furthermore, tissue biopsy specimens and pleural effusion obtained from the pleural cavity will provide us with the scientific rationale to study T cell persistence and phenotypic changes and will advance our ability to understand and improve T cell therapy to benefit patients with MPD.[40]

From the combined experience of protocols IRB# 12-169 and IRB# 15-007, the majority of patients screened for eligibility (50-60%) have a fused pleural space. Patients with pleural disease and effusion are most commonly drained and undergo pleurodesis by primary/pulmonary physicians for immediate symptomatic relief. This procedure results in no effusion in the pleural cavity by the time they are considered for the trials. With this rationale, the MSK mesothelioma working group discussed with the Interventional Radiology (IR) group to facilitate direct administration into the pleural space adjacent to the tumor. This procedure is already in clinical practice by the MSK IR.

To date, 43 patients have been treated with our iCasp9M28z-transduced T cells with this protocol and IRB #16-040. No adverse events or reactions were noted in these patients following infusion; thus, patients will be allowed to be treated on consecutive days. Patients will continued to be monitored. Patient staggering of 4 weeks will be followed between cohorts.

3.2 Mesothelin

3.2.1. Rationale for targeting mesothelin

Mesothelin is an immunogenic cell surface antigen[41,42] that is expressed at high levels in MPD and mesothelioma.[42-47] In normal tissues, it is expressed only in the pleura, pericardium, and peritoneum, at low levels.[42,48] Mesothelin is involved in cell proliferation,[53] adhesion,[49,50] cell signaling,[54] and metastases.[56] It has uniform and strong expression in 80% of patients with mesothelioma.[42,46,47,51,52] Serum SMRP secreted by mesothelin-expressing MPD tumors can be measured both in humans[46,47,53-58] and in mice and has been shown to correlate with therapy response and prognosis. Our laboratory has shown—both in preclinical mouse models and in patients—that overexpression of mesothelin in mesothelioma cells promotes invasion and is associated with secretion of MMP-9. In patients with LAC, overexpression of mesothelin is associated with reduced recurrence-free and overall survival and is an independent factor of poor prognosis. In patients with triple-negative breast cancer, overexpression of mesothelin is associated with reduced disease-free and overall survival, as well as increased incidence of distant metastases.

Our laboratory investigation of primary lung and breast cancer tumors revealed that 60% of patients with LAC and 16% of patients with breast cancer (including 36% of patients with triple-negative breast cancer) have tumors that overexpress mesothelin.[99, 100]

Antimesothelin recombinant immunotoxin SS1P and MorAb-009 have shown *in vivo* specificity and significant antitumor activity,[42,59,60] with no off-target toxicities noted. In a trial of pancreatic cancer vaccine, patients with a survival advantage had strong and consistent CD8+ T cell responses to mesothelin epitopes, which was associated with a vaccine-induced delayed-type hypersensitivity response.[61] Specific T cell epitopes derived from mesothelin have been shown to activate human T cells to efficiently lyse human tumors expressing mesothelin.[62] *Thus, there is strong evidence that adoptive immunotherapy with a mesothelin receptor will be tumor specific.*

3.2.2. Mesothelin CAR T cells

CAR-mediated mesothelin antigen recognition offers distinct advantages—for instance, receptor specificity is easily generated, as the *human Fab* we are using as part of the mesothelin CAR is highly specific and has high affinity. As these receptors can transduce both CD4+ and CD8+ T cells (*Adusumilli et al. Science Translational Medicine, Nov 5;6(261)ra151)*, transduction of a patient's T cells with CARs could generate helper and CTL responses, possibly resulting in a sustained antitumor response. CAR-modified CD4+ T cells exhibit direct cytolytic activity against tumor cells, indicating that genetically targeted CD4+ T cells serve as both helper cells and effector cells. Finally, soluble tumor antigens (CEA, HER-2, and Lewis Y) that are known to decrease the efficacy of monoclonal antibody therapies have been shown to have no effect on CAR T cells.[63-65] Given the toxicities associated with chemotherapy, alternative therapies are viewed favorably. Several recent clinical trials have suggested that immune-based therapies with vaccines, either alone or in combination with cytokines, might provide a more targeted approach for treating patients with MPD, with fewer resulting AEs.

3.2.3 Targeted elimination of MPD by genetically directed human lymphocytes

The genetic engineering of T cells is a novel strategy designed to accelerate the generation of tumor-specific T cells and remedy the biological limitations that constrain the antitumor functions of normal T cells.[26-28] Unlike the physiologic TCR, CARs encompass immunoglobulin-variable regions or receptor ligands as antigen-recognition elements, thus permitting T cells to recognize cell-surface tumor antigens in the absence of HLA expression.[30] T cell activation is mediated by the cytoplasmic domain of the CAR, which is typically derived from the CD3ζ chain or the FcRIγ chain (Figure 1). Our group has shown that ζ chain–based CARs can induce strong activation capable of sustaining T cell proliferation and permitting secondary antigenic restimulation *in vitro*, provided that antigen is presented in the context of CD28-mediated costimulation.[26,27,29,31,34-36] In an effort to determine whether human T cells expand in this manner, whether they can mediate tumor eradication *in vivo*,

and whether further *in vivo* costimulation is needed to sustain their function, tumor models using SCID-bg/bg and NOD-SCID mice were developed. These models showed that mesothelin-targeted T cells can effectively eliminate MPD. Our orthotopic mouse model of mesothelioma was extensively characterized to resemble human mesothelioma. T cells were transduced with iCasp9M28z, a CAR that targets human mesothelin. The M28z receptor encompasses the ζ chain of the CD3 complex as its activation domain and specifically redirects *in vitro* cytolysis against mesothelin-positive tumor cells. The tumor models used included orthotopic and subcutaneous MPD. Tumor eradication was directly proportional to the *in vivo* effector-to-tumor cell ratio and mesothelin expression. The administration of iCasp9M28z-transduced T cells induced objective responses in all mice and cured a substantial portion of them (*Adusumilli et al. Science Translational Medicine, Nov 5;6(261)ra151*). The data strongly support the feasibility of targeting MPD with autologous T lymphocytes directed against mesothelin by a transduced ζ chain–based receptor. MSKCC investigators have previously shown that similar technology and approaches can be used to treat other diseases, including hematologic malignancies ("A Phase I Trial for the Treatment of Purine Analog-Refractory Chronic Lymphocytic Leukemia Using Autologous T Cells Genetically Targeted to the B Cell Specific Antigen CD19," MSKCC IRB protocol 06-138, J. Park, PI; "A phase I trial of Precursor B Cell Acute Lymphoblastic Leukemia (B-ALL) Treated with Autologous T cells Genetically Targeted to the B Cell Specific Antigen CD19", MSKCC IRB protocol 09-114, J. Park, PI).

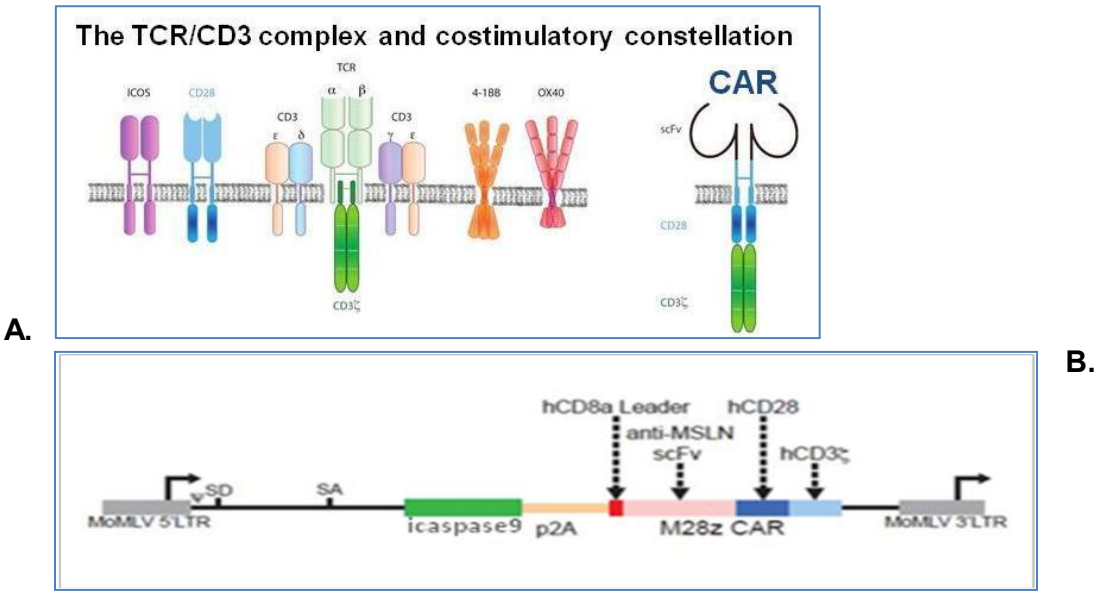


Figure 1. Schematic representation of (A) the physiological TCR and the scFV-ζ chain chimeric antigen receptor (CAR) structure, and (B) iCasM28z CAR

3.2.4 Rationale for using genetically redirected, adoptively transferred T cells

The advent of effective methods for gene transfer in T cells has provided a new means for creating tumor-specific T cells. In principle, genetic reprogramming could be used to improve T cell survival, augment T cell expansion, generate memory lymphocytes, and offset T cell death, anergy, and immune suppression. Such genetic alterations are distinct from redirecting antigen specificity and may eventually prove to be critical for sustaining immunity to the tumor. CARs that comprise both CD28 and CD3 cytoplasmic domains have been shown, by our group[26-28,31,66,67] and others,[68-70] to better support T cell stimulation by target cells that present antigen in the absence of activating costimulatory ligands. The M28z receptor has thus been selected for clinical investigation. Because of the potential risk of initiating any immune response against normal tissues that express very low

levels of mesothelin, the ICaspase-9 gene will be cotransferred with the cDNA encoding the M28z receptor, by use of the M28z gamma-retroviral vector. Constitutive expression of ICaspase-9 has been extensively investigated by our group and others and has been shown to render T cells sensitive to AP1903, providing a means to eliminate T cells if required. Furthermore, expression of ICaspase-9 enables elimination of >90% of T cells, as demonstrated by our group's preclinical studies and by other groups' clinical studies.[77,97] Intravenously administered AP1903 eliminated intrapleural intratumoral iCasp9M28z T cells within 1 hour.

3.2.5 Rationale for combining T cell therapy with cyclophosphamide

The rationale for combining genetically modified T cell therapy with cyclophosphamide chemotherapy in Cohorts 2 to 8 is two-fold. First, lymphodepleting chemotherapy may enhance the ability of adoptively transferred tumor-specific T cells to proliferate *in vivo* through homeostatic proliferation. Second, pretreatment with cyclophosphamide may transiently reduce the numbers of patient CD4+CD25+ regulatory T cells, which would otherwise suppress the function of adoptively transferred tumor-specific T cells. Ongoing clinical trials of adoptive T cell therapy have established the benefit of cyclophosphamide as a preparatory lymphodepleting agent. The infused T cells comprise both CD4+ and CD8+ T cells, both of which will be targeted to mesothelin. In studies in patients with melanoma, both subsets have been shown to possess antitumor activity. The T cell doses proposed for this study are within the range of T cell doses administered in other comparable studies. In this study, 1 dose of cyclophosphamide will be administered in Cohorts 2 to 8 (as a lymphodepleting agent) before infusion of T cells.

3.2.6 Rationale for combing T cell therapy with Pembrolizumab

Mesothelin (MSLN)-targeted CAR T cells can eradicate large tumor burdens in preclinical mouse models when administered either at a higher dose or regionally (By contrast, following administration of a single low dose of MSLN-targeted CAR T cells (low doses mimic "stress" that CAR T cells face within solid tumors with high antigen loads as seen in our phase I clinical trial), tumor cells upregulate PD-L1 in response to T-cell-secreted cytokines and CAR T cells upregulate PD-1. Upregulated PD-L1 on tumor cells binds to and inhibits T cells. To overcome such tumor-mediated adaptive resistance following CAR T-cell administration, we have shown that treatment with anti-PD-1 agents rescues exhausted CAR T cells. Mice treated with a single dose of MSLN CAR T cells and subsequent anti-PD-1 demonstrated enhanced antitumor efficacy and functional persistence of CAR T cells (Cherkassky *Journal of Clinical Investigation* 2016). Pembrolizumab is approved second line therapy for MPM patients in NCCN guidelines.[102]

Furthermore, we have noted similar observations in this clinical trial patients that received CPB agents several weeks after CAR T-cell administration (following documentation of no unexpected and definitely related, or expected and >Grade 3 adverse events as well as no dramatic therapeutic response at the doses administered). Detection of CAR T cells in peripheral blood (by PCR for vector copy numbers) associated with decrease in tumor burden as demonstrated by decrease in serum SMRP and / or complete or partial metabolic responses in patients who received CPB agents after CAR T-cell therapy provides provoking supportive data to investigate the combination therapy.

3.3 Anti-PD-1 Agents

After successful completion of the Intervention Phase, patients will then undergo pembrolizumab starting 4 weeks after intrapleural administration of CAR T cells. Given the limited efficacy of cytotoxic chemotherapy in malignant mesothelioma, anti-PD-1 agent has been an active arena of research. Many agents have demonstrated safety and modest to substantial efficacy. Based on these data, in July 2017, pembrolizumab and nivolumab with or without ipilimumab were added to the NCCN Guidelines for Malignant Pleural Mesothelioma for use in the 2nd line setting or beyond.[102]Additional studies to further characterize the optimal use of these agents are ongoing.

Pembrolizumab: Despite the disappointing results with DETERMINE, data with other agents is promising. Pembrolizumab, a humanized monoclonal antibody targeting PD-1 was assessed in KEYNOTE-028, a multi-cohort, non-randomized phase Ib basket trial (Alley *Lancet Oncol* 2017). In this study, 25 patients with PD-L1 positive advanced pleural mesothelioma received treatment with pembrolizumab (10 mg/kg IV q21 days). While 64% of patients reported a treatment-related adverse event, only 20% reported grade 3 treatment-related adverse events. Three patients required dose interruption because of immune-related adverse events: 1 patient with grade 3 rhabdomyolysis, 1 patient with grade 3 iridocyclitis, and grade 2 infusion-related reaction. There were no treatment related deaths or discontinuations. The objective response rate was 20% (95% CI 6.8-40.7) with five patients achieving a partial response and 52% had stable disease. The median duration of response was 12 months (95% CI 3.7-not reached).

Subsequently, preliminary results from a phase II trial of pembrolizumab (200 mg IV q21 days) in patients with previously treated malignant mesothelioma were presented (Kindler *JCO WCLC 2016 abstract*). In particular, this study was structured to facilitate identifying a PD-L1 expression threshold for patient selection. Among 35 patients, a PD-L1 threshold could not be established. The response rate was similar to the Alley study at 21% and a disease control rate of 76%. The second phase of this trial is ongoing.

Nivolumab: Nivolumab is a fully human IgG4 monoclonal antibody targeted against the PD-1 receptor on activated T and B lymphocytes. Nivolumab has shown single-agent activity in a variety of solid tumors. The pharmacokinetics, clinical activity and safety of nivolumab have been assessed in completed phase I and ongoing phase 2 or 3 studies in subjects with lung cancer, melanoma and renal cell cancer and other solid tumors.

A small phase II trial is assessing the disease control rate at 12 weeks with nivolumab (NIVOMES Josine Quispel-Janssen *iMig 2016*), a human monoclonal antibody targeting PD-1. Preliminary data were presented at the 2016 International Mesothelioma Interest Group Meeting showing that 50% of 18 patients achieved disease control at 12 weeks. Of these patients, 5 achieved a partial response. Interestingly, two patients had pseudo-progression prior to a partial response.

In a multicenter randomized but non-comparative phase 2 trial in France, 125 patients with malignant pleural mesothelioma were assigned to receive either nivolumab or nivolumab plus ipilimumab (Scherpereel Lancet Oncol 2019). The 12-week disease control rate was 40% and 52% for nivolumab and nivolumab plus ipilimumab, respectively. Fourteen percent and 26% of patients experienced grade 3-4 toxicities in the nivolumab and nivolumab plus ipilimumab arms, respectively. The most frequent grade 3 adverse events were asthenia, asymptomatic increase in aspartate aminotransferase or alanine aminotransferase, and asymptomatic lipase increase.

MSK Experience with anti-PD-1 agents: Prior to the addition of pembrolizumab as well as nivolumab with or without ipilimumab to the NCCN guidelines for MPM in July 2017, patients who were ineligible for clinical trials with these agents were able to receive therapy through compassionate use mechanisms. Additionally, since the change in the NCCN guidelines, many patients receive these agents as standard therapy for recurrent and/or progressive disease. As a result, the MSK Mesothelioma Program has substantial experience administering these agents and has treated more than 50 patients off-study with these agents. Furthermore, the MSK Mesothelioma Program currently has two open clinical trials using CPB agents and a third study pending final IND approval. Finally, MSK and the Thoracic Disease Management Team have pioneered the field of immuno-oncology and have tremendous experience administering these drugs and managing immune-related adverse events as well as helping to define immune-related toxicity algorithms and immune response criteria

3.4 Rationale for a Phase II Design

Our preclinical studies show that following administration of mesothelin-targeted CAR T cells in orthotopic pleural mesothelioma mice, CAR T cells infiltrate the tumor and are functionally exhausted after initial cytolytic activity. This is due to repeated antigen activation induced upregulation of PD-1 in CAR T cells with simultaneous overexpression of PD-L1 and PD-L2 on the cancer cell surface in response to effector cytokine secretion. We have demonstrated that the administration of anti-PD1 agents rescued functionally exhausted CAR T cells and that the resulting reactivation induced additional cancer cell cytolysis. Based on this rationale, following confirmation of safety, the anti-PD1 agent, pembrolizumab, was administered in patients who tolerated the initial intrapleural administration of mesothelin-targeted CAR T cells. Anti-tumor activity along with evidence of neoantigen responses as well as epitope spreading were observed. Based on these combined preclinical and clinical observations, conducting a phase II study with intrapleurally administered mesothelin-targeted CAR T cells followed by anti-PD1 agent administration is warranted.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a phase I/II dose-escalation study to assess the safety and tolerability of administering increasing doses of engineered autologous T cells that have been targeted to mesothelin, without (Cohort 1) and following (Cohorts 2-9) pretreatment with cyclophosphamide. Patients diagnosed with malignant pleural mesothelioma, lung or breast cancer who have evidence of pleural disease are eligible for initial screening. Patients will be potentially eligible to progress to the treatment phase of this trial if laboratory testing confirms that their tumors express the target protein mesothelin. Patients with MPM in cohorts 9-11 will undergo three stages of the study- 1) screening, 2) treatment, 3) treatment with pembrolizumab

The second part of this study will be a Phase II study at the established recommended phase II dose of mesothelin-targeted CAR T cells in combination with pembrolizumab to assess efficacy of the combination therapy.

4.2 Intervention

To conduct a clinical trial of adoptive therapy with genetically modified T cells in patients with MPD, we have established an *ex vivo* transduction and expansion protocol that is capable of generating clinical-grade iCasp9M28z T cells for treatment. Autologous peripheral blood lymphocytes will first be isolated by leukapheresis. CD4+ and CD8+ T cells will then be activated, to allow for the efficient retroviral transduction of the iCasp9M28z CAR, using a highly efficient gamma-retroviral T cell transduction methodology. Cells will then be expanded for a short period (9 to 16 days), as required by the dose-escalation scheme.

These T cells will be engineered using a “second-generation” CAR (with tandem CD28 and CD3zeta signaling domains). The transduction and expansion protocol will be conducted using a closed system developed at MSKCC that has been previously approved for ongoing clinical trials of chronic and acute lymphocytic leukemia (CLL and ALL) (“A Phase I Trial for the Treatment of Purine Analog-Refractory Chronic Lymphocytic Leukemia Using Autologous T Cells Genetically Targeted to the B Cell Specific Antigen CD19,” MSKCC IRB protocol 06-138, J. Park, PI; “A phase I trial of Precursor B Cell Acute Lymphoblastic Leukemia (B-ALL) Treated with Autologous T cells Genetically Targeted to the B Cell Specific Antigen CD19”, MSKCC IRB protocol 09-114, J.Park, PI).

The proposed trial will test 7 levels of T cell doses. The dose-escalation scheme is described in detail below (section 9.1.4). In Cohorts 2-8, on the basis of our experience in an ongoing study of CLL, pretreatment with lymphodepleting cyclophosphamide will be administered at 1.5 g/m^2 2 - 7 days before infusion of T cells.

Patients who undergo leukapheresis or collection of PBMCs from whole blood on protocol will only be able to receive the CAR T cell treatment on protocol if they meet treatment eligibility as outlined in section 6.1 & 6.2.

4.3 Treatment of on-target, off-tumor toxicity

Patients will be monitored throughout the trial for potential on-target, off-tumor reactions in response to the genetically engineered T cells including signs of inflammation within vital organs. If such reaction is suspected, consideration will be given to administer corticosteroid therapy as clinically indicated. If unacceptable toxicity occurs that is probably or likely related to anti-mesothelin CAR T cells, AP1903, a dimerizing agent, may be administered to mediate clearance of the genetically engineered cells and resolve toxicity. AP1903 will be considered for the following: Any grade 4 toxicity that is felt to be probably or likely related to the mesothelin-CAR T cells will trigger administration of AP1903 if in the judgment of the Principal Investigator the syndrome cannot be adequately treated with routine supportive care including corticosteroids, and/or clinically available agents for neutralizing inflammatory cytokines (anti-TNF, IL1R antagonists, anti-IL6R mAbs). In the event that the PI is unavailable this judgment will be made by one of the Co-Principal Investigators, or a member of the Cell Therapy team. Persistent Grade 3 toxicity may also trigger administration of AP1903 if, in the judgment of the Principal Investigator, the risk of long-term morbidity is high. Any other Grade 3 or 4 toxicity may trigger administration of AP1903 if, in the judgment of the Principal Investigator, the risk for short-term or long-term morbidity is high and the toxicity is not responsive to standard supportive care, including administration of corticosteroids. AP1903 will be administered per pharmacy guidelines. Where feasible, mesothelin-CAR persistence studies will be obtained from the start of the infusion, within 3 hours of the start of the infusion, and at 24 hours after infusion of AP1903.

The decision for use of AP1903 will be made by the study PI. Following administration of corticosteroids, if the patient clinical condition is not improving, a discussion will be initiated among study PI, Co-PIs and the CTC team regarding the use of AP1903. In case study PI is not available, study Co-PI, a member of the Cell Therapy Team or the attending in charge will make the judgment. In case of disagreement regarding the use of AP1903, the CAR Oversight committee should be approached for recommendation.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Cyclophosphamide

- Nitrogen mustard derivative alkylating agent; converted to activated alkylating metabolites by hepatic microsomal enzymes. It interferes with DNA replication and RNA transcription.
- Common indications: Hodgkin's disease, lymphoma, multiple myeloma, CLL, acute lymphocytic leukemia, acute myeloblastic leukemia, mycosis fungoides, neuroblastoma, retinoblastoma, mesothelioma, ovarian carcinoma, and breast carcinoma.
- Supplied as lyophilized powder: 200-mg, 500-mg, and 2000-mg vials.

The drug is reconstituted with sterile water to result in a concentration of 20 mg/mL. It is stable for 24 hours at room temperature or for 6 days refrigerated. No significant toxicity has been noted at daily

doses of 1.5 g/m^2.[78-82] Baseline renal and hepatic function will be assessed before administration of cyclophosphamide.

5.2 Mesothelin-targeted T cells

Patients with therapy-refractory MPD will be treated by intrapleural infusion of autologous T cells genetically modified *ex vivo* to express the iCasp9M28z chimeric antigen receptor. This section of the protocol will describe the iCasp9M28z retroviral vector; the protocol for isolation, transduction, and expansion of iCasp9M28z T cells; and the analysis performed on the transduced T cells to measure gene expression, function, purity, and microbial sterility before infusion.

5.2.1 The vector

The iCasp9M28z retroviral vector is based on SFG, a splicing vector in which transgene expression is under the control of the Mo-MuLV long terminal repeat (LTR). The vector encodes a CAR consisting of a mesothelin-specific human scFv[44] fused to the transmembrane and cytoplasmic signaling domain of CD28, fused to the cytoplasmic signaling domain of the CD3ζ chain. It is linked to the ICaspase-9 gene through a P2A site derived from porcine teschovirus-1. Expression of both ICaspase-9 and M28z is driven by the retroviral LTR. The M28z vector will be transfected into the 293GP-R30 packaging cell line (RD114 envelope),[71] and cell-free supernatant will be used to infect the 293GP-GLV9 packaging cell line (GaLV pseudotyped).[71] The titration of individual 293GP-GLV9 clones is performed by infecting T cells with serial dilutions of the vector stocks and subsequently determining (by real-time PCR [RT-PCR] using sequences specific for the SFG vector) the average vector copy number in the genomic DNA of T cells. A high-titer 293GP-GLV9-iCasp9M28z packaging cell clone has been selected. The absence of replication-competent retroviruses (RCRs) has been ascertained using the S+L- assay, after amplification of 293 cells, in accordance with Federal Drug Administration (FDA) guidelines. A master cell bank (MCB) of the resulting 293GP-GLV9-iCasp9M28z clone and clinical lots of the retroviral vector M28z supernatant derived from the MCB were produced in the Cell Therapy and Cell Engineering Facility at MSKCC (CTCEF), in accordance with FDA and NIH recommendations and guidelines (see Appendix 3).

5.2.2 Generation and isolation of iCasp9M28z patient T cells for adoptive therapy

Please refer to the IND application for further details regarding the iCasp9M28z$^+$ retroviral vector and the generation/safety testing of a clinical grade packaging cell line used in this protocol.

5.2.3 Quality control for release of transduced cells

Before infusion, the transduced CD3+ T cells will be quality tested for number, purity, viability, and sterility. Please refer to the IND application for details regarding analyses performed on iCasp9M28z$^+$ T cells to measure gene expression, function, purity, and sterility prior to infusion.

5.2.4 Turnover of cell preparations

Depending on scheduling, patients will likely undergo infusion of their autologous transduced T cells 3 to 6 weeks after leukapheresis. T cells will be stored after leukapheresis. After transduction of thawed T cells, the transduced cells will either be immediately infused into the patient or frozen for future infusion.

5.2.5 AP1903 DIMERIZING AGENT

Availability: Supplied by Bellicum Pharmaceuticals.
Preparation: Formulated at 5 mg/ml in 25% Solutol HS15 (a non-ionic stabilizer).
Description: AP1903 is a lipid-permeable molecule with homodimerizing activity. Dimerizer drug AP1903 homodimerizes an analogue of human protein FKBP12 (Fv) which contains a single acid substitution (Phe36Val) so that AP1903 binds to wild-type FKBP12 with 1000-fold lower affinity. Upon binding of AP1903 to the engineered FKBP12, caspase 9 activation ensues leading to

endogenous caspase 3 activation and cellular apoptosis, beginning within 30 minutes and peaking at 3 hours.
Chemical Formula*:* C78H98N4O20
Stability: Stable for at least 24 months when stored at 2-8°C.
Administration: Premedicate with Tylenol and Benadryl using standard dosing 15-30 minutes prior to AP1903. AP1903 should be warmed to room temperature prior to dilution. AP1903 will be prepared and administered as per pharmacy guidelines.
Pharmacology: T1/2 3.6 hours in mice
Toxicity: none expected, the no observed effect level in dogs was 1000mg/kg, which is much beyond the prescribed 0.4 mg/kg dose. Urticaria and flushing observed in one patient, which did not occur with subsequent AP1903 administration administered after premedication. In the same trial, one patient experienced a cytokine release reaction after receiving AP1903 following dendritic cell infusions. Please refer to the Investigative Brochure on AP1903 provided by the Bellicum Pharmaceuticals. In the event that the AP1903 is used, we will report the use to Bellicum Pharmaceuticals along with any relevant clinical data regarding use of the agent.

5.3 Pembrolizumab

5.3.1 Preparation and Administration
Pembrolizumab will be given as 200 mg flat dose infusion intravenously.

5.3.2 Formulation, Packaging, and Labelling
Pembrolizumab is supplied commercially as 100mg/4ml solution for injection and will be handled in accordance with standard regulatory requirements.

5.3.3 Storage and Handling Requirement
Clinical supplies must be stored in a secure, limited- access location under the storage conditions specified on the label. Receipt and dispensing of medication for this study must be recorded by an authorized person at the site.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

Patients deemed eligible for the study are patients with MPD. Patients with malignant pleural effusion and MPD will undergo insertion of an intrapleural catheter as clinically indicated. All patients with a functional pleural catheter are eligible for the study, as long as there are no clinical concerns of infection. In patients with documented pleural disease, but no significant effusion for insertion of a pleural catheter, CAR T cells will be administered by intervention radiology-guided intrapleural injection. After obtaining a screening consent, patients' tumor and/or blood will be tested for presence of tumor marker – mesothelin. If mesothelin positivity is confirmed, then patients may have a leukapheresis product obtained from peripheral blood. When the patient is ready to proceed to the treatment phase of the study, treatment consent will be obtained. For patients with no free-flowing pleural effusion, an interventional radiologist will review radiological imaging to assess the feasibility of intrapleural administration. For patients with pleural fluid, all obtainable pleural fluid will be drained from the chest through a pleural catheter and will be preserved for analysis. Subsequently, a single dose of mesothelin-targeted T cells will be instilled via the catheter into the pleural space or by intervention radiology guidance into the pleural cavity. If a patient requires a thoracic surgical procedure (e.g. VATS) for any clinical indication after instillation of CAR T cells, pleural biopsies will be obtained. Patients with PleurX catheters for management of MPD for whom VATS is not considered are eligible for this study.

6.1 Subject Inclusion Criteria

1. Patients with MPD aged ≥18 years
2. Karnofsky performance status ≥70%
3. Patients with malignant pleural disease (MPD), pathologically confirmed at MSKCC (radiographic confirmation is acceptable for screening phase eligibility), and defined as one of the following (patients who have not yet received treatment may enroll in the screening portion only):
    a. Malignant pleural mesothelioma – previously treated with at least one prior treatment regimen.
    b. Non-small cell lung cancer metastatic to the pleura—previously treated with at least one prior treatment regimen (chemotherapy, surgery, or targeted agent) and documented progression of disease. Patients with disease outside of the pleura will be discussed among study PI and Co-PIs prior to be considered eligible for the study. Disease outside of the pleura must not require any immediate therapy per PI's discretion.
    c. Breast cancer metastatic to the pleura— previously treated with at least one prior treatment regimen (chemotherapy, surgery or targeted agent) and documented progression of disease. Patients with disease outside of the pleura will be discussed among study PI and Co-PIs prior to be considered eligible for the study. Disease outside of the pleura must not require any immediate therapy per PI's discretion.
4. Only patients with a diagnosis of malignant pleural mesothelioma will be included in cohort 9 and in the Phase II portion of the study. Expression of mesothelin must be confirmed by meeting one of the following criteria.
    a. Mesothelin expression (>10% of the tumor expressing mesothelin) by immunohistochemical (IHC) analysis
    b. Elevated serum SMRP levels (>1.0 nM/L).
5. A) Free flowing pleural effusion requiring management by placement of a pleural catheter. Patients with a functional pleural catheter already in place are eligible for the study, as long as there are no clinical concerns of infection.
   OR
   B) No free-flowing pleural effusion: an Interventional Radiologist has agreed that radiology-guided intrapleural or peritumoral injection of the CAR T cells is feasible.
6. Chemotherapy, targeted therapy (such as a tyrosine kinase inhibitor) or therapeutic radiotherapy must have been completed at least 14 days prior to administration of T cells. *Continuation of hormonal therapy (i.e. for breast cancer) is acceptable. Prior immunotherapy with checkpoint blockade (i.e. PD1 inhibitor, PDL1 inhibitor or CTL4-antagonist or similar agent) must have been completed more than 1 month prior to the T cell infusion.*
    a. Chemotherapy must have been completed at least 7 days prior to leukapheresis
    b. Palliative radiotherapy must be completed at least 2 days prior to administration of cyclophosphamide.
7. Any major thoracic (thoracotomy with lung or esophageal resection) or abdominal (laparotomy with organ resection) operation must have occurred at least 28 days before study enrollment. Patients who have undergone diagnostic VATS or laparoscopy can be included in the study.
8. All acute toxic effects of any previous therapeutic or palliative radiotherapy, chemotherapy, or surgical procedures must have resolved to grade I or lower according to CTCAE (version 4.0).
9. Lab requirements (hematology)
    - White blood cell (WBC) count ≥3000 cells/mm^3

- Absolute neutrophil count ≥1500 neutrophils/mm^3
- Platelet count ≥100,000 platelets/mm^3

10. Lab requirements (serum chemistry)
    - Bilirubin ≤1.5x upper limit of normal (ULN)
    - Serum alanine aminotransferase and serum aspartate aminotransferase (ALT/AST) ≤5x ULN
    - Serum creatinine ≤1.5x ULN or Cr > 1.5x ULN, but calculated clearances of >60
11. Negative screen for human immunodeficiency virus (HIV), hepatitis B virus (HBV) antigen, and hepatitis C virus (HCV). *If testing was performed during the previous 3 months, there is no need to repeat testing, as long as documentation of results is provided to the study site. Subjects must receive counseling and sign a separate informed consent form for HIV testing.*
12. Subjects and their partners with reproductive potential must agree to use an effective form of contraception during the period of drug administration and for 4 weeks after completion of the last administration of the study drug. An effective form of contraception is defined as oral contraceptives plus 1 form of barrier or double-barrier method contraception (condom with spermicide or condom with diaphragm).
13. Subjects must be able to understand the potential risks and benefits of the study and must be able to read and provide written, informed consent for the study

6.2 Subject Exclusion Criteria

1. Untreated or active CNS metastases (progressing or requiring anticonvulsants or corticosteroids for symptomatic control); patients with a history of treated CNS metastases are eligible, provided that all of the following criteria are met:
    - Presence of measurable or evaluable disease outside of the CNS;
    - Radiographic demonstration of improvement upon completion of CNS-directed therapy and no evidence of interim progression between completion of CNS-directed therapy and the screening radiographic study;
    - Completion of radiotherapy ≥8 weeks prior to the screening radiographic study;
    - Discontinuation of corticosteroids and anticonvulsants ≥4 weeks prior to the screening radiographic study.
2. Non-small cell lung cancer metastatic to the pleura that extends outside of the pleura requiring immediate therapy
3. Breast cancer metastatic to the pleura that extends outside of the pleura requiring immediate therapy
4. Prior history of seizure disorder
5. Patients currently receiving treatment for concurrent active malignancy. *Continuation of hormonal therapy (i.e. for breast cancer) is acceptable. Prior immunotherapy with checkpoint blockade (i.e. PD1 inhibitor, PDL1 inhibitor or CTL4-antagonist or similar agent) must have been completed more than 1 month prior to the T cell infusion.*
6. Autoimmune or antibody-mediated disease, including but not limited to systemic lupus erythematosus, rheumatoid arthritis, ulcerative colitis, Crohn's disease, and temporal arteritis (Patients with a history of hypothyroidism will not be excluded)
7. History of myocarditis or congestive heart failure (as defined by New York Heart Association Functional Classification III or IV), as well as unstable angina, serious uncontrolled cardiac arrhythmia, uncontrolled infection, or myocardial infarction 6 months prior to study entry

8. Subjects with left ventricular ejection fraction (LVEF) less than 50%
9. Patients with active interstitial lung disease (ILD)/pneumonitis or a history of ILD/pneumonitis requiring treatment with systemic steroids
10. Baseline pulse oximetry is less than 92% on Room air
11. Pregnant or lactating women
12. Known active infection requiring antibiotic treatment before the start of treatment (day 0)
13. A requirement for daily systemic corticosteroids for any reason or a requirement for other immunosuppressive or immunomodulatory agents. Topical, nasal, and inhaled steroids are permitted.
14. Administration of live, attenuated vaccine within 8 weeks before the start of treatment (day 0) and throughout the study
15. Any other medical condition that, in the opinion of the PI, may interfere with a subject's participation in or compliance with the study

7.0 RECRUITMENT PLAN

This study will be conducted at MSKCC. A minimum of 4 and a maximum of 54patients will be enrolled in this study. All patients will be recruited through the Thoracic Surgical, Thoracic Medical Oncology, or Breast Medical Oncology Service of MSKCC. Only patients with mesothelioma must be aged 18 years or older and have completed at least one prior treatment regimen will be eligible for the treatment phase of this trial. Patients aged 18 years or older who have metastatic breast or lung cancer and have been previously treated with at least one prior treatment regimen (chemotherapy, surgery, or targeted agent) and documented progression of disease will be eligible for this trial.

Table 2: Race/Ethnicity

Sex	White, not of Hispanic origin	Black, not of Hispanic origin	Hispanic	Asian or Pacific Islander	Unknown	Total
Male/ Female	60	6	0	6	0	54

Potential research subjects will be identified by a member of the patient's treatment team, the PI, or the research team at MSKCC. If the PI is a member of the treatment team, he or she will screen his or her patients' medical records for suitable research study participants and discuss the study and the patient's potential for enrolling in the research study with the patient. Potential subjects contacted by their treating physician will be referred to the PI/research staff to record appropriate contact information, so that these patients can be approached about enrolling in the study.

The PI/research staff may also screen the medical records of patients they do not have a treatment relationship with, for the limited purpose of identifying patients who are eligible.

During the initial conversation between the PI/research staff and the patient, the patient may be asked to provide certain health information that is necessary for the recruitment and enrollment process. The PI/research staff may also review portions of the patient's medical records at MSKCC to further assess eligibility. They will use the information provided by the patient and/or the medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study or declines to participate, the research staff will destroy all information collected on the patient during the initial conversation and review of medical records, except for any information that must be maintained for screening log purposes.

Subjects will be required to sign a statement of informed consent that meets the requirements of the Code of Federal Regulations ([CFR] Federal Register Vol. 46, No. 17, Jan. 27, 1981, Title 21, Part 50) and the Institutional Review Board (IRB) of MSKCC. The medical record will include a statement that written, informed consent was obtained, along with the date that written consent was obtained—thereby documenting that written, informed consent was obtained before the subject was enrolled in the study.

7.1 Mailed Screening Consent Option

Select established patients at MSKCC seen within the last 12 months who fit the inclusion criteria as stated in Section 6.1 are eligible to receive the Screening Informed Consent via the mail. These patients also must have tissue available for IHC mesothelin level testing to be performed at MSKCC. After an introductory phone discussion about the Screening Informed Consent & purpose with the patient by their MD, consenting professional or PI, prospective participants will be mailed/faxed/emailed a copy of the Informed Screening Consent with a cover letter (Appendix III). If mailing, a stamped return envelope will be supplied to the participant. A time for the consent discussion will be coordinated with the patient and the appropriate consenting professional will follow up with a phone call to the prospective patient. If the patient would like to participate the consenting professional will instruct the patient to sign and date the consent and return it within 60 days. Upon receipt by the consenting professional, s/he will sign and date (day received). The research team will mail a completed copy back to the participant for his/her records. The consenting process must be documented in the participant's EMR. The patient will be registered to the screening informed consent portion of the protocol. This mailed screening consent process will only be used for selected established patients who fit the inclusion criteria and who have a tissue sample available.

8.0 PRETREATMENT EVALUATION

The study calendar for this protocol can be found in section 10. Documented cycle delays that occur outside of the allowable window of variance (+/-3 days, unless otherwise noted) because of holidays, weekends, weather, or other unforeseen circumstances will constitute a protocol deviation and will be reported to IRB.

There are 2 phases to the study: the Screening Phase and the Intervention Phase. Patients must meet inclusion criteria 1-3, 5 and exclusion criteria 1-6 and 12 to be eligible for the Screening Phase. Patients must meet all inclusion and exclusion criteria to enroll in the Intervention Phase.

Screening Phase: After signing the Screening Informed Consent, the patient's mesothelin tumor expression will be determined as previously described. In order to be eligible for this protocol, the patient's carcinoma must express the mesothelin protein detectable by IHC analysis of banked (paraffin embedded) or fresh biopsied tumor and/or the patient must have elevated serum mesothelin levels. Patients with epithelioid or biphasic mesothelioma are not required to sign the Screening Informed Consent as all these patients' tumors express mesothelin.[98]

After signing Screening Informed Consent, if a patient's tumor and/or serum is found to express mesothelin, patients may undergo leukapheresis for the collection of PBMC. (Patients with epithelioid or biphasic mesothelioma are not required to sign Informed Consent (Screening Informed Consent) as all these patients' tumors express mesothelin.[98]) Subsequently, the leukapheresis product will be used to generate the iCasp9M28z$^+$ genetically-modified T cells. It is noted in the Screening Informed Consent that being leukapheresed does not guarantee treatment as results from additional testing performed may show that modified T cells are not suitable for infusion (quality control testing) and/or that patient clinical condition might have changed.

Intervention Phase: Following leukapheresis, patients must sign Treatment Informed Consent before being receiving treatment on the study. Patients who undergo leukapheresis or collection of PBMCs from whole blood on protocol will only be able to receive treatment on protocol if they meet treatment eligibility as outlined in section 6.1 & 6.2.

To be performed prior to leukapheresis
- Echocardiogram

To be performed before study treatment:
- Leukapheresis
- Successful generation of T cells

To be performed within 4-6 weeks before the start of treatment:
- A complete history and physical examination
- Concomitant medication inquiry
- ECG

Laboratory tests to be performed within 4-6 weeks before the start of treatment:
- Hematology blood tests: CBC, serum chemistry: electrolytes (Na, K, Cl, $C0_2$), BUN, glucose, creatinine, bilirubin, AST, ALT, calcium, phosphorus, uric acid, total protein, albumin, LDH, PT/PTT, troponin and SMRP
- Negative screen for HIV, HBV antigen, and HCV. If testing was performed during the previous 3 months, there is no need to repeat testing, as long as documentation of results is provided to the study site.
- Research blood tests
  - Cytokine profile
  - RCR testing
- Urinalysis and culture & sensitivity (C&S; urinalysis includes specific gravity, pH, ketones, sugar, protein, bilirubin, blood, WBC, and microscopic examination of sediment if clinically indicated)

Patients in cohorts 9 and in Phase II will receive pembrolizumab 4 weeks (+3/- 1 week window) following CAR T cell administration.

To be performed prior to pembrolizumab:
- Patients in cohorts 9 and in the Phase II will begin treatment with pembrolizumab 4 weeks (+3/ -1 week) after completing CAR T administration. Prior to receiving the first dose of pembrolizumab, patients will be evaluated in the Medical Oncology clinic and will undergo standard of care assessments required for pembrolizumab

9.0 TREATMENT/INTERVENTION PLAN

9.1.1 Production of genetically modified T cells

Following enrollment, leukapheresis product will be obtained in the blood donor facility at MSKCC and cryopreserved in the Cell Therapy and Cell Engineering Facility (CTCEF). Before protocol treatment, the leukapheresis product will be thawed, and T cell isolation, transduction, and expansion of iCasp928z T cells will be performed in the MSKCC CTCEF. It is estimated that it will take approximately 3 to 6 weeks to generate T cells for treatment.

9.1.2 Pretreatment

In Cohorts 2 to 9, patients will receive cyclophosphamide intravenously (at 1.5 g/m^2) , 2 – 7 (Day (-7) – (-2)) days before T cell infusion. Standard MSKCC antiemetic therapy that is being used in CAR T cell therapy protocols (IRB# 09-114) will be administered prior to chemotherapy to prevent nausea/vomiting.

9.1.3 Infusion of iCasp9M28z genetically modified T cells

Administration through the pleural catheter

On Day 0, patients will be treated with genetically modified T cells. Thirty to 60 minutes before T cell infusion, patients will be given 650 mg of acetaminophen orally and 25-50 mg of diphenhydramine orally or intravenously, to prevent infusion-related reactions. It is expected that the genetically modified T cells will be infused for at least 15 minutes and no more than 2 hours through the indwelling pleural catheter depending on the volume of the T cells, but this length of time may vary. A physician will be available during the infusion. Please note, during formulation of iCasp9M28z T cells, under or over estimation of CAR modified T cells may occur. Patient may receive an altered fractionation of the total dose or up to 35% over or under total cell dose with approval of the PI. Patients who do not have enough cells to match the current dose cohort will be treated in the cohort in which they have cells available

Administration with Interventional Radiology

The above-mentioned regimen will be followed, with the exception that CAR T cells will be administered in the IR suite with the assistance of an Interventional Radiologist. The interventional radiologist will access the pleural cavity in the standard fashion. The genetically modified T cells will be administered into the pleural space. Following completion of CAR T cell administration, patients will be monitored in the IR suite, and transferred to the BMT, CTC, or Leukemia services. Patients may be transferred to the PACU in the interim if necessary. A physician will be available during the infusion and when the patient is being monitored in the intervention radiology suite.

Modified T cells may be infused in a fractionated manner. Approximately half of the dose may be administered on the first day and the remaining dose 2-5 days later. The exact dose as well as timepoints of the first and any subsequent infusion of T cells will be determined by the study PI with consideration to the patient's clinical status.

9.1.4 Treatment scheme

A minimum of 4 patients and a maximum of 54 patients will be treated with escalating CAR T cell doses. Cohorts 1-8 (Table 3) will receive CAR T cell treatment and followed weekly. Cohorts 9 (Table 3) will receive CAR T cell infusion, followed by pembrolizumab. Patients will receive 3 doses of pembrolizumab followed by reassessment. Those responding or deriving clinical benefit, without unacceptable toxicity, will continue on pembrolizumab. Since only limited published data are available regarding the safety of genetically modified T cells in patients with cancer, the proposed treatment doses in this study are based on safety data from previously published studies that used cloned autologous CD4+ or CD8+ T cells in patients with melanoma. The safety of the lymphodepleting cyclophosphamide dose proposed in this study (1.5 g/m^2) is based on our previous experiences at MSKCC with high-dose cyclophosphamide in the treatment of patients with melanoma and CLL. Ongoing trials at MSKCC have delivered higher initial doses of genetically modified T cells via IV infusion (e.g., CLL trial: 3×10^6 CAR+ T cells/kg; ALL trial: 3×10^6 to 1×10^6 CAR+ T cells/kg). To our knowledge, this proposed trial is the first at MSKCC and other centers to deliver modified T cell intrapleurally. A conservative starting dose of 3×10^5 was chosen to ensure patient safety. An additional infusion of modified T cells may be considered for patients who have obtained clinical benefit from the initial T cell therapy and did not experience any non hematologic grade 4 toxicities.

Table 3

Cohort	Dose **
-1*	1×10^5
1	3×10^5
2	3×10^5+Cyclophosphamide
3	1×10^6+Cyclophosphamide
4	3×10^6+Cyclophosphamide
5	6×10^6+ Cyclophosphamide
6	1×10^7+Cyclophosphamide
7	3×10^7+Cyclophosphamide
8	6×10^7+Cyclophosphamide
9	6×10^7+Cyclophosphamide + pembrolizumab

*Necessary only if toxicity is encountered at the initial dose level.
**Mesothelin-targeted T cells/kg; intermediate dose levels may be evaluated, if indicated. Patient may receive an altered fractionation of the total dose or up to 35% over or under total cell dose with approval of PI.

At least 3 patients will be treated starting at dose level 1 with a projected accrual of 1 patient per month. Patient staggering of 4 weeks will be followed between cohorts. However, patients can be prescreened to be ready for the next enrollment.

Dose-escalation scheme
The dose-escalation scheme is as follows.

- If none of the initial 3 patients at this dose level experiences a dose limiting toxicity (DLT), the next T cell dose will be administered to the next cohort of 3 patients
- If 1 of the initial 3 patients at a dose level experiences a DLT, then up to 3 additional patients will be treated at the same dose level. If then one in 6 of the patients at the same dose level experiences a DLT, the next dose level will be investigated. Thus, dose escalation will only proceed with 0 of three, or 1 of six, patients experiencing a DLT per dose level.
- In the event that 2 of 3 patients, or 2 of 6 patients in the first cohort (dose level 1) experiences DLT, then the next cohort of patients will be treated at a lower dose level (dose level -1) with 1×10^5 T cells/kg. Three patients will be enrolled at this dose level; if 0 or 1 patients experiences a DLT, a further 3 patients will be enrolled at this dose level. If overall 0 or 1 patient experiences a DLT at this dose level, this dose will be established as the MTD.

CAR T cell oversight committee: Decisions to escalate to the next level or, when appropriate, to deescalate to a lower level will be recommended to the institutional CAR T cell Oversight Committee by the principal investigator (PI). The CAR T cell Oversight Committee will make the final decision of whether to proceed to the next dose level. In addition, the PI will coordinate regular meetings/communication with CAR T cell Oversight Committee to

a. present the data for each cohort of patients and study progress
b. discuss and obtain recommendations prior to escalating to the next dose level or if appropriate deescalating the dose level
c. report any significant toxicities associated with the CAR T cell intervention.
d. present any proposed significant changes in the protocol.

9.2 DLTs
DLT is defined as any of the following occurring within 30 days from the infusion of the iCasp9M28z$^+$ T cells.

- Grade 4 leukopenia (WBC < 1000/ml) lasting 30 days or more from the time of infusion (in patients with a pre-treatment WBC of > 1000/ml).

- New grade 3 or 4 non-hematologic toxicities lasting more than 48 hours (excluding renal or hepatic toxicities that are part of the CRS event), which is probably or definitively attributed to T cell infusion, not attributed to any chemotherapy received or persistent disease.
- Grade 3 neurotoxicity (encephalopathy). (*Transient cognitive disturbances lasting less than 24 hours that do not require any intervention will not be considered as DLT. Transient cognitive disturbances may arise from general anesthesia or sedatives used in patients undergoing any intervention procedures or surgical procedure for the management of their pleural effusions*.)
- Neurotoxicity / encephalopathy leading to intubation for airway management
- Grade 4 or greater severe cytokine release syndrome (CRS) as defined in the section below not improving after 48 hours of systemic steroids
- Grade 3 EEG-confirmed seizures or Grade 4 multiple seizures that are not responsive to the neurological treatment by the Neurology team.
- Any grade 5 toxicity including death, attributable (definitely, probably, or possibly) to T cell infusion.
- Any adverse event that is not responding to infusion reaction management as defined in the protocol (section 9.4) leading to permanent discontinuation of iCasp9M28z+ T cells infusion.
- As patients will be followed weekly for 3 weeks after the CAR T cell infusion, any adverse events will be documented and reported. After the patient starts pembrolizumab, the investigator of the study will analyze the correlative data to determine whether AEs are related to CAR T cells or related to the combined treatment. This is to determine whether pembrolizumab will be continued or stopped. Patients will receive 3 doses of pembrolizumab, followed by reassessment. The decision to continue pembrolizumab will be made by the Thoracic Medical Oncology service in discussion with the principal investigator of this protocol. Patients will continued to be monitored and any adverse events after 3 doses of pembrolizumab will be documented. All events will be presented to the CAR oversight committee.

9.3 Stopping Rules for Delayed Toxicity:
Dose escalation will proceed based on DLT experienced within the treatment and observation periods as described. Delayed toxicities (any of the toxicities specified above seen after 30 days) which are likely or definitely related to treatment with genetically-modified T cells will be collected and evaluated by the investigators and reported to the MSKCC IRB, Investigational Drug Committee, and the FDA. Accrual might be held pending analysis of adverse events and will be restarted only after approval of the IRB and FDA.

Death other than death related to progressive disease or pre-existing condition that occurs within 30 days of the CAR T cell infusion will trigger a pause to the study, whereupon the study will be paused to enrollment and treatment until an appropriate evaluation of the cause of the toxicity is determined and a plan of correction if necessary is established. In all cases, the detailed report of death including the attributable cause will be presented to the CAR T cell Oversight Committee and an appropriate action determined.

Study stopping will be deferred to the independent CAR T cell Oversight Committee.

9.4 Management of Infusion-Related Reactions:

- If a grade 1 infusion-related reaction occurs, CAR T cell infusion may be continued at the same dose and rate of administration.

- Fever, chills, and rigors may be treated with acetaminophen orally, diphenhydramine IV or orally (or alternative antihistamine), meperidine IV. Nausea and vomiting may be treated using previously established MSKCC guidelines.
- If a grade 2 infusion-related reaction occurs, treatment may be continued with a reduction in the infusion rate of T cells. Symptoms may be treated as above, as clinically indicated.
- If a grade 3 infusion-related reaction occurs, the infusion of CAR T cells will be interrupted. Symptomatic treatment, as outlined above, will be administered as clinically necessary, and the infusion can be resumed at a reduced rate after resolution of symptoms. If grade 3 reactions recur after resumption of the infusion, T cell infusion will be discontinued.
- If a grade 4 infusion-related toxicity recurs, T cell infusion will be discontinued. Symptoms will be treated using the guidelines above, as well as established MSKCC adult hypersensitivity guidelines, and no further T cells will be administered.

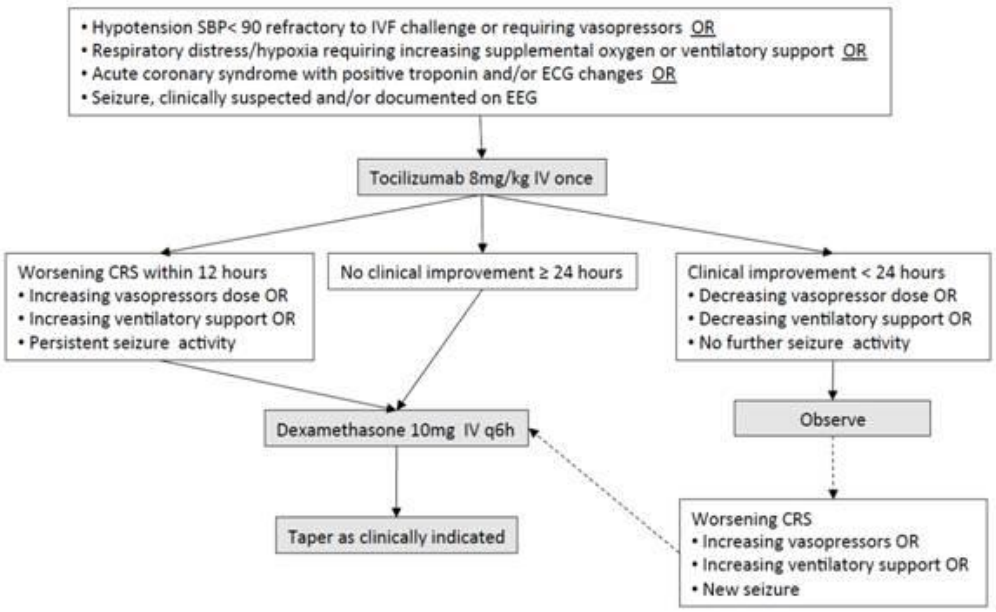
9.5 Management of Cytokine Release Syndrome (CRS): Following infusion of iCasp9M28z^{+} T cells, patients may develop severe CRS. Severe CRS is defined by the presence of one of the following clinical and laboratory parameters, not expected in the post-operative period if the patient underwent a surgical procedure

- Hypotension: SBP<90 refractory to IV fluids or requiring at least one vasopressors
- Respiratory distress/hypoxia requiring increasing supplemental oxygen (not related to post-operative oxygen requirement) or ventilator support
- Acute coronary syndrome (ACS) with positive troponin and/or ECG changes concerning for A
- Seizure, clinically suspected and/or documented on EEG

In order to ensure adequate monitoring and treatment of any CRS or other serious toxicity in patients receiving modified T cells, patients will be admitted to Memorial Hospital as inpatients prior to their infusion of modified T cells, and will remain hospitalized for at least 2 days.

In an effort to reduce complications related to these CRS toxicities, the following management algorithm will be used:

Severe Cytokine Release Syndrome (CRS) Management Algorithm



If the patient is not responding to the above measures, or worsening despite the treatment proposed, PI will make the judgment to infuse AP1903 as described in section 4.3. Following administration of corticosteroids, if the patient clinical condition is not improving, a discussion will be initiated among study PI, Co-PIs and the CTC team regarding the use of AP1903. In case study PI is not available, study Co-PI, a member of the Cell Therapy Team or the attending in charge will make the judgment. In case of disagreement regarding the use of AP1903, CAR Oversight committee should be approached for recommendation.

9.6 Management of Neurological Toxicities: Patients with severe CRS may develop neurological complications including confusion, delirium, expressive aphasia, obtundation, myoclonus and seizure-like activity. Some of the previously treated patients required ICU stay due to these side effects, and few patients required intubation. All of these side effects were observed in patients with morphologic ALL at the time of CAR T cell infusion. The following specific measures will be taken to minimize toxicity in treated patients.

- Upon admission, all patients' baseline neurological examination will be documented in the history and physical examination prior to CAR T cell infusion.
- At the PI's discretion and in consultation with a neurologist if necessary, patients will receive levetiracetam (Keppra®) for seizure prophylaxis prior to CAR T cell infusion. In cases of allergic reactions to levetiracetam, alternative anti-seizure medications will be used under the guidance of the Neurology team.
- After CAR T cell infusion, if patient develops neurological signs, a Neurology consult will be requested. These patients will be followed by the Neurology team, at least 3 times a week (or as clinically indicated) for the first 2 weeks or until neurological symptoms resolve. If neurological toxicities including seizure are observed, these symptoms will be managed as per the institutional neurology guidelines.

9.7 Management of 'On-target, Off-tumor' Toxicities: patients may develop 'on-target, off-tumor' toxicity due to very low levels of mesothelin expression in the normal pleura, pericardium or peritoneum. Patients will be monitored for evidence of pleuritis, pericarditis or peritonitis. 'On-target, Off-tumor' toxicity will be defined as:

- Symptoms of persistent pleuritis that may worsen with deep inspiration, pain not relived by routine pain management and is unrelated to post-operative incisional or chest pain
- Symptoms of pericarditis diagnosed by sharp, persistent, retrosternal chest pain that may become worse with deep inspiration and is associated with new EKG changes and/or new pericardial effusion diagnosed by echocardiogram.
- Symptoms of peritonitis diagnosed by diffuse abdominal pain that may worsen following deep inspiration, or cough with rebound tenderness on examination, that may be associated with previously unknown ascites.

Patients with suspected 'on-target, off-tumor' toxicity will be closely monitored as clinically indicated. If patient continues to demonstrate above criteria, AP1903 will be administered as indicated in section 4.3.

9.8 Supportive Treatment:

- Patients will receive antibiotics, red blood and platelet cell transfusions according to MSKCC standard care guidelines.
- Patients may receive subcutaneous filgrastim or pegfilgrastim at the discretion of the treating physician following conditioning chemotherapy and infusion of modified T cells.

- The patient may be discharged from the hospital, at the discretion of the treating physician if the patient is clinically stable and there is no evidence of significant tumor lysis, transfusion reactions, or unforeseen adverse reactions after T cell infusion.
- The ICU attending will be made aware of patients treated with T cells on this protocol if patient status is worsening with increased risk of adverse events or of an unexpected admission suspected to be related to T cell infusion
- In the event of symptoms related to an adverse reaction to infused T cells including fever, hypotension, renal failure, or hypoxia, the ICU staff will immediately be consulted to provide close monitoring and assessment for possible transfer to the ICU for closer observation.
- If there is laboratory evidence of acute renal failure (increased creatinine of 1.5x over pre-T cell infusions levels), the MSKCC renal service will be consulted

If the patient develops fevers, chills or other evidence of infection on outpatient followup, he/she will be admitted to the inpatient service for further evaluation and IV antibiotics as clinically indicated, ID service will be consulted.

Management of tumor lysis syndrome per institutional guidelines (may include allopurinol).

9.9 Pembrolizumab toxicity

Other side effects not attributable to the T cells or the potential synergy of pembrolizumab with the T cells will be managed according to Institutional Standard Practices for the management of toxicity associated with checkpoint inhibitor therapy (see Section 11 below for additional details regarding dose delays and cessation of pembrolizumab).

10.0 EVALUATION DURING TREATMENT/INTERVENTION

STUDY CALENDAR

Procedure									
	Pre-treatment	**Day (-7) - (-2)**	**Day -1**	**Day 0 Rx Day**	**Days 1, 2**	**Day 3[16]**	**3 days after discharge**	**Weeks 1-4**	**Day 28 (+21/-7 days) and every 3 weeks[17]**
Medical history[1]	X*						X	X	X
Physical examination	X*		X				X	X	X
AE inquiry			X	X	X	X	X	X	X
Concomitant medication inquiry	X*		X						X
Weekly communication[2]								X	
Vital signs[3]				X					X
Hematology[4]	X*		X		X	X		X+	X
Serum chemistry[5]	X*		X		X	X		X+	X
HIV testing, serologic testing for HBV and HCV[6]	X								
Serum C-reactive protein[7]			X		X+	X+		X+	
Serum SMRP[8]	X							X+	X
Cytokine profile[9]	X				X	X+	X+	X+	
RCR test[10]	X		X						
Urinalysis and C& S[11]	X				X+	X+	X+	X+	
Troponin[12]	X		X	X	X	X+	X+	X+	X+
Echocardiogram[13]	X								
ECG[14]	X		X	X					X+
Leukapheresis	X								
Administration of cyclophosphamide		X							

Administration of CAR T cells				X					
Pembrolizumab									X
Chest X-ray			X+		X+	X+	X+	X+	
PET/CT[15]									X

In cases of study procedures occurring on weekends/holidays or in cases of other logistical issues, an allowance of +/-3 days is considered to be included for all time points stated in this protocol, if not stated otherwise.

*Within 4 – 6 weeks of study treatment (day 0)
+As clinically indicated or at the discretion of the PI

[1] Record complete medical history
[2] Weekly communications will be performed by the PI, co-PIs, CTC MDs, nurse, or research team.
[3] Vital signs (i.e., blood pressure, temperature, and pulse rate) will be charted approximately 15 minutes (+/10 minutes) prior to the CAR T cell infusion, 15 minutes during infusion, 30, 60 minutes (+/10 minutes) after treatment is completed.
[4] CBC
[5] Electrolytes (Na, K, Cl, $C0_2$), BUN, glucose, creatinine, bilirubin, AST, ALT, calcium, phosphorus, uric acid, total protein, albumin, PT, PTT, and LDH
[6] If testing was performed within the previous 3 months, there is no need to repeat testing, as long as documentation of results is provided to the study site.
[7] Following T cell administration, C-reactive protein titers will be performed as clinically indicated
[8] SMRP will be drawn for screening purposes (prior to CAR T cell administration), at the week 1 post treatment visit, and at PI discretion at other time points.
[9] Cytokine profile to be completed prior to T cell infusion and once during admission following T cell infusion. Additional samples will be collected at post treatment protocol time points at the discretion of PI.
[10] RCR samples will be obtained prior to T cell infusion, 3 months, 6 months, 12 months after T cell administration and yearly thereafter (see 10.4)
[11] Urinalysis includes specific gravity, pH, ketones, sugar, protein, bilirubin, blood, WBC, and microscopic examination of sediment as clinically indicated
[12] Troponin will be tested prior to T cell infusion, Days 1 and 2, and if clinically indicated at subsequent time points. Troponin is not required on Day 0 but may be obtained at PI discretion
[13] A baseline echocardiogram will be performed prior to leukapheresis. A repeat echocardiogram will be performed in clinically indicated
[14] A baseline ECG will be performed prior to the T cell infusion, Days 1 and 2, and if clinically indicated at subsequent time points. An ECG will be performed on Day 0 within 4 hours following T cell infusion
[15] PET or CT chest or CT Chest, Abdomen, Pelvis with IV contrast (as clinically indicated) will be obtained 28 days (+/- 7 days) after T cell administration. A scan will be repeated 2 weeks (+/- 7 days) after the third dose of pembrolizumab.
[16] Day 3 assessments are only indicated if the patient remains admitted
[17] Patients will continue on pembrolizumab every 3 weeks for as long as they are responding and/or deriving clinical benefit and do no have unacceptable toxicity.

Specimen handling: collecting blood samples

A. Venous blood samples (~7 mL/tube) for biochemical and metabolic markers will be collected, stored at room temperature until processing, and forwarded to the Hematology/Clinical Chemistry laboratories. PBMCs will be collected in cell preparation tubes and kept on ice until distribution to either Dr. Riviere's or Dr. Adusumilli's laboratory. Samples will then be stored as indicated in Section 5.0.

B. Labeling blood/pathologic samples
Submitted patient documents/samples will have a unique identifier consisting of a study number derived from the site identification and the order in which the patient was enrolled in the study, followed by the sample time point, vial #, date of processing, and the initials of who processed the sample. This identifier will be used for the purposes of the study only and will be distinct from the patient identification (medical records) number used. Each sample will be accompanied by a sample log. Pathologic specimens will be stored at -80°C in Dr. Adusumilli's freezer in the Schwartz or Zuckerman buildings.

C. Collection of blood and blood products carries low risks for the patient. Minimal discomfort and bruising at the site of needle entry are common. Leukapheresis is performed once and has the same risks of discomfort and bruising at the site of needle entry as does collection of peripheral blood.

Details of specialized tests are provided below.

10.1 T cell infusion evaluation

Temperature, pulse, respirations, and blood pressure will be measured and recorded in the patient's chart. Patients will be evaluated clinically by vital signs approximately 15 minutes (+/10 minutes) prior to infusion, 15 minutes during infusion, 30, 60 minutes (+/10 minutes) after treatment is completed. All infusions of modified T cells will be administered by MD, LIP, or RN under MD supervision. Infusions in the Interventional Radiology suite will be administered by the Interventional Radiology attending. A physician will always be available during the time of the infusion.

Chemistry profiles and CBCs will be obtained daily for at least 2 days (days 1 and 2). Following T cell administration, C reactive protein titers will be performed as clinically indicated. Serum troponin and ECG testing will be performed on Days 1 and 2, and if clinically indicated at subsequent time points. For the first cohort, patients will be treated in the Intensive Care Unit, and they will remain inpatients for 2 nights, unless undue toxicity or unexpected AEs occur; maximum expected inpatient stay is 2 nights. Protocol assessments past Day 2 are only required if clinically indicated and if the patient is still admitted. A physician will be available during the time of the infusion. Patients will remain as an inpatient for at least 2 days after any T cell infusion. They will be seen daily during the inpatient stay by an attending from the BMT or Leukemia Services with consults from Cellular Therapeutics Center and Thoracic Surgery. A Cellular Therapeutics Center attending will always be contactable via the CTC pager.

The research blood work will be stored at room temperature. The Clinical Research Coordinator (CRC), study investigator, research fellow, or MSK messenger will bring research samples to the laboratory of Dr. Adusumilli or the CTCEF as needed to be processed. An RSA will be assigned to the study to facilitate coordination of specimen collection and delivery. Leftover samples will be banked and stored for future research purposes in the CTCEF or Dr. Adusumilli's laboratory. For patients who are no longer on-study, the T cells may be used in the laboratory for research purposes or discarded.

10.2 Follow-up evaluation

Patients will be seen as an outpatient within 3 days (+3 days window) of discharge. Patients will have at least one weekly communication with the study team for toxicity assessment in addition to being seen in clinic weekly for at least 3 weeks following the CAR T cell infusion. The patient will receive 3 doses of pembrolizumab administered every 3 weeks. If the patient responds or derives clinical benefit, the patient will be followed for an additional 3 cycles of pembrolizumab. After the completion of 6 doses of pembrolizumab, the patient will be taken off study for the completion of study treatment. However, if patient continues to derive clinical benefit, the patient may receive pembrolizumab off study. Thereafter, the frequency of further follow-up will be determined by the treating physician, as clinically indicated. At each follow-up visit, patients will undergo a complete history and physical examination. CBCs will be obtained as clinically indicated and AE assessments will be performed at each clinic visit. Clinical assessments, including labs and radiographical images, will be collected at each visit even if the patient is off protocol. Every effort will be made to collect all relevant clinical information if the patient is receiving care outside of MSK.

10.3 Assessment of CAR T cell survival

To assess and quantify the survival of genetically modified T cells after infusion, peripheral blood analysis will be performed 1 to 3 days post infusion, at week 4, and subsequently as indicated by the PI until the CAR T cells become undetectable in Dr. Adusumilli's laboratory or in Cell Therapy and Cell Engineering Facility. Biopsies will be performed for selected patients on the basis of availability and access to the involved tumor site, as well as the willingness of the patient to undergo the procedure.

10.4 RCR testing

At the time of T cell infusion, the retroviral vector copy number of the infused T cells will be assessed by RT-PCR. Samples will undergo RCR testing at the time of infusion. Peripheral blood samples will undergo RCR testing prior to T cell infusion, 3 months, 6 months, 12 months after treatment and archived yearly thereafter. The recommendation for RCR testing was established by the FDA in the communication: *"Guidance for Industry: Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Following-up of Patients in Clinical Trials Using Retroviral Vectors (November 2006)"*. All efforts will be made to collect blood samples per these guidelines; missed RCR samples due to patients being off-study or other logistic issues will not constitute a protocol violation.

10.5 Pathologic sample archiving

Pathologic samples (both frozen and paraffin-embedded) obtained after any clinically indicated surgical procedure will be archived. Specimens will be analyzed for mesothelin-expressing tumor, CD3 infiltration, and necrosis as needed.

10.6 Long-term follow-up plan

In compliance with CBER/Biological Response Modifiers Advisory Committee recommendations for gene-transfer research protocols, long-term follow-up of enrolled patients will include:

1) A minimum of 1 physical examination per year, with documentation of significant-interval medical history, to be performed at MSKCC for 15 years after completion of treatment.

2) All information obtained during follow-up will be compiled by the research study assistant assigned to this study and reviewed by the PI. The PI will provide an annual summary of long-term follow-up results, which will be provided to the FDA in conjunction with each investigational new drug (IND) annual report. In the unlikely event that a plan change becomes necessary, this information will also be provided.

10.7 Contingencies for patients lost to follow-up

As the long-term follow-up extends to 15 years, it is likely that some patients will be lost to follow-up. To minimize this risk, enrolled patients will be requested to provide forwarding information if they move out of the area. Furthermore, we will make every effort to obtain contact information of close relatives of patients at the time of enrollment. The research study assistant will be responsible for tracking and locating patients enrolled in this study during the follow-up period.

10.8 Contingencies for loss of the PI

In the event that the PI leaves the institution, the role of the PI and the responsibilities for further patient follow-up associated with this title will be transferred to a co-PI or other member of the MSKCC Thoracic Surgery Service, at the discretion of the service chief and with approval of the institution's IRB.

10.9 Contingencies for death of a patient during study

In the event that a patient dies during the study period, the patient's family will be requested to have an autopsy performed, regardless of the cause of death. If an autopsy is permitted by the family, samples of blood, all tumor sites, brain, liver, kidney, lung, and gonads, as well as marrow and spleen, will be obtained. These tissues will be analyzed histochemically for evidence of residual tumor cells and for evidence of residual iCasp9M28z T cells. In addition, samples will be obtained for PCR-amplified analysis of tissue fractions to detect for evidence of residual iCasp9M28z+ T cells. DNA from the tissue samples will be obtained to assess vector sequences detectable in the tissues

and to define sites of insertion of the vectors, if possible. In addition, attempts will be made to expand iCasp9M28z T cells from the bone marrow, blood, and spleen cell populations. The expanded cell populations will be further analyzed for appropriate vector sequence and insertion site.

11.0 TOXICITIES/SIDE EFFECTS

Adverse Event: An adverse event is any noxious, pathologic, or unintended change in anatomical, physiologic, or metabolic functions, as indicated by physical signs, symptoms, and/or laboratory changes occurring in any phase of the clinical trial, whether associated with drug or placebo and whether or not considered drug related. All of the following are to be considered adverse events:

- An exacerbation of a pre-existing condition
- An intercurrent illness
- Any drug interaction
- Any event related to a concomitant medication
- Development of an abnormal laboratory value or a significant change from baseline in a laboratory value within the range of normal, considered by the investigator to be clinically important
- An unexpected significant worsening of the cancer under treatment. Anticipated day-to-day fluctuations in the activity of the cancer or the anticipated progression of the cancer (other than death) should not be considered an adverse event.

Serious Adverse Event: A serious adverse event is one that is fatal or life-threatening (see below), is temporarily or permanently disabling, requires inpatient hospitalization (initial or prolonged), or is associated with a congenital anomaly, a new cancer or a drug overdose (either accidental or intentional). In addition, any event suggesting a significant hazard, contraindication, side effect or precaution should also be considered serious.

Life-threatening: means an immediate risk of death from the reaction as it occurred. Life- threatening does not include a reaction that, had it occurred in a more serious form, might have caused death. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening even though drug-induced hepatitis can be fatal
Toxicity will be graded on a scale of I to V, as described in version 4 of the CTCAE (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

11.1 Dose delay criteria

The table below provides a full list of adverse events that warrant CPB dose delay. Dose delay criteria apply for all drug-related adverse events. All study drugs must be delayed until treatment can resume. Finally, any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication also applies. Adverse events are to be determined to be related or clinically significant to pembrolizumab, by the treating physician and/or PI. If the adverse event is determined to be unrelated, pre-existing, or not clinically significant, treatment will continue. Subjects may be dosed +/- 7 days from their scheduled treatment date. Dose given outside of the +/- 7-day window will be considered a dose delay. Treatment may be delayed for up to a maximum of 6 weeks from the previous dose. In patients with mild or moderate infusion reactions to pembrolizumab, the rate of infusion can be interrupted or slowed. However, pembrolizumab should be permanently discontinued in patients with severe or life-threatening infusion reactions. In all cases, when treatment is held, treatment should only be resumed when the adverse event improves to Grade 0 or 1. Disease status assessments by CT should continue as per protocol even if dosing is delayed.

Guidelines for Withholding Pembrolizumab

Table 6

ADVERSE EVENT (determined to be related, pre-existing, or clinically significant by treating physician and/or PI)	SEVERITY**	DOSE MODIFICATION
Colitis	Grade 2 or 3 diarrhea or colitis	Withhold dose*
	Grade 4 diarrhea or colitis	Permanently discontinue
Pneumonitis	Grade 2 pneumonitis	Withhold dose*
	Grade 3 or 4 pneumonitis	Permanently discontinue
Hepatitis	AST or ALT >3x ULN but ≤5x ULN OR bilirubin >1.5x ULN but ≤3x ULN	Withhold dose*
	AST or ALT >5x ULN OR bilirubin >3x ULN	Permanently discontinue
Hypophysitis	Grade 2 or 3 hypophysitis	Withhold dose*
	Grade 4 hypophysitis	Permanently discontinue
Adrenal Insufficiency	Grade 2 adrenal insufficiency	Withhold dose*
	Grade 3 or 4 adrenal insufficiently	Permanently discontinue
Type I diabetes mellitus	Grade 3 hyperglycemia	Withhold dose*
	Grade 4 hyperglycemia	Permanently discontinue
Nephritis and Renal Dysfunction	Serum creatinine >1.5x ULN but ≤6x ULN	Withhold dose*
	Serum creatinine >6x ULN	Permanently discontinue
Skin	Grade 3 rash or suspected Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN)	Withhold dose*
	Grade 4 rash or confirmed SJS or TEN	Permanently discontinue
Encephalitis	New-onset moderate or severe neurologic signs or symptoms	Withhold dose*
	Immune-mediated encephalitis	Permanently discontinue
Other***	First occurrence of all other Grade 3 adverse events	Withhold dose*
	Recurrence of the same Grade 3 adverse events	Permanently discontinue
	Life-threatening or Grade 4 adverse reaction	Permanently discontinue
	Requirement for ≥ 10 mg per day prednisone or equivalent for >12 weeks	Permanently discontinue
	Persistent Grade 2 or 3 adverse event lasting >12 weeks (except for fatigue)	Permanently discontinue

*Resume treatment when the adverse event improves to Grade 0 or 1.
**Adverse event severity was graded per National Cancer Institute Common Terminology Criteria for Adverse Events. Version 4.0 (NCI CTCAE v4).

***Any adverse event, laboratory abnormality or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued vaccination or pembrolizumab dosing. These patients will then be removed from the study.

Criteria to Resume Treatment: If the criteria to resume treatment are met as outlined in Table, the patient should restart treatment at the next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol, the next scheduled time point will be delayed until dosing resumes.
If treatment is delayed >6 weeks, the subject must be permanently discontinued from study therapy except for the following:

- Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting >6 weeks, the Principal Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted.
- Dosing interruptions >6 weeks that occur for non-drug-related reasons may be allowed if approved by the Principal Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting >6 weeks, the Principal Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted.

Management of Immune-Related Adverse Events: For management of immune-related adverse events, we will follow established algorithms included in the section below.

11.2 Autoimmunity

Autoimmune reactions mediated by the infused autologous T cells are a theoretical possibility if anergic autoimmune T cells are stimulated by activation through the M28z receptor or if normal mesothelin-positive tissues (pleura, pericardium, and peritoneum) are targeted. Patients who develop evidence of either hematologic or unforeseen autoimmune reactions during therapy will be treated with standard autoimmune regimens, including corticosteroids and/or AP1903 if elimination of transduced T cells is indicated. As a secondary alternative, AP1903 may be administered to specifically delete the infused T cells.

11.3 Insertional oncogenesis

In a previous study in a pediatric population with X-linked SCID, wherein patient bone marrow stem cells were retrovirally transduced with the common γc chain gene, 5 cases of subsequent T cell acute lymphoblastic leukemia thought to be related to the insertion of the retroviral vector at or near a cellular protooncogene were reported. In at least 4 cases, the insertion of the retroviral vector was at or near the LMO-2 protooncogene. Unfortunately, 1 of the first 3 patients diagnosed with T cell leukemia has subsequently died of complications related to GvHD associated with allogeneic bone marrow transplantation therapy for this leukemia. The results from this trial illustrate the significant risk of insertional oncogenesis related to retroviral gene therapy in stem cells. However, several significant differences exist between the X-linked SCID trial and our proposed adoptive T cell trial. First, whereas the X-linked SCID trial involved gene transfer into undifferentiated pluripotent stem cells, our trial involves modification of fully differentiated T cells. Second, whereas stem cells undergo a high number of recombination events during maturation after retroviral transduction (potentially creating secondary mutations that could enhance the potential of generating a leukemic clone), mature T cells no longer undergo further differentiation, and recombination thereby minimizes the risk of further oncogenic mutations. Viewed from another perspective, patients enrolled in the X-linked SCID trial had favorable long-term survival. The subsequent T cell leukemias developed >3 years after treatment. In our patient population, prolonging survival to >3 years may represent a significant benefit from therapy, thereby mitigating the potential risk associated with this treatment. Third, it is worth noting that, to date, of the >300 SCID-beige mice treated with retrovirally

modified human T cells in our preclinical studies—with >100 surviving for >1 year—none has had any evidence of clonal human T cell expansion or human T cell leukemia. Fourth, patients treated with T cells genetically modified to express LNGF-R, HSV-tk, neomycin, adenosine deaminase, or an anti–HIV-1 *tat* ribozyme have not developed any evidence of T cell clonal expansion after up to 10 years of follow-up.[62,66-68] Nevertheless, we plan to monitor for insertional oncogenesis in treated patients. Specifically, we will closely monitor T cell number and, when appropriate, assess clonal T cell expansion by use of PCR, FACS, and/or immunoscope analysis, as described above. Finally, in over 100 patients treated with CAR therapy including more than 50 patients at MSKCC, none has to date shown evidence of insertional oncogenesis. Furthermore, our CAR has iCasp9 suicide gene that can be turned on when needed.

11.3.1 Generalized inflammatory response generated by infused genetically modified T cells

The potential toxicities include transfusion reactions at the time of T cell infusion. Although all previously published data regarding the infusion of *ex vivo* expanded T cells have reported it to be safe, the infusion of T cells may nevertheless elicit a generalized inflammatory response. There is a theoretical possibility that the patient will develop fever, chills, arthralgias and myalgias, or diarrhea. Another possibility includes anaphylaxis or immediate hypersensitivity reaction characterized by facial swelling, itching, or difficulty breathing. For this reason, we have chosen an initial T cell dose (3×10^5 CAR T cells/kg) that is significantly lower than those cited in the published literature, thereby minimizing the risk of unanticipated inflammatory reactions. To avoid the possibility of reactions, patients will be premedicated with 650 mg of acetaminophen orally and 25-50 mg of diphenhydramine orally or intravenously. Furthermore, if any untoward cytokine storm occurs, >90% of the infused T cells can be eliminated by intravenous injection of 1 dose of AP1903.

11.3.2 Description of reported DLTs after CAR treatment

A thorough description of expected DLTs, based on previous data on use of CARs, can be found in the following publications.

1) Adverse events following infusion of T cells for adoptive immunotherapy: a 10-year experience. *Cytotherapy.* **2010**;12:743-749.

Summary: The authors reviewed infusion-related AEs following administration of *ex vivo* expanded T cell products (antigen-specific CTLs, allo-depleted T cells, and genetically modified T cells) in IND studies. From 1998 to 2008, 381 T cell products were administered to 180 recipients, enrolled in 18 studies, receiving T cells targeting malignancies or posttransplant viral infections. There were no grade III or IV infusion reactions during initial monitoring or the 24-hour follow-up. Twenty-four mild (grade I to II) AEs occurred in 21 infusions, either during or immediately after infusion (up to 6 hours); the most common AEs were nausea and vomiting (10/24; 41.6%), probably because of the dimethyl sulfoxide cryoprotectant, and hypotension (20.8%), attributable to premedication with diphenhydramine. Twenty-two additional nonsevere events were reported within 24 hours of infusion; most common among these were culture-negative fever, chills, and nausea. An increased risk of AEs was associated with age (incidence rate ratio [IRR], 0.98; 95% confidence interval [CI], 0.96 to 1.00; $P < 0.05$), while the risk of immediate infusion-related events was higher in patients reporting allergies (IRR, 2.72; 95% CI, 1.00 to 7.40; $P < 0.05$). Sex, disease type, and T cell source (allogeneic or autologous) had no effect on the frequency of AEs. The authors conclude that infusion of these T cell products was safe in the outpatient setting and was associated with no severe reactions, so monitoring for 1 hour after infusion is probably sufficient. As many of the AEs were attributable to premedication with diphenhydramine, a lower dose (0.25 mg/kg) should be selected.

2) Case report of a serious adverse event following the administration of T cells transduced with a CAR recognizing ERBB2. *Mol Ther.* **2010**;18(4):843-851.

Summary: An optimized CAR vector containing CD28, 4-1BB, and CD3ζ signaling moieties was assembled in a γ-retroviral vector and used to transduce autologous peripheral blood lymphocytes from a patient with colon cancer that was metastatic to the lungs and liver and refractory to multiple standard treatments. The gene transfer efficiency into autologous T cells was 79% CAR+ in CD3+ cells, and these cells demonstrated high specific reactivity in *in vitro* coculture assays. Following completion of nonmyeloablative conditioning, the patient received 1×10^{10} cells intravenously. Within 15 minutes after infusion, the patient experienced respiratory distress and displayed a dramatic pulmonary infiltrate on chest X-ray. She was intubated, and despite intensive medical intervention, the patient died 5 days after treatment. Serum samples obtained after cell infusion showed marked increases in IFN-γ, GM-CSF, TNF-α, IL-6, and IL-10, consistent with a cytokine storm. The authors speculated that the large number of administered cells localized to the lung immediately after infusion and were triggered to release cytokine by the recognition of low levels of ERBB2 on lung epithelial cells.

3) CARs on track in the clinic. *Mol Ther.* **2011**;19(3):432-438.

Summary: A patient death related to treatment with anti-CD19 CAR occurred shortly after the patient received cyclophosphamide for lymphodepletion and infusion of CAR-transduced cells. Although the precise etiology of this patient's death remains uncertain, it was consistent with an inflammatory cytokine cascade after administration of cyclophosphamide, which worsened after infusion of T cells, to give a clinical picture of acute sepsis, renal failure and resultant shock, and adult respiratory distress syndrome. Importantly, this patient's death did not appear to be directly caused by the cellular product, and this trial has been reopened

4) Toxicity management of CAR T cell therapy at MSK: Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med 2014 Feb 19:6(224).

Summary: The Center for Cell Engineering group reported on 16 patients with relapsed or refractory B cell acute lymphoblastic leukemia (B-ALL) treated with autologous T cells expressing the 19-28z chimeric antigen receptor (CAR) specific to the CD19 antigen. The overall complete response rate was 88%, which allowed to transition most of these patients to a standard-of-care allogeneic hematopoietic stem cell transplant (allo-SCT). Through systematic analysis of clinical data and serum cytokine levels over the first 21 days after T cell infusion, our group has defined diagnostic criteria for a severe cytokine release syndrome (sCRS), with the goal of better identifying the subset of patients who will likely require therapeutic intervention with corticosteroids or interleukin-6 receptor blockade to curb the sCRS. Additionally, we found that serum C-reactive protein, a readily available laboratory study, can serve as a reliable indicator for the severity of the CRS. This data provides a strong rationale in monitoring CAR T cell therapy patients and expected toxicity management.

The use of 19-28z gene-modified T cells in adults with CLL

We have infused 19-28z gene-modified T cells in eight adults with CLL. The first three adults were treated with 19-28z gene-modified T cells (3×10^7 T cells/kg) and no DLTs were noted. All three patients have died from complications from progressive CLL. The fourth patient was pre-treated with cyclophosphamide (1.5 gm/m^2), which was followed by a single dose of gene-modified T cells (3×10^7 T cells/kg). Unfortunately, this patient died approximately two days after receiving the T cells. The protocol was placed on hold to allow a comprehensive evaluation of this death. In addition, an autopsy was granted and performed. After a thorough review of all clinical data by institutional and federal regulators the leading suspicion for cause of death is sepsis. However, we cannot rule out that death was related to infusion with the gene-modified T cells so we have updated the consents to include the above information. In addition, we have modified the protocol to 1) split the dose and 2) reduce the

total dose of T cells (1 x 10^7 T cells/kg). Since these changes, six other patients have been treated with cyclophosphamide and split-dose, reduced dose T cells without any significant complications to date.

Nonetheless, it remains possible that these anti-mesothelin targeted chimeric antigen receptor T cells used here could result in serious cytokine release syndrome, or in off-tumor, on-target effects on mesothelin expressing tissues. In addition, two patients had neurological events after receiving CAR T cells. To monitor cytokine release syndrome and possible neurological events, a treatment algorithm is developed as outlined in section 9.5. On-target, Off-tumor toxicity is a major reason for the incorporation of an inducible suicide gene as described below which would be used to eradicate the genetically engineered cells if substantial, unexpected autoimmune toxicity was observed. If Grade 4 toxicity is observed, or if Grade 3 toxicity is observed and believed to be causing substantial risk to the patient, then the AP1903 drug will be used as a suicide switch to deplete the genetically engineered cells. The proof-of-principle of this approach has been used in a graft-versus-host disease clinical setting and substantial preclinical data demonstrates that this agent is capable of rapid, efficient eradication of >99% of transduced T cells. Given the steep dose response curves with chimeric antigen receptors, we are confident that such two log depletion would be sufficient to prevent substantial clinical toxicity.

11.3.3 INCORPORATION OF CASPACIDE CELL THERAPY SAFETY SWITCH AS A SAFETY MEASURE

Given the theoretical risk of toxicity in this trial due to on-target, off-tumor effects on mesothelin+ non-malignant tissues, we have incorporated a suicide switch into the genetic vector that would allow rapid killing of the engineered cells in the event of unacceptable toxicity. The transgene iCasp9 consists of the sequence of the human FK506-binding protein (FKBP12; GenBank number, AH002818) with an F36V mutation, connected through a Ser-Gly-Gly-Gly-Ser linker to the gene encoding human caspase 9 (CASP9; GenBank number, NM001229), which has had its endogenous caspase activation and recruitment domain deleted. FKBP12-F36V binds with high affinity to an otherwise bioinert small-molecule dimerizing agent, AP1903. In the presence of AP1903, the iCasp9 promolecule dimerizes and activates the intrinsic apoptotic pathway, leading to cell death. The safety and efficacy of the transgene was initially tested *in vitro* and in small-animal models, and subsequently shown to mediate efficient apoptosis and clearance of GVHD inducing T cells in humans following allogeneic stem cell transplantation. Indeed, a single dose of the drug (0.4 mg/kg as a 2 hour infusion), given to four patients who developed GVHD, led to elimination of more than 90% of the genetically engineered T cells within 30 minutes of administration, followed by a subsequent reduction of 0.5-1.0 log during the subsequent 24 hours. GVHD resolved and did not recur. The dimerizing inducing agent is clinically inert and lacks biologic activity other than inducing apoptosis of genetically engineered cells. Experience in humans comprises the study by DiStasi noted above, a follow on study of an additional 10 patients, 3 of whom similarly responded rapidly and completely with resolution of GvHD after administration of AP1903, as well as a pharmacokinetic analysis of AP1903 in 28 healthy male volunteers. Doses of 0.01-1.0 mg/kg resulted in plasma concentrations ranging from 10-1275 ng/ml with a short T1/2 (plasma levels of 18% of maximum 30 minutes after infusion and 7% of maximum 2 hours after infusion)[74] The drug was well tolerated without significant toxicity. In a third trial, 18 men with progressive prostate cancer were enrolled in a dose escalation trial of dendritic cells that had been engineered to express prostate specific membrane antigen (PSMA) and iCASP9, with a total of 6 cycles/patient. 24 hours after each dendritic cell injection, the men received AP1903 to induced death of the injected dendritic cells. One patient exhibited a cytokine reaction following AP1903, presumably related to the dendritic cell lysis and a second patient experienced urticarial and flushing after AP1903. Subsequently, all patients received Benadryl and Tylenol premedication and significant toxicity was not observed (ASCO, Abstract #4670, 2011). In all trials, the dose of AP1903 has been 0.4 mg/kg

infused IV over two hours. In this trial, patients would receive AP1903 in the event of unacceptable toxicity.

11.4 Risk of infection

There is a small risk of cotransmission of microbial pathogens during the infusion of cultured T cells. For this reason, cultures of infused preparations will be obtained before infusion, to exclude transmission of viral, bacterial, and fungal pathogens. This testing typically takes 2 weeks following T cell production.

11.5 Risk of the generation of RCRs

To minimize the risk of RCRs, all vector stocks will be tested in accordance with FDA and RAC guidelines before use in T cell transduction. Furthermore, patient T cells will be tested for RCRs at the time of treatment, and peripheral blood T cells from patients will be tested for RCRs prior to T cell infusion, 3, 6, 12 months and yearly after for 15 years , samples will be archived if the RCR testing are negative during the first year.

11.6 Risk of insertional mutagenesis in genetically modified T cells

The risk that random retroviral insertion may result in adverse mutations in modified T cells is likely very low. Furthermore, this remote risk is markedly less significant than the risks of hormone-refractory MPD, for which this treatment is designed. To date, no deleterious mutational events have been reported in patients treated with retrovirally modified T cells. The inclusion of the ICaspase-9 "suicide gene," which confers sensitivity to the prodrug AP1903, provides a potential safeguard as a treatment option if a T cell malignancy were to arise.

11.7 Retroviral transmission to offspring

This is not applicable in this patient population. Although this AE has not been observed in other trials that involved transduction of retrovirally modified T cells, barrier methods of contraception will be strongly recommended to all patients enrolled in the study.

11.8 Cyclophosphamide toxicity

Patients will be closely monitored for the following toxicities and will be treated with appropriate medical management in these settings.

- Nausea, vomiting (patients will receive antiemetics as detailed in section 9.1.2 before and during treatment, to prevent this side effect), diarrhea
- Acute water retention attributable to inappropriate antidiuretic hormone release (hydration will be closely monitored and acute water retention treated with diuretics; hydration will be maintained to ensure adequate urine output)
- Cardiomyopathy (pretreatment cardiac function will be assessed to define eligibility); patients will be monitored during treatment to ensure against cardiac dysfunction; as only 1 dose of cyclophosphamide will be administered, the likelihood of cyclophosphamide-induced cardiomyopathy is minor
- Hemorrhagic cystitis (patients with a history of treatment with cyclophosphamide or any other chemotherapy will be excluded)
- Fatigue, loss of fertility, nail changes, mouth sores, secondary malignancies

Common Toxicities Associated with Cyclophosphamide

Hematologic	leukopenia, thrombocytopenia, anemia
Gastrointestinal	anorexia, nausea and vomiting (common with doses > 600mg/m^2), diarrhea
Hepatic	hepatitis, elevations in SGOT and SGPT
Renal	hemorrhagic cystitis
Cardiac	cardiotoxicity with high doses (> 120 mg/kg)
Respiratory	high doses may cause interstitial pulmonary fibrosis
Dermatologic	alopecia, facial flushing
Miscellaneous	nasal stuffiness, syndrome of inappropriate anti-diuretic hormone (SIADH) induced hyponatremia, fever after high-dose therapy

Fever in the setting of neutropenia will be managed in the Thoracic Surgery Inpatient Service at MSKCC with broad-spectrum intravenous antibiotic treatment. Patients will remain hospitalized until neutropenia and fevers have resolved. Patients with radiographic evidence of infection at the time of admission will be discharged, at the treating physician's discretion, once neutrophil reconstitution and radiographic evidence of resolving infections are established.

11.9 Risks of leukapheresis

To obtain sufficient quantities of T lymphocytes for the production of T cells for use in this trial, patients will undergo a leukapheresis procedure. The required volume, generally 250 to 270 mL, is obtained during a 3-hour leukapheresis procedure. The risks of leukapheresis can involve occasional vasovagal responses to venipuncture and minimal hemodynamic alterations associated with single-unit phlebotomies. To protect against these risks, leukapheresis will be conducted in the Blood Bank Donor Room, with full medical and nursing supervision and support systems to prevent and/or address AEs.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

12.1 Criteria for Response Assessment

The clinical benefit rate will be defined as the proportion of patients with a response of complete response (CR), partial response (PR), and stable disease (SD) at 12 weeks following the first dose of pembrolizumab as measured by mRECIST criteria.

Patients will receive a CT chest with IV contrast at baseline prior to the administration of preconditioning cyclophosphamide. Thereafter, patients will receive a serial CT chest with IV contrast 4 weeks (+/- 7 days) following the CAR T cell administration and two weeks (+/- 7 days) following the third dose of pembrolizumab. Additional scans may also be performed at any time based on the investigator and/or treating physician's discretion.

Modified RECIST (mRECIST) criteria for mesothelioma will be used to assess pleural mesothelioma using CT (with contrast) scans of the chest.[103] At the level of the pleura, tumor thickness perpendicular to the chest wall or mediastinum is measured in 2 positions at 3 separate levels on transverse cuts of T scans. The sum of measurements define a pleural unidimensional measure. Transverse cuts, at least 1 cm apart and related to anatomical landmarks in the thorax are chose to

allow reproductive assessments at later time points. If the measure tumor is presents, transverse cuts in the upper thorax, above the level of division of the main bronchi are preferred. Nodal, subcutaneous, and other bidimensionally measurable lesion are measured unidimensional per RECIST criteria. Unidimensional measurements are added to obtain the total tumor measurement

Modified RECIST criteria for pleural mesothelioma are as follows:

Complete Response: Complete response (CR) as defined as the disappearance of all target lesions with no evidence of tumor elsewhere.

Partial Response: Partial response is defined as at least a 30% reduction in the total tumor measurement

Stable Disease: Stable disease is defined as subjects who fulfilled the criteria for neither PR nor PD

Progressive Disease: Progressive disease is defined as an increase of at least 20% in the total tumor measurement over the nadir measure, or the appearance of one or more new lesions

12.2 Criteria for Non-Measurable Lesions

Response in patients who do have not mesurable pleural disease by mRECIST criteria will be assessed by the PI and treating physician. Response will be determined by considerations of clinical assessment (no additional treatments other than CAR T cells and pembrolizumab were administered), radiographic review (the absence of POD) and serum SMRP (<2 fold change from baseline).

12.3 Considerations of Pseudoprogression

An initial increase in tumor burden of appearance of new lesions could precede immunotherapy-induced tumor regression (Wolchok et al.2009). According to this model of response, subjects initially assessed as POD by modified RECIST or iRECIST criteria for mesothelioma, in the absence of significant clinical deterioration warranting discontinuation of study treatment will continue treatment and receive a confirmatory scan at least 4 weeks later. The following criteria will be used to determine if study treatment is continued:

- If the tumor burden at the confirmatory scan is more than 20% larger than the tumor burden at the initial PD scan, the subject will be considered to have confirmed PD and will be discontinued from study treatment
- If the tumor burden at the confirmatory scan is within 20% of the tumor burden at the initial scan, the subject will be considered to have SD and will continue treatment until the next scheduled scan 3 months after the initial PD. Any subsequent scheduled tumor assessment visit showing that the tumor burden is more than 20% larger than the tumor burden at the initial PD will be considered as confirmed PD, and the subject will be discontinued from study treatment

12.4 Exploratory Efficacy Assessments

Exploratory immune monitoring assays: processing and testing

Assessment of the presence and quantity of immune responses to mesothelin will be performed by Dr. Adusumilli's laboratory, in collaboration with Dr. Jedd Wolchok at the Ludwig Institute for Cancer Research's Immune Monitoring Facility and Dr. Isabelle Riviere at the Cell Therapy and Cell Engineering Facility.

Flow cytometric assay

This assay detects antibodies that recognize native cell-surface mesothelin on 3T3 cells that have been transfected with mesothelin. Controls for this assay consist of nontransfected 3T3 cells or mesothelin-negative cells: 1×10^6 cells from either cell line are washed in phosphate buffered saline (PBS) containing 0.25% (w/v) bovine serum albumin (staining buffer). Serum is then added (1:100 dilution in PBS) and incubated on ice for 30 minutes. After washing in staining buffer, the cells are incubated with antihuman IgG–fluorescein isothiocyanate (Caltag) for 30 minutes on ice. Cells are washed again in staining buffer and analyzed by flow cytometry using a FACSCalibur instrument (Becton-Dickinson). A positive response is defined as an increase in mean fluorescence intensity that is 5 standard deviations above the mean value for the preimmune serum (assayed in triplicate).

Cellular immunity (mesothelin-specific T cell assay)
All pre- and postimmunization PBMC samples will be analyzed by a mesothelin-specific IFN-γ enzyme-linked immunosorbent spot assay (Immune Monitoring Facility) and by cytotoxic activity (Cell Therapy and Cell Engineering Facility). This analysis will be completed when possible by the CTCEF.

Persistence of genetically modified T cells
The quantification of persisting, genetically modified T cells in peripheral blood, and any other cellular specimens will be performed by quantitative PCR (Dr. I. Riviere, Cell Therapy and Cell Engineering Facility).

12.5 Bioeffect summaries
The following measure will be summarized by dose level:

Changes in the biomarker SMRP, assessed at screening and at the week 1 post operative visit.

13.0 CRITERIA FOR REMOVAL FROM STUDY
Withholding of CAR T cell administration
If a subject experiences a DLT, as defined in Section 9.2, or if a diagnosis of encephalitis is made, no further study medication will be administered to that subject. Other reasons for the termination of treatment include:

- Initiation of alternative therapy for MPD
- Subject withdraws consent to continue in the trial
- Subject develops an AE or intercurrent illness (regardless of a DLT) that precludes further treatment
- The investigator withdraws the subject, in the subject's best interests
- Subject is lost to follow-up (defined as the inability to contact the subject on 3 separate occasions during a period of 2 months)
- Administrative reasons (e.g., the subject is transferred to hospice care or the subject is unwilling to continue or comply)

Emergency situations
To date, life-threatening events have been rare in a similar MSKCC trial treating patients with CLL ("A Phase I Trial for the Treatment of Purine Analog-Refractory Chronic Lymphocytic Leukemia Using Autologous T Cells Genetically Targeted to the B Cell Specific Antigen CD19," MSKCC IRB protocol 06-138, J. Park, PI). The potential problems could include headache, fever, and chills, which may not be directly relevant to the clinical trial, to cytokine release syndrome which occurs close to infusion while the patient is in-house. Patients will be asked to contact our office in the event of any untoward or unusual symptoms. Patients will be instructed at the time of initiation of the trial

that, if they experience any untoward event(s) and are not able to contact our office, they are to proceed to the nearest emergency room for triage and possible treatment. Dr. Adusumilli or his designee will be the contact person should this occur.

Replacement of Patients
Patients who are unable to complete 3 doses of pembrolizumab for reasons other than adverse events related to the study treatment will be replaced for the assessment of efficacy. However, all patients, including those replaced, will be followed for safety monitoring and the outcomes recorded.

14.0 BIOSTATISTICS

14.1 Phase I

Dose-escalation algorithm
It is anticipated that 11 dose levels will be evaluated in this treatment protocol, as summarized below. Patients will be treated in sequential groups of 3 to 6 patients per T cell dose. The projected trial size for this study is a minimum of 4 and a maximum of 54 patients. The trial will proceed using the dose escalation scheme described in section 9.1.4.

Table 4*

Cohort	Dose **
-1*	1×10^5
1	3×10^5
2	3×10^5+Cyclophosphamide
3	1×10^6+Cyclophosphamide
4	3×10^6+Cyclophosphamide
5	6×10^6+Cyclophosphamide
6	1×10^7+Cyclophosphamide
7	3×10^7+Cyclophosphamide
8	6×10^7+Cyclophosphamide
9	6×10^7+Cyclophosphamide + cyclophosphamide

*Necessary only if toxicity is encountered at the initial dose level.
**Mesothelin-targeted T cells/kg; intermediate dose levels may be evaluated, if indicated. Patient may receive an altered fractionation of the total dose or up to 35% over or under total cell dose with approval of PI.

Escalation to the next dose level is probable if the risk of DLT is low and if the likelihood of escalation decreases as the risk of DLT increases, as shown below.

True risk of toxicity	0.10	0.20	0.30	0.40	0.50	0.60
Probability of escalation	0.91	0.71	0.49	0.31	0.17	0.08

Analysis of Secondary Objectives Endpoints
The secondary aims of the study will be addressed by descriptive exploratory statistical analyses, since the sample size is not known in advance and there are no formal hypotheses being tested. These analyses may include descriptions of time patterns for continuous variables measured repeatedly, both at the individual level and aggregated by dose level.

Changes in serum levels of the biomarker SMRP will be assessed at screening and at the week 1 post operative visit.

14.2 Phase II
Efficacy of the final dose established in Phase I will be assessed in Phase II using a Simon's two-stage Minimax design. The primary endpoint in Phase II is clinical benefit rate (see Section 12.1) at 12 weeks following the first dose of pembrolizumab.

Determination of Sample Size in Phase II
The null and desired clinical benefit rates are 20% vs 45%. In the first stage, 13 patients will be enrolled; if one or fewer patients clinical benefit at 12 weeks, then accrual will be halted due to futility. Otherwise, the trial will continue to Stage 2 to enroll 8 additional subjects for a total of 21 patients. At the end of Stage 2, if 6 or fewer patients are determined to have clinical benefit in total, then there is insufficient evidence of efficacy. If 7 or more patients are determined to have clinical benefit among all 21 evaluable patients, then there is sufficient evidence to warrant further study. This two-stage design yields 80% power and a type I error of 5%. The expected sample size is 16.99, and the probability of early termination is 0.502 under the null. The six patients treated at the final dose in Phase I will also be included in this Phase II component of the trial. Hence we need to accrue 15 additional evaluable patients.

Analysis of primary and secondary endpoints
We will summarize the primary efficacy endpoint of clinical benefit rate with descriptive analysis, including exact 95% confidence intervals. Phase II patients who do not receive both T cells and 3 doses of pembrolizumab will not be evaluable for efficacy and will be replaced (Section 13.0). The total number of enrolled patients in Phase II component depends upon the number of observed responses and ranges from 13 – 21 evaluable patients, including the 6 patients treated at the final dose in Phase I.

Secondary endpoints: The toxicity profile of the combination therapy will be further assessed based on Phase II patients. Progression-free survival (PFS) (defined from time from first dose of treatment to progression or death due to any cause) will be estimated using Kaplan-Meier method at 6, 12, 18 and 24 months. A 1-year PFS rate of 40% will be considered of interest. Overall survival (OS) will be summarized similarly, along with median PFS and median OS.

Response rate (CR + PR) by mRECIST and iRECIST criteria and best response will be summarized as proportions, along with exact 95% confidence intervals. Duration of best response (for responders with measurable disease) is defined as the time from initial CR or PR to documented disease progression or death due to any cause, whichever occurs first, and summarized descriptively using the Kaplan-Meier approach. Duration of response will be evaluated for patients with response of CR or PR. Patients without disease progression or death due to any cause before the data cutoff date will be right censored at the time of last tumor assessment date before the data cutoff.

Longitudinal trajectories of patient's weight/BMI and serum SMRP (measured periodically) will be assessed using linear mixed-effects models with patient-level random effects. The models will include fixed effect of time since first dose of the treatment and the baseline measurements (measured prior to lymphodepleting cyclophosphamide).

To prevent excessive DLT rate among the additionally enrolled patients at the MTD level in Phase II, we will continue safety and toxicity monitoring. Whenever >=4 DLTs are observed at the recommended Phase II dose, the enrollment will be halted, and investigators will thoroughly review all cases and toxicities. All patients from all dose levels will be analyzed for secondary objectives. All toxicity profiles will be summarized and tabulated by dose level. Even if the trial is halted due to

futility after Stage 1 of the Phase II trial, all available patients will be assessed for efficacy, toxicity profiles, and secondary endpoints.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures. During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming that the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

15.2 Randomization

Not applicable.

16.0 DATA MANAGEMENT ISSUES

A clinical research coordinator will be assigned to the study. The responsibilities of the research study assistant include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol study team.

The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

16.1 Data entry

Data collected during this study will be entered into a secure electronic database: the MSKCC Clinical Research Database (CRDB). The CRDB system is compliant with regulations as required by 21 CFR 11. Data entered into the CRDB are stored indefinitely.

16.1.1 Source documents

Study personnel will record clinical data in each patient's source documents (i.e., the patient's medical record). Source documentation will be made available to support the electronic database. Source documentation is stored in the patient's electronic medical record indefinitely. The electronic medical record is the MSKCC gold standard for clinical care. This includes but is not limited to electronic signatures, optical images of patient records, consent forms, and electronic data provided from MSKCC source clinical systems. The compliance requirements for all of the above are stated in "*Part 11, Electronic Records, Electronics Signatures-Scope and Application*."

16.1.2 Record retention

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator will maintain all source documents, study-related documents, and the electronic data.

16.2 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period, and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team at a minimum of 2 times per year, and more frequently if indicated.

16.3 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) plans at MSKCC were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials," which can be found at http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM plans at MSKCC were established and are monitored by the Clinical Research Administration. The MSKCC DMS plans can be found on the MSKCC intranet at https://one.mskcc.org/sites/pub/clinresearch/Documents/MSKCC Data and Safety Monitoring Plans.pdf

There are several different mechanisms by which clinical trials are monitored for data, safety, and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research quality assurance) and departmental procedures for quality control, plus there are 2 institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees—the Data and Safety Monitoring Committee for Phase I and II clinical trials and the Data and Safety Monitoring Board for Phase III clinical trials—report to the center's research council and IRB.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group) will be addressed, and the monitoring procedures will be established at the time of protocol activation.

The mesothelin CAR T cell infusion-related reactions, adverse events occurred and the management will be reported to the CAR T cell oversight committee. Any protocol changes or deviations will be immediately reported to the CAR T cell oversight committee.
Decisions to escalate to the next level or, when appropriate, to deescalate to a lower level will be recommended to the institutional CAR T cell Oversight Committee by the principal investigator (PI). The CAR T cell Oversight Committee will make the final decision of whether to proceed to the next dose level. In addition, the PI will coordinate regular meetings/communication with CAR T cell Oversight Committee to

a. present the data for each cohort of patients and study progress
b. discuss and obtain recommendations prior to escalating to the next dose level or if appropriate deescalating the dose level
c. report any significant toxicities associated with the CAR T cell intervention.
d. present any proposed significant changes in the protocol.

The CAR T cell Oversight Committee will not replace the IRB. Any AEs, protocol deviations, etc., still need to be reported to OCR/IRB as well as the CAR T cell Oversight Committee

16.4 External Collaboration

MSKCC is collaborating with Atara Biotherapeutics to develop the next-generation mesothelin targeted-CAR product (the product being tested in 15-007 is the first generation product). To facilitate the next generation product development, deidentified patient level clinical data including but not limited to patient demographics, disease characteristics, clinical safety and efficacy data, radiographic data including deidentified scans and time point assessment of response, as well as correlative data for all patients treated on trial may be shared. Data may also be shared with any potential sublicensees of Atara Biotherapeutics.

17.0 PROTECTION OF HUMAN SUBJECTS

Inclusion of Children in Research: This protocol/project does not include children because the MPD is not seen in children. This statement is based on exclusion 4b of the NIH Policy and Guidelines on the Inclusion of Children as Participants in Research Involving Human Subjects.

Risks and possible toxicities/side effects: Discussed in Section 11.

Potential benefits: There is a potential benefit to society as a whole, in that a successful phase I study could lead to a larger phase II trial which would improve our understanding of the treatment of these diseases and potentially extend survival for patients with MPD. Participation in this study may not provide any benefit to the individual participant, and may or may not provide information which will ultimately benefit others.
Adverse event reporting: Discussed in section 17.2.

Alternative treatment options may include standard chemotherapy off-study, participation in a different clinical trial, or best supportive care/ palliative care.

Financial Costs/Burdens: Patients will be responsible for the costs related to treatment and complications of treatment. Costs to the patient (third party insurer) will include cost of supportive medications and their administration, cost of T cell administration, hospitalizations (including intensive care unit), routine blood and urine tests, diagnostic studies, office visits, EKG, imaging studies, physician services, adult day hospital and outpatient costs. Patients will not be charged the cost of analysis for the research correlates. Costs of generating genetically-modified T cells will not be billable to research participants.

Voluntary Nature of the Study: Participation in this study is voluntary. Whether or not a patient chooses to participate in this study will not affect the availability of standard, supportive, or other investigational treatment at MSKCC.

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the PI and approved by the IRB and Privacy Board.

The consent indicates that individualized de identified information collected for the purposes of this study may be shared with other qualified researchers. Only researchers who have received approval from MSK will be allowed to access this information which will not include protected health information, such as the participant's name, except for dates. It is also stated in the Research Authorization that their research data may be shared with other qualified researchers.

17.2 Serious Adverse Event (SAE) Reporting

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

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- 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

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant's last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

All SAEs must be submitted in PIMS. If an SAE requires submission to the HRPP office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be submitted within 5 calendar days of the event. All other SAEs must be submitted within 30 calendar days of the event.

The report should contain the following information:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment(s)
- If the AE was expected
- Detailed text that includes the following
    - An explanation of how the AE was handled
    - A description of the participant's condition
    - Indication if the participant remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

SAE requiring AP1903 administration will be reported to Bellicum Pharmaceuticals.

17.2.1 SAE Reporting to the FDA

For IND/IDE protocols: The SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the IND Office

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are performed, consenting professionals will explain full details of the protocol and study procedures, as well as the risks involved, to participants before their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB-/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the IRB/Privacy Board of this center. The consent form will include the following:

1. The nature and objectives, potential risks, and benefits of the intended study
2. The length of study and the likely follow-up required
3. Alternatives to the proposed study (this will include available standard and investigational therapies; in addition, patients will be offered an option of supportive care for therapeutic studies)
4. The name of the investigator(s) responsible for the protocol
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time

Before any protocol-specific procedures can be performed, consenting professionals will fully explain the aspects of patient privacy concerning research-specific information. In addition to signing the IRB informed consent form, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 APPENDICES

APPENDIX I: GUIDELINES FOR THE GENE TRANSFER AND MANUFACTURING
APPENDIX II: Mesothelin – targeted CAR T Cell Flowsheet
APPENDIX III: Mailed Screening Informed Consent Cover Letter

CAR T-Cell Manufacturing
Details of the CAR T-cell manufacturing process (28) and analyses are described in the protocol (available on request).

The leukapheresis product is processed by the Cell Therapy and Cell Engineering facility (CTCEF) according to CTCEF SOPs. The leukapheresis product is washed in PBS with 1% HSA using a closed system cell washer (either COBE 2991 or Cell Saver 5+). Post-wash and prior to cryopreservation, the content in $CD3^+$, $CD4^+$, $CD8^+$, $CD28^+$ and CD45+ cells is determined by flow cytometry by the MSKCC Cellular Immunology laboratory. Cells are aliquoted in cryobags and frozen using a controlled rate freezer (Cryomed or equivalent) and stored in vapor phase liquid nitrogen (-150 °C). A viability assay is performed on sentinel vials to determine post-thaw viability.
T cells are isolated and activated by Dynabeads® ClinExViVo CD3/CD28 and transduced with cell-free clinical grade ICasM28z viral vector stocks produced in the CTCEF. T cells are transduced in X-Vivo 15 media by spinoculation in tissue culture bags coated with Retronectin (Takara Biomedicals, Japan) as previously described (28). Briefly, the transduced T cells are expanded in the Wave®/Xuri bioreactor (Cytiva Lifesciences, MA) and grown in X-Vivo 15 media (Cambrex Bio Science Walkersville, Inc., MD) supplemented with 5% AB serum (Gemini Bio-Products, Inc., CA) with IL-2 (Aledsleukin Proleukin ®, Chiron). Perfusion of complete media is started around day 4 to 7 at rates calculated on the basis of cell density. Viability, cell count and phenotype of the transduced $CD3^+$ T cells (based on the expression of CD3, CD4, CD8, M28z CAR) are determined at the end of the expansion when the cell dose is reached.
Cells are washed with 1% HSA in 1x plasmalyte A using a closed system cell washer.

For apheresis product containing ≥40% CD14+ cells, initially CD14 cells are depleted by surface adherence since we have observed that the CD14+ cells phagocyte the Dynabeads, preventing activation of the CD3+ cells and decreasing transduction efficiency (manuscript submitted). The apheresis is washed and resuspended in X-VIVO 15 with IL-2 and split into 10 Solo flasks. Cells are incubated at 37°C with 5% CO_2 for approximately 1.5 h. Then, non adherent cells are collected and the volume is reduced to 100 mL. For apheresis product containing <40% CD14+ cells, the T cells are selected with Dynabeads ClinExVivo CD3/CD28 as described above. Contaminating beads in the final culture are removed before infusion by magnetic separation with the ClinExVivo (Dynal) magnet separator as per manufacturer's recommendation and CTCEF SOP.

Upon bead removal, the bulk cellular product (EOP) will be prepared by concentrating the volume of T lymphocyte in Plasmalyte A with 1 % HSA based on the patient dose and aliquoted in cryobags (OriGene or equivalent). The samples are collected from the bulk cellular product (EOP) for release quality control tests.