PHASE 1 STUDY OF HUMAN CHIMERIC ANTIGEN RECEPTOR MODIFIED T CELLS IN PATIENTS WITH MESOTHELIN EXPRESSING CANCERS

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LIST OF ABBREVIATIONS

AE, adverse event
CAR, chimeric antigen receptor
CCI, Center for Cellular Immunotherapies
CD137, 4-1BB costimulatory molecule
CFR, Code of Federal Regulations
CRF, case report form
CRM, Continual Reassessment Method
CRS, Cytokine Release Syndrome
CTCAE, common terminology criteria for adverse events
CTL, cytotoxic T lymphocyte
CTX, cyclophosphamide
CVPF, Clinical Cell and Vaccine Production Facility at the University of Pennsylvania
DLT, dose-limiting toxicity
DSMB, Data and Safety Monitoring Board
EOC, epithelial ovarian cancer
FDA, Food and Drug Administration
GCP, Good Clinical Practices
GMP, Good Manufacturing Practices
HACA, human anti-CAR antibody
HAMA, human anti-mouse antibody
IBC, Institutional Biosafety Committee
IP, intrapleural administration
i.p., intraperitoneal administration
IRB, Institutional Review Board
irRC, immune related response criteria
MABEL, Minimum Anticipated Biological Effect Level
MAS, Macrophage Activation Syndrome
MPM, malignant pleural mesothelioma
MPM, MyPennMedicine (UPenn patient portal)
OBD, optimal biologic dose
PBMC, peripheral blood mononuclear cells
PDA, pancreatic ductal adenocarcinoma
PDAE, protocol-defined adverse event
PDSAE, protocol-defined serious adverse event
PDCS, Product Development and Correlative Sciences laboratory
ROA, route of administration
SAE, serious adverse event
SS1, mesothelin specific scFv
TCR, T cell receptor
TCSL, Translational and Correlative Studies Laboratory

UADE, Unanticipated Adverse Device Effect
UPenn, University of Pennsylvania

STUDY SUMMARY

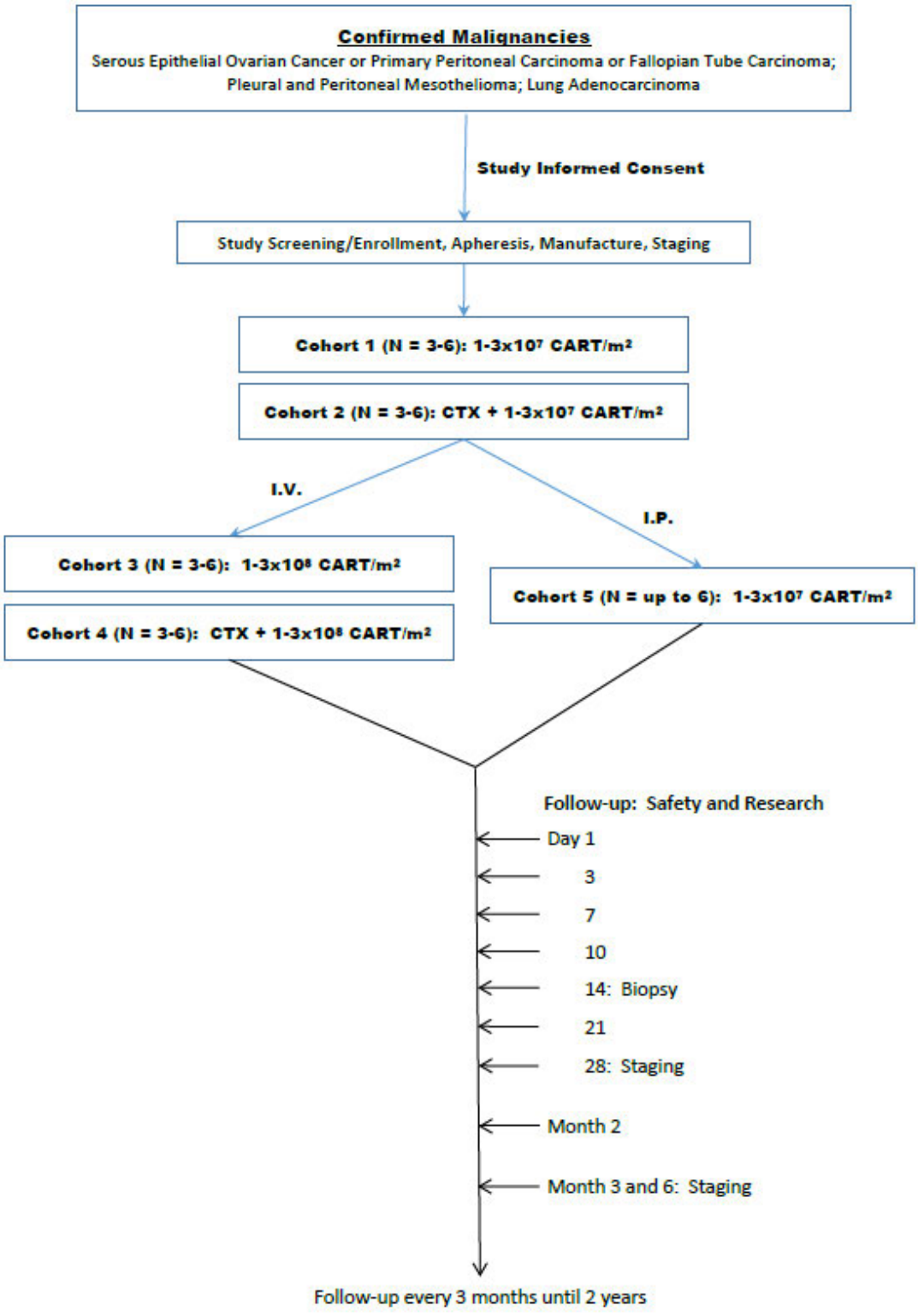
Title	Phase I study of human CAR modified T cells in patients with mesothelin expressing cancers
Short Title	huCART-meso in mesothelin expressing cancers
Protocol Number	UPCC# 02916; IRB# 826085; [redacted]
Phase	Phase 1 - Safety and Proof of Concept
Methodology	Phase I study to establish safety and feasibility of both intravenous administration and local delivery of lentiviral transduced huCART-meso cells with or without lymphodepletion.
Study Duration	This protocol will require approximately 6-8 years. Each subject will be followed for up to 2 years post study treatment on this study. Subject will then be asked to enroll; into a separate long-term follow-up study and followed for up to 15 years post-infusion.
Study Center(s)	University of Pennsylvania
Number of Subjects	Up to 27 evaluable subjects. Evaluable subjects are those who receive the target dose of huCART-meso cells as per their cohort assignment.
Study Design	This is a Phase I study evaluating the safety and feasibility of both intravenous administration and local delivery of lentiviral transduced huCART-meso cells with and without lymphodepleting chemotherapy. • Cohort 1 (N = 3-6): will receive a single dose of 1-3x10^7 huCART-meso cells/m^2 on day 0 without any conditioning chemotherapeutic regimen. • Cohort 2 (N = 3-6): will receive a single dose of 1-3x10^7 huCART-meso cells/m^2 on day 0, following a flat dose of 1 gram/m^2 of cyclophosphamide administered 2-4 days prior to huCART-meso cells (day -4 to day -2). • ~~Cohort 3 (N=3-6): will receive a single dose of 1-3x10^8 /m^2 lentiviral transduced huCART-meso cells on day 0 without any conditioning chemotherapeutic regimen.~~ **Cohort 3 permanently closed with Protocol V5.** • ~~Cohort 4 subjects (N=3-6) will receive a single dose of 1-3x10^8 /m^2 lentiviral transduced huCART-meso cells on day 0, following a flat dose of 1 gram/m^2 of cyclophosphamide administered 2-4 days prior to huCART-meso cells (day -4 to day -2).~~ **Cohort 4 permanently closed with Protocol V5.** • Cohort 5 (N = up to 6): will receive a single dose of 1-3x10^7 huCART-meso cells/m^2 day 0 by intrapleural infusion (IP) through an indwelling pleural catheter without any conditioning chemotherapeutic regimen. The safety of this dose level has been established by Cohorts 1 and 2. • Cohort 6 (N = up to 6): will receive a dose of 1-3x10^7huCART-meso cells/m^2 via IV infusion on Day 0, following a flat dose of 1 gram/m^2 of cyclophosphamide administered 2-4 days prior to huCART-meso cells (~Day -4 to -2). This initial infusion may be followed by up to two additional IV infusions of huCART-meso cells at the same dose level, given between 21-42 days apart, if the subject meets eligibility to receive additional infusions (See **Section 6.8.2**). Cyclophosphamide will not be repeated prior to subsequent doses of huCART-meso cells. Cohort 6 was activated with Protocol V6. Enrollment into Cohort 6 will occur in parallel with Cohort 5.

	• Cohort 7 (N = up to 6): will receive a single dose of 1-3x10^7 huCART-meso cells/m^2 via intraperitoneal (i.p.) administration, following lymphodepleting chemotherapy with cyclophosphamide 300 mg/m^2/day and fludarabine 30 mg/m^2/day given over 3 days by intravenous infusion. Lymphodepleting chemotherapy will be scheduled such that the last day of chemotherapy is 3 days (+/- 1 day) prior to the 1st infusion of huCART-meso cells. This initial i.p. infusion may be followed by up to two additional infusions of huCART-meso cells via intravenous (IV) administration at the same dose level, given between 21-42 days apart. The subject must meet eligibility to receive additional infusions (See **Section 6.9.2**). Lymphodepleting chemotherapy will not be repeated prior to additional infusions of huCART-meso cells. Infusion #1 for the first three subjects in Cohort 7 will be staggered by at least 21 days to allow for the assessment of DLTs. Enrollment into Cohort 7 will occur in parallel with Cohort 5 and Cohort 6. The Maximum Tolerated Dose (MTD) is defined as the dose at which 0-1 DLT occurs in 6 evaluable subjects tested within the dose range of this study. As of Protocol Amendment V5, the maximum tolerated dose has been established as 1-3x10^7 huCART-meso cells/m^2. Adverse events will be collected and evaluated during the protocol specified adverse event reporting period outlined in **Section 9.1**.
Objectives	**Primary objective:** Determine the safety and feasibility of intravenous administration and local delivery of lentiviral transduced huCART-meso cells, with and without lymphodepleting chemotherapy in the target population. **Secondary objectives:** Clinical objectives: 1. Assess the clinical anti-tumor effect by standard criteria (RECIST [modified RECIST for mesothelioma] and immune-related response criteria [where feasible]) for each tumor type. 2. Assess progression-free survival (PFS) and overall survival (OS). Correlative objectives: 1. Evaluate huCART-meso cells engraftment and persistence in peripheral blood and body fluid. 2. Determine the bioactivity of huCART-meso cells in peripheral blood and body fluid. 3. Evaluate the development of anti-CART immune responses favoring rejection of huCART-meso cells (where scientifically relevant). 4. Evaluate the development of secondary cellular and humoral anti-tumor responses as a consequence of epitope spreading. 5. Where tumor material or body fluids can be obtained: a. Measure trafficking of huCART-meso b. Evaluate mesothelin expression on tumor cells to assess for antigen-escape c. Evaluate genetic editing (if feasible)

	d. Analyze tumor microenvironment and cell interactions (if feasible)
Study Population and Main Inclusion Criteria	Patients 18 years of age and older diagnosed with one of the following malignancies that have progressed after at least one prior regimen of standard therapy: • Persistent or recurrent serous epithelial ovarian cancer or primary peritoneal carcinoma or fallopian tube carcinoma • Metastatic or recurrent lung adenocarcinoma- **Recruitment of NSCLC patients suspended with Protocol Amendment V10** • Malignant pleural or peritoneal epithelial mesothelioma.
Investigational Agent(s), Dose, Route, Regimen	Investigational Agent(s): • huCART-meso cells: autologous T cells lentivirally transduced with chimeric anti-mesothelin immunoreceptor M5 scFv fused to the 4-1BB and CD3ζ signaling domains. • Cyclophosphamide and fludarabine: cytotoxic chemotherapy agents used for lymphodepletion prior to huCART-meso cell administration in targeted cohorts. Dose: • huCART-meso: o 1-3x10^7 huCARTmeso cells/m^2 (Cohort 1-2, 5-7) ▪ In the event of 2 DLTs at this dose level, we will dose de-escalate by 10-fold. o 1-3x10^8 huCART-meso cells/m^2 (Cohort 3 and 4). **Cohorts 3 and 4 permanently closed with Protocol V5.** • Cyclophosphamide: o Cohort 2 and Cohort 6: Flat dose of 1 gram/m^2 o Cohort 7: 300 mg/m^2/day given over 3 days • Fludarabine: o Cohort 7: 30 mg/m^2/day given over 3 days Route of Administration: • huCART-meso: o Intravenous (Cohorts 1, 2, 6, and 7; **Cohorts 3 and 4 permanently closed with Protocol V5**) o Intrapleural (Cohort 5) o Intraperitoneal (Cohort 7) • Cyclophosphamide: intravenous (Cohorts 2, 6 and 7; **Cohort 4 permanently closed with Protocol V5)** • Fludarabine: Intravenous (Cohort 7 only) Regimen**:** • huCART-meso cells: o Cohorts 1, 2 and 5: Single dose on Day 0 o Cohort 6: The initial IV infusion may be followed by up to two additional IV infusions of huCART-meso cells at the same dose level, between 21-42 days apart.

	○ Cohort 7: Intraperitoneal (i.p) infusion followed by up to two additional infusions of huCART-meso cells given via IV administration at the same dose level, given between 21-42 days apart. • Cyclophosphamide: single dose administered 2-4 days (~Day -4 to -2) prior to huCART-meso cells (Cohort 2), and Cohort 6 (prior to first infusion only); **Cohort 4 permanently closed with Protocol V5.** • Cyclophosphamide and Fludarabine (Cohort 7 only): given over 3 days; scheduled so that the last dose of chemotherapy falls 3 days (+/- 1 day) prior to CAR T-cell infusion (Day 0).
Duration of administration-huCART-meso cells	Based on the total volume to be infused and the recommended infusion rate of 10-20 ml per minute.
Reference Therapy	None
Statistical Methodology	This is a phase I safety and feasibility study. The statistical analysis will be primarily descriptive in keeping with the exploratory nature of the study. Descriptive statistics will be applied to determine the relative persistence and trafficking to blood (and optionally tumor) of the huCART-meso cells. Data regarding the number of CAR T cells in blood, and the tracking of soluble biomarker levels will be presented graphically. Correlations with radiographic and other standard measures of tumor burden will be determined. We will compute 95% confidence intervals for proportions and means. Adverse events will be collected and evaluated for all subjects during the protocol specified adverse event reporting period outlined in **Section 9.1**. AEs will be graded for severity using the National Cancer Institute (NCI) – Common Terminology Criteria (v4.03). All adverse events will be described and exact 95% confidence intervals will be produced for adverse event rates, both overall and within major categories. The data will be monitored on an ongoing basis for evidence of excessive toxicity. Results will be tabulated and summarized. Rates of clinical responses will be summarized in exact 95% confidence intervals. Distributions of progression-free and overall survival and duration of clinical response will be presented graphically using Kaplan-Meier curves. The two-year survival rates will be presented.

FIGURE 1-1: STUDY SCHEMA (COHORTS 1-5*)



***Cohort 3 and 4 permanently closed with Protocol V5**

FIGURE 1-2: STUDY SCHEMA (COHORT 6)

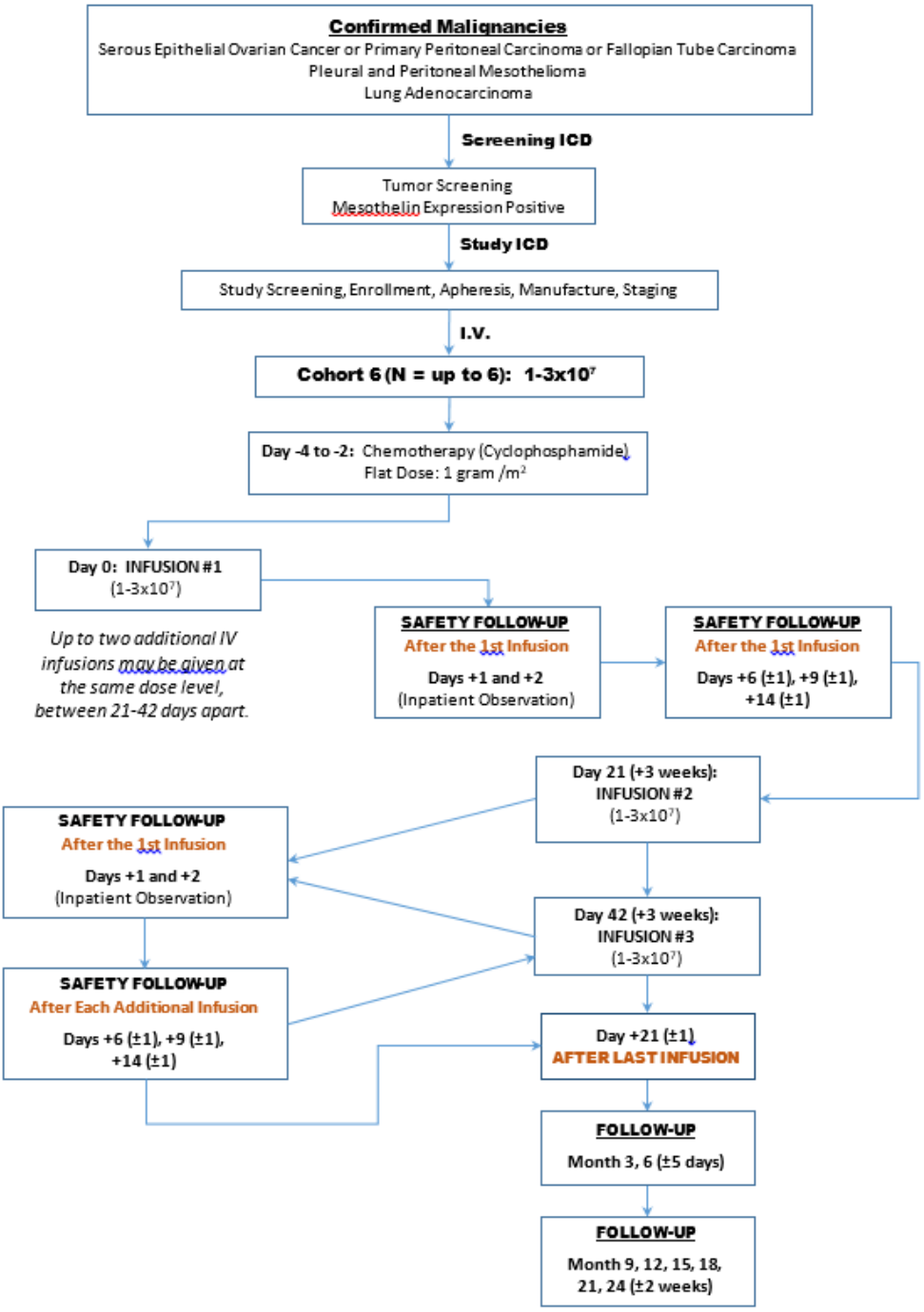
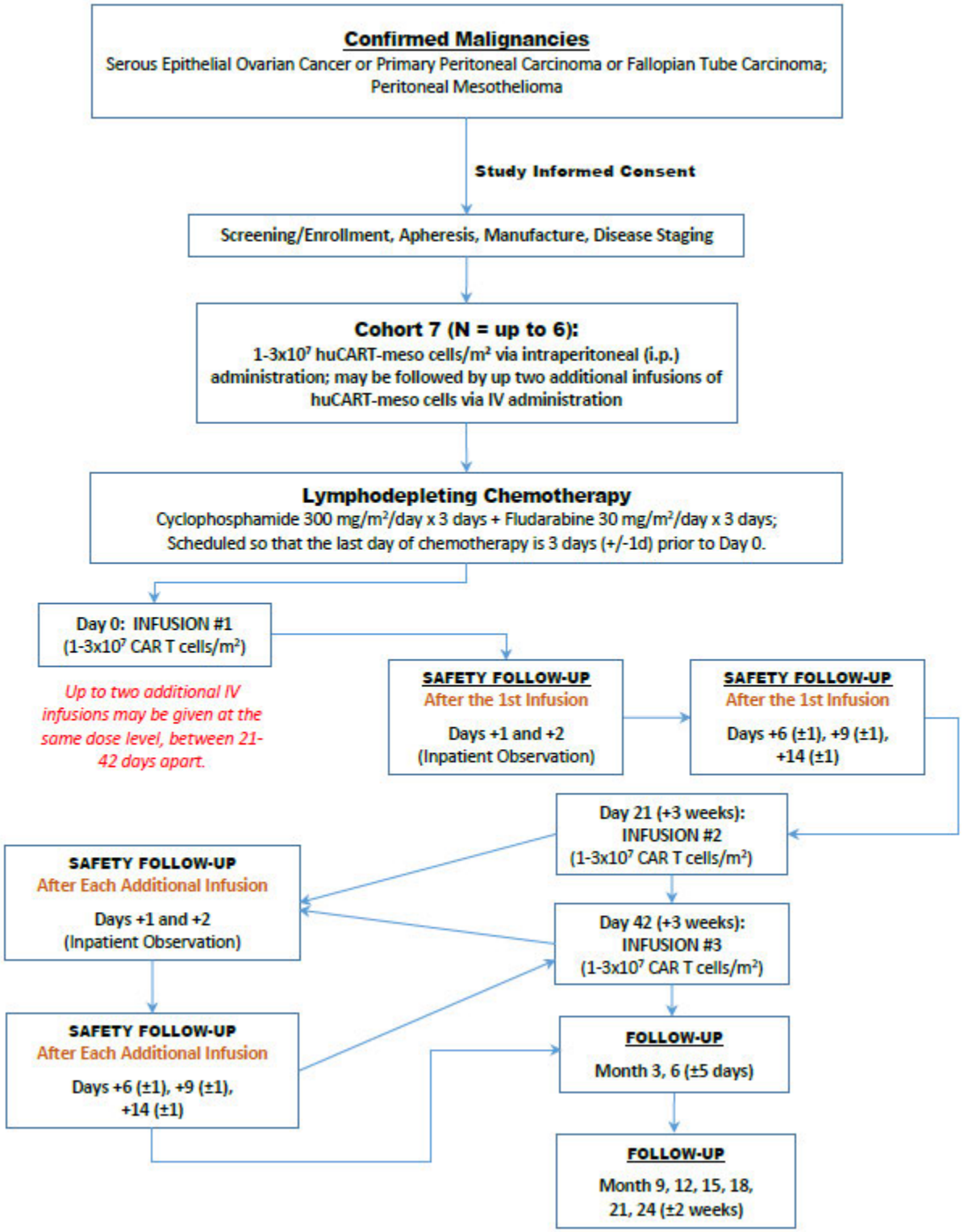


FIGURE 1-3: STUDY SCHEMA (COHORT 7)



1 INTRODUCTION

1.1 Background

1.1.1 Mesothelin

Mesothelin was originally identified by Pastan and colleagues as a tumor associated antigen due to its limited expression by normal tissues and overexpression on tumors (Chang et al., 1992; Chang and Pastan, 1996). The mesothelin gene encodes a precursor 71-kDa protein that is processed to yield the 40-kDa protein, mesothelin, which is anchored at the cell membrane by a glycosylphosphatidyl inositol (GPI) linkage and an amino-terminal 31-kDa shed fragment, called megakaryocyte potentiating factor (MPF). Both fragments contain N-glycosylation sites. A soluble splice variant of the 40-kDa carboxyl-terminal fragment called "soluble mesothelin/MPF-related" has been found in the sera of patients with PDA(Johnston et al., 2009). Mesothelin is currently being explored both as a therapeutic target as well as a bio-marker for disease activity and therapeutic response (Argani et al., 2001).

Mesothelin is a differentiation antigen that is also present on normal tissues. Using the mouse anti-human mesothelin antibody K1 that was developed by the Pastan group, strong K1 reactivity has been demonstrated within mesothelial cells that line the peritoneal, pleural, and pericardial cavities, although at lower levels than usually seen for malignant tissues(Chang et al., 1992). Weak K1 reactivity has been detected within the Fallopian tube epithelium, tracheal basal epithelium and tonsils epithelium. However, K1 reactivity has not been detected in the majority of normal tissues including the liver, kidneys, spleen, bone marrow, lymph nodes, thymus, cardiac muscle, tongue, skeletal muscle, skin, cerebral cortex, cerebellum, spinal cord, peripheral nerve, pituitary, adrenal, salivary gland, mammary gland, thyroid, parathyroid, testis, prostate, epididymis, cervical epithelium, lung parenchyma, esophagus, small-bowel epithelium, colon epithelium, bladder epithelium, gall-bladder epithelium(Chang et al., 1992). Of note, K1 antibody has lower affinity and it stains frozen tissues but not formalin fixed tissue, probably due to the destruction of the epitope upon tissue fixation (Pastan and Hassan, 2014). Mesothelin has also been found on all layers of the cornea(Jirsova et al., 2010).

Epithelial MPM universally expresses mesothelin while sarcomatoid MPM does not express mesothelin (Chang and Pastan, 1996), suggesting that anti-mesothelin targeted therapies will not be successful in this histologic subtype.

Mesothelin is also expressed on a large number of lung cancers, however, in most cases, expression is low. Approximately 25% of lung adenocarcinomas express mesothelin strongly on >25% of cells. Interestingly, this population of high mesothelin expressors is enriched in the Kras-positive patients (Kachala et al., 2014; Thomas et al., 2015).

Most serous epithelial ovarian carcinomas, and the related primary peritoneal carcinomas, express mesothelin (Chang et al., 1992). Mesothelin is a target of a natural immune response in ovarian cancer(Ho et al., 2005), and has been proposed to be a target for cancer immunotherapy(Hassan et al., 2004). The presence of mesothelin-specific CTLs in patients with pancreatic cancer correlates with overall survival (Thomas et al., 2004). In addition, Pastan and coworkers have used soluble antibody fragments of an anti-mesothelin antibody conjugated to immunotoxins to treat cancer patients with mesothelin-positive tumors. This approach has demonstrated adequate safety and some clinical activity in pancreatic cancer (Hassan et al., 2007a; Hassan et al., 2007b). In ovarian cancer, this therapeutic strategy produced one minor response by RECIST criteria and stable disease in a second patient who also had complete resolution

of their ascites. Fallopian tube carcinoma is a rare type of carcinoma that is similar to epithelial ovarian cancer from the clinical and histological perspective. The standard of care also follows the principles applied to the epithelial ovarian cancer patients (Pectasides et al., 2006). Weak expression of mesothelin was detected on this tumor type using the mouse anti-human mesothelin antibody K1 (Chang et al., 1992). Additional supportive preclinical data for targeting mesothelin has been published (Stromnes et al., 2015).

1.1.2 Lung Adenocarcinoma, Epithelial Ovarian Cancer, and Mesothelioma

Subjects with one of these three cancers have been chosen as the study population here because of the frequent expression of the target antigen mesothelin in these three types of malignancies. Each malignancy, including the current conventional and immunotherapeutic approaches for treating it, is discussed below.

1.1.2.1 Lung Adenocarcinoma

Lung cancer is the leading cause of cancer related mortality in the United States. NSCLC (non-small cell lung cancer) accounts for approximately 85% of all lung cancers and encompasses different histologies including squamous cell carcinoma, large cell carcinoma, and adenocarcinoma (Siegel et al., 2015). Most NSCLC patients present with loco-regionally advanced or metastatic disease where response rates and median overall survival remain dismal. Platinum-based chemotherapy is the mainstay of treatment for NSCLC in both adjuvant and metastatic disease. Over the last few years, we have made improvements in overall survival of patients with metastatic disease due to the availability of immune therapies and targeted therapies based on patients' individual molecular profile(Borghaei et al., 2015; Mok et al., 2009; Sandler et al., 2006; Topalian et al., 2012). Anti-PD1 antibodies have been approved in Japan and USA, based on potent activity in a variety of tumors. In 2015, pembrolizumab (anti-PD1 Keytruda made by Merck) was FDA approved for non-small cell lung cancer and nivolumab (anti-PD1 Opdivo made by Bristol-Myers Squibb) was approved for non-small cell lung cancer and metastatic renal cell carcinoma. Combination of checkpoint inhibitor therapies such as nivolumab (fully human IgG4 anti-PD-1) with or without ipilimumab (anti-CTLA-4) for treatment of recurrent small cell lung cancer (**NCT1928394**) has been shown to be tolerable and to induce durable responses (2015 ASCO Abstract# 7503). We have also witnessed the advent of maintenance strategies and antiangiogenesis approaches that have improved patient outcomes (Patel et al., 2009). However, despite these advances in treatment, overall survival of patients with metastatic NSCLC remains poor at 12 to 18 months, and there are virtually no long term survivors.

Even after response to first line therapy, patients eventually develop progressive disease and require additional treatment. The management of patients with advanced NSCLC who have relapsed after their initial treatment is a difficult problem. The approach depends upon the initial systemic therapy administered and upon whether symptoms relate to widely disseminated metastatic disease or whether symptoms are related to involvement of a single location. Treatment in this setting is largely palliative in this refractory population and there is a major, immediate need for new therapies that have the potential for long term remission, disease control and can improve quality of life and overall survival.

The Sponsor has decided to suspend enrollment of NSCLC patients into Cohort 5 as of Amendment 10. This decision was based on the following considerations:

- Limited recruitment of this disease population to date; only 1 evaluable subject infused to date.
- No current plans to further develop this investigational product in this disease population.
- Logistical challenges of re-introducing pre-screening/mesothelin expression testing back into the study for one targeted disease population within Cohort 5 only.

1.1.2.2 Ovarian Cancer

Ovarian cancer is the 5th most common cancer in women and, in the United States in 2015, 21,290 women were diagnosed with ovarian cancer and 14,180 women died of the disease (2015). Despite efforts studying different cytotoxic and targeted agents to treat ovarian cancer, no major improvement in cure rates has been seen (Huang et al., 2008). Surgery and platinum-based therapies are the current standard of care for ovarian cancer management. Thus, new approaches to ovarian cancer treatment are desired. As ovarian tumors have previously been shown to be immunogenic (Zhang et al., 2003) immunotherapeutic strategies, including vaccines, adoptive cell transfer, and immunomodulatory drugs, have recently been presented as possible methods of combating ovarian cancer (Coukos et al., 2016).

In an early cell transfer trial by Fujita et al., administration of autologous tumor infiltrating lymphocytes (TILs) to ovarian cancer patients in the adjuvant setting (after surgical resection and subsequent cisplatin-chemotherapy) resulted in a prolonged disease-free survival and increased the 3-year survival rate, supporting the notion that T cell transfer can actively inhibit ovarian tumor growth (Fujita et al., 1995). Moreover, administration of TILs (alone or in combination with cisplatin-containing chemotherapy) after a single dose of cyclophosphamide was shown to induce objective cancer regressions in some patients with existent chemotherapy-naïve or recurrent ovarian cancer as well (Chang and Pastan, 1996). The mechanism of tumor eradication appeared to be cell-mediated as suggested by the increased CD8+ T cell percentages, activation of cellular immunity and enhanced natural killer activity detected in the blood from treated patients (Johnston et al., 2009).

1.1.2.3 Malignant Mesothelioma

Malignant mesothelioma is the most serious effect of exposure to asbestos. It is an aggressive cancer affecting the membrane lining of the lungs or peritoneum. There are three major types of mesothelioma depending on the organ affected: pleural mesothelioma, peritoneal mesothelioma, and pericardial mesothelioma.

Peritoneal mesothelioma is a primary malignancy of the mesothelial surface of the peritoneum and represents about 20% of the mesothelioma cases. It is a locally progressive tumor that encases the abdominal organs and often is diagnosed at a late stage due to nonspecific patient symptoms. While up front responses with cisplatin and pemetrexed only have about a 25% response rate with overall survival around one year, centers of excellence employing a multimodality approach of cytoreductive surgery with hyperthermic intraperitoneal chemotherapy have demonstrated 5-year survival rates of 39-63%. However, peritoneal mesothelioma remains incurable and novel therapeutic options are needed.

Malignant pleural mesothelioma (MPM) is a primary neoplasm of the mesothelial lining of the pleura space. MPM is usually staged using the IMIG/AJCC staging system. At the time of diagnosis, stage II disease is most common (50%), stages I (18%) and III (28%) are less frequent, and stage IV disease is very infrequent (4%). Thus, in most cases, MPM is a diffuse but locoregional disease at the time of diagnosis. The tumor grows in a locally-invasive fashion, often invading thoracic structures such as chest wall, pericardium, myocardium, and great vessels. Distant, blood-borne metastases occur very late in the disease (Sterman et al., 1999).

Traditional MPM therapy has included three modalities that have been used individually or in combination: surgery, irradiation, and chemotherapy. Unfortunately, to date, none of these modalities has proven curative. Surgical resection allows the removal of gross tumor but cannot provide microscopically-negative margins. Radiation is limited by the large tumor volume and the radiation

intolerance of adjacent intrathoracic organs. The most effective combination chemotherapy, pemetrexed (Alimta)/cisplatin, has yielded overall response rates of up to 40%, but with improvements in overall median survival of only 3.5 months(Vogelzang et al., 2003). Because of these shortcomings, several clinical trials utilizing aggressive surgical approaches (i.e. extrapleural pneumonectomy) coupled with radiation treatment and pre- or post-operative chemotherapy have been conducted in good performance status patients. Peri-operative morbidity from this multimodality therapy has been significant, and there remains a peri-operative mortality rate of 5%, even in the most experienced centers.

After cisplatin/pemetrexed, there is no well-accepted second-line chemotherapy (for review, see(Ceresoli et al., 2009), but based on activity in front-line patients, most centers use either vinorelbine or gemcitabine, either alone or in combination with a platinum agent if disease progression has occurred shortly after first line therapy. There is surprisingly little data about the patient response to second-line therapy; however, in the best study published to date(Zucali et al., 2008), the activity and toxicity of combination gemcitabine/vinorelbine in pemetrexed-pretreated MPM patients was assessed. Thirty consecutive patients who had been pretreated with pemetrexed were given gemcitabine (1000 mg/m^2) and vinorelbine (25 mg/m^2) intravenously on Days 1 and 8 every three weeks. Most patients were PS 0 or 1 (83%). Only three patients had a partial response (10%); there were no complete responses. The median time to progression was 2.8 months (range 0.8 - 25 months). Progression-free survival at three months was 45% (95% CI, 26.4 - 64.3%). The median survival rate was 10.9 months (range 0.8 - 25.3 months). Novel therapeutic approaches are clearly needed in this disease.

1.1.3 Adoptive immunotherapy

Breaking tolerance to self-antigens is a major obstacle in the application of immunotherapy to solid malignancies. Vaccine strategies aimed at harnessing endogenous anti-tumor T cells are limited by the T cell receptor (TCR) repertoire which can be deleted within the thymus as part of central tolerance or rendered non-functional by post-thymic mechanisms of peripheral tolerance. One approach designed to overcome these obstacles is adoptive immunotherapy, a term used to describe the transfer of immune cells for the treatment of cancer or infectious disease (June, 2007). With adoptive immunotherapy, therapeutically effective T cells can be engineered to recognize tumors.

Penn has continued to pioneer new adoptive immunotherapeutic strategies such as producing genetically engineered T cells referred to as chimeric antigen receptors (CARs). The significant advancement with such therapies is that CARs combine the effector functions of T lymphocytes with the ability of antibodies to recognize distinct surface antigens in a non-MHC restricted manner. As a result, CARs can be used to redirect T cells to recognize an intact membrane protein independent of antigen processing.

The *in vivo* persistence, expansion, and functional capacity of adoptively transferred engineered T cells is dependent on two discrete signals mediated by cell surface receptors. The primary “activation” signal is produced by ligation of the TCR with an MHC-peptide complex. The second “costimulatory” signal is generated by ligation of a costimulatory molecule on the T cell surface with its cognate ligand on the surface of an antigen presenting cell.

We and others have observed that 4-1BB, a T cell co-stimulatory receptor induced by TCR activation, is critical for long-term proliferation of CD8 cells whereas CD28 is essential for sustained CD4 cell proliferation(Maus et al., 2002). Consistent with this finding, we have found that “bipartite receptors” comprised of TCRζ and either CD28 or 4-1BB signaling modules substantially improve the function and proliferation of T cells *in vivo*. (Kalos et al. 2011)(Porter et al. 2011) (Grupp et al. 2013a) (Maude et al.

2014) (Porter et al. 2015)(Milone et al. 2009). In previous studies, we found that adoptive immunotherapeutics, such as CART19 administered intravenously, can persist for more than 5 years in some patients with advanced, chemotherapy-resistant leukemia.

Based on this broad experience, we pursued a similar approach in developing a CAR specific for mesothelin protein (Carpenito et al., 2009). Here, we have used the same type of lentiviral transduction system and the same type of CAR construct, comprised of a mesothelioma specific scFv fused to the 4-1BB and CD3z signaling domains, to generate CART-meso cells. The main difference between the anti-CD19 CAR and anti-mesothelin CAR constructs rely on the origin of the scFv portion of the construct which is specific to the target protein.

1.1.4 Lymphodepletion

Adoptive immunotherapy strategies may be able to capitalize on homeostatic T cell proliferation (Dummer et al., 2002), a recent finding that naive T cells begin to proliferate and differentiate into memory-like T cells when total numbers of naive T cells are reduced below a certain threshold(Goldrath and Bevan, 1999; Surh and Sprent, 2000). Lymphodepletion eliminates regulatory T-cells and other competing elements of the immune system that act as “cytokine sinks”, enhancing the availability of cytokines such as IL-7 and IL-15 (Klebanoff et al., 2005). This hypothesis has been tested clinically in patients with metastatic melanoma refractory of conventional treatments (Dudley et al., 2002). The patients received a lymphodepleting conditioning regimen consisting of cyclophosphamide (60 mg/kg x 2 days) and fludarabine (25 mg/m^2 x 5 days) prior to adoptive transfer of T cells. Patients with myeloma, NHL, and CLL have been treated with infusions of ex-vivo co-stimulated and expanded autologous T cells after lymphodepleting chemotherapy, and observed improved engraftment (Laport et al., 2003) (Kalos et al., 2011; Porter et al., 2011; Rapoport et al., 2005) (Porter et al., 2015).

Based on the reported data as well as the data collected from our recently closed study (UPenn UPCC# 31213, **NCT02159716**) that tested the safety of an earlier generation mesothelin directed CART cells in a similar patient population indicated that preconditioning using intravenous cyclophosphamide (1.5 g/m^2) was safe and enhanced CART expansion in peripheral blood. Neutropenia was observed in all subjects with a subset being febrile at 10-14 day post-infusion which interfered with obtaining the protocol recommended tumor biopsy in that timeframe. These data informed our perspective on lymphodepletion in this study, and we initially proposed to lower the cyclophosphamide dose to 1 g/m^2 which we believed would limit toxicity in the target patient populations.

However, preconditioning with cyclophosphamide and fludarabine in combination (Cohort 7 only) with adoptively transferred lymphocytes for the treatment of neoplasia induces modulation of several cytokines and homeostatic proliferation of the transferred lymphocytes, promotes homing of those lymphocytes to secondary lymphoid organs, and contributes to *in vivo* T cell persistence. This provides a rationale for combining adoptive immunotherapy with chemotherapy.(Bracci et al., 2007; Moschella et al., 2011) The main goal of using both cyclophosphamide and fludarabine is to achieve lymphodepletion that may enhance engraftment of adoptive T cells, while minimizing complications from neutropenia/myelosuppression. Therefore, provided the previously published data as well as the preliminary data from early cohorts in this study, we hypothesize the use of cyclophosphamide and fludarabine in preparation for local administration will improve the engraftment of the huCART-meso cells without increasing the risk to subjects.

1.1.5 Local versus systemic administration of CAR T cells

The route of CART administration is an area of interest (Katz et al., 2016; Tilley et al., 2014) due to the finding that trafficking of CART cells to solid tumors is limited (Moon et al., 2011). A recent paper published by the Memorial Sloan Kettering Cancer Center indicates that intrapleural (IP) delivery of anti-mesothelin redirected CAR T cells in mice is superior to systemic delivery (de Biasi et al., 2014). This publication was followed by opening of a clinical trial testing the safety of anti-mesothelin redirected CAR T cells (iCasp9M28z T cells) with or without cyclophosphamide preconditioning (1.5 g/m^2) in patients with malignant pleural disease from mesothelioma, lung cancer, or breast cancer by intrapleural administration (NCT02414269). Other studies are also testing the merits of local/intratumoral route of administration (NCT02498912, NCT01818323) of CAR T cells in solid tumors.

In addition, based on our prior experience with mesothelin-directed CAR T cell therapies, we hypothesize that local delivery of the maximum tolerated dose (MTD) of huCART-meso cells (established via IV infusion in early cohorts on this study, 1-3x10^7 huCART-meso cells/m^2) may reduce the risk to subjects by mitigating the potential for off-tumor, on-target toxicities such as those previously discussed. Brown et al., based on in silico modeling of CAR T cell delivery to peripheral organs, maintains that the typical CAR T cell dose administered intravenously in solid tumor clinical trials (approximately 10^8 cells) is likely insufficient to promote an appreciable anti-tumor effect (Brown et al, 2019; doi: http://dx.doi.org/10.1101/759167). Therefore, local administration allows for delivery of the huCART-meso cells directly to the tumor, minimizing their peripheral distribution, reducing the potential for off-tumor, on-target toxicity, and maximizing the number of CAR T cells that extravasate into the tumor microenvironment. Therefore, local administration of huCART-meso cells may increase the overall therapeutic index of this investigational product.

Thus, we propose to test the safety and feasibility of local administration, test the CART escape and persistence into the blood, and directly compare to the same parameters following IV administration of the same dose.

Finally, in an effort to further balance both toxicity and efficacy, the Cohort 7 will permit repeat IV infusions of huCART-meso cells every 21-42 days (at the clinical discretion of the physician-investigator) after the initial local dose. We hypothesize that repeat IV infusions will bolster the anti-tumor effect of persistent huCART-meso cells which may lead to improved clinical outcomes while mitigating risk of toxicities observed at higher IV doses.

1.2 Investigational Agent

The investigational agent in this protocol is huCART-meso cells, which are autologous T cells transduced with chimeric antigen receptor (CAR) composed of anti-mesothelin M5 scFv fused to 4-1BB and TCRzeta signaling domains. huCART-meso cells will be manufactured using the lentiviral transduction platform, the same as multiple other investigational cellular products tested at UPENN. The same technology that is used to transduce CART19 cells will be used to transduce huCART-meso cells (lentiviral transduction). The M5 scFv with specificity for mesothelin is newly derived from a human scFv library by Novartis Inc. The scFv is expected to redirect specificity of the transduced T cells to mesothelin expressing cells. The intracellular signaling domain of the CAR molecule is comprised of the TCRζ and 4-1BB signaling modules previously tested in clinical studies (Grupp and June, 2010; Hassan et al., 2010a; June et al., 2009; Kreitman et al., 2009; Romeo and Seed, 1991), both entirely of the native human sequences. The CAR receptors are "universal" in that they bind antigen in an MHC-independent fashion, thus, one receptor construct can be used to treat a population of patients with mesothelin antigen-positive tumors.

Clinical grade engineered T cells will be manufactured at the UPENN Clinical Cell and Vaccine Production Facility (CVPF). Please refer to the current version of the huCART-meso Investigator's Brochure for additional details.

1.3 Preclinical data

Please refer to the current version of the huCART-meso Investigator's Brochure for additional details.

1.4 Clinical data to date

Please refer to the current version of the huCART-meso Investigator's Brochure for additional details.

1.5 Toxicities associated with CART cells

Please refer to the current version of the huCART-meso Investigator's Brochure for complete details.

1.6 Trial Rationale

Immunotherapy is a novel and promising approach for the treatment of solid tumors; immunotherapy with CART cells in particular has the potential advantage of targeted therapies that can invoke a rapid tumor response, and the advantage of long-lived responses that are the hallmark of engagement of the adaptive immune system such as memory T cells.

This Phase I study poses the hypothesis that targeting the mesothelin antigen that is widely and frequently expressed in multiple tumor types, particularly epithelial ovarian cancer and mesothelioma will be safe, feasible, and result in anti-tumor responses. Furthermore, this study addresses the questions of whether a human construct persists better *in vivo* than a murine based CAR construct, whether lymphodepletion is necessary for engraftment and persistence of huCART-meso directed T cells in patients with solid tumors, and whether intrapleural (IP) and intraperitoneal (i.p.) administration has benefits compared to IV administration.

As of Protocol Amendment V5, the maximum tolerated dose has been established as $1\text{-}3x10^7$ huCART-meso cells/m^2. While the safety of this dose level has been established using IV administration, there was a lack of clear clinical efficacy at this dose level in Cohorts 1 and 2. Therefore we believe that exploration of a different dosing regimen is warranted. In pre-clinical studies, we have seen both safety and increased efficacy of multiple CAR T cell doses. Therefore, we plan to explore the safety and feasibility of administering up to three (3) infusions of huCART-meso cells at the MTD (given a minimum of 21 days apart) in Cohort 6 (intravenous administration) and Cohort 7 (intraperitoneal administration on Day 0, followed by repeat IV administration). As a secondary objective, we will also evaluate the anti-tumor response of this regimen.

1.7 Potential Risks and Benefits

This protocol is designed to determine the safety and feasibility of both intravenous administration and local delivery of permanently modified CAR T cells that target mesothelin, with or without lymphodepletion.

1.7.1 huCART-meso Cells

Please refer to the current version of the huCART-meso Investigator's Brochure for complete details.

1.7.1.1 Intrapleural Administration of CAR T cells (Cohort 5)

It is anticipated that subjects with pleural effusion will already have a catheter in place. However, in the event a catheter must be placed for IP administration, the risks associated with this include infection, bleeding, local pain at insertion site, pneumothorax, subcutaneous emphysema, and post drainage pain. There is also a risk associated with the moderate sedation used at the time of catheter placement which includes over sedation, nausea, vomiting, respiratory compromise, irregular heart rhythm, and low blood pressure. The subject will be closely monitored by a licensed provider per institutional standard practice. The timing of placement and subsequent removal of the catheter will be per clinical discretion.

The risks associated with pleural instillation are the same as those associated with IV administration. However, there is also a higher risk of pleuritis given the direct administration into the pleural cavity.

1.7.1.2 Intraperitoneal Access and Administration of CAR T cells (Cohort 7)

Some subjects may already have a catheter in place; however, in the event a catheter must be placed for Intraperitoneal (i.p.) Instillation, placement of the catheter will be performed by Interventional Radiology (IR) or other appropriately licensed provider. Location (IR or bedside) of the paracentesis procedure will depend on the ascites volume (moderate/large amount of ascites, i.e. at least 3 cm pocket is required for bedside placement). The timing of placement and subsequent removal of the catheter will be per clinical discretion.

Percutaneous techniques will be utilized in this protocol. Percutaneous insertion with radiologic guidance has the advantage of minimally invasive and correct catheter positioning in the lower abdomen. Safely accessing the intra-abdominal space for an individual patient may or may not require administration of a small volume of saline or contrast.

Risks associated with the procedure include, but are not limited to, pain or discomfort at the needle insertion site, bleeding at the site, internal bleeding, injury to a blood vessel, organ puncture, and infection which may result in an infection of the blood stream. The development of any infection may result in the need for intravenous antibiotics. X-ray contrast material may be used at the time of catheter insertion. Risks associated with the X-ray contrast material include an allergic reaction.

Moderate sedation may be used during the catheter placement. Risks of the medications used for the moderate sedation include aspiration (inhaling food or liquid into the lungs), respiratory depression, over-sedation, nausea, vomiting, respiratory compromise, irregular heart rhythm, and low blood pressure. In addition to the potential risks associated with the procedure, the X-ray contrast material, and the moderate sedation, there may be other unpredictable risks including death.

Subjects will be asked to sign a standard hospital consent form for this procedure. The subject will be closely monitored during/after the procedure by a licensed provider per institutional standard practice.

The risk of peritoneal inflammation with IP administration is anticipated to be higher than that of IV administration given the direct administration into the peritoneal cavity.

1.7.1.3 Cyclophosphamide

In over 10% patients, cyclophosphamide has dermatologic reactions (alopecia beginning 3-6 weeks) and may cause sterility; thrombocytopenia and anemia are less common than leukopenia and neutropenia with an onset at 7 days, nadir at 10-14 days, and recovery at 21 days. Nausea and vomiting occur more

frequently with large doses, usually beginning 6-10 hours after administration; subjects will be offered antiemetic prophylaxis and therapy. Acute hemorrhagic cystitis, believed to be a result of chemical irritation of the bladder by acrolein, a cyclophosphamide metabolite, occurs in 7% to 12% of patients and has been reported in up to 40% of patients in some series. Patients will be encouraged to drink plenty of fluids during and after therapy (most adults will require at least 2 L/day), and void frequently. With large IV doses, IV hydration is usually recommended. The use of mesna and/or continuous bladder irrigation is rarely needed for doses <2 g/m^2. Less frequent reactions (1-10%) such as facial flushing, headache, and skin rush may occur. Nasal congestion occurs when IV doses are administered too rapidly (large doses via 30-60 minute infusion); patients experience runny eyes, rhinorrhea, sinus congestion, and sneezing during or immediately after the infusion. If needed, a decongestant or decongestant/antihistamine (e.g., pseudoephedrine or pseudoephedrine/triprolidine) can be used to prevent or relieve these symptoms.

The above describes the risks of cyclophosphamide when given as part of routine care. We expect similar side effects when administered as part of this study.

1.7.1.4 Fludarabine

Fludarabine may induce myelosuppression, including anemia, thrombocytopenia, neutropenia, and lymphopenia. Severe, often reversible, myelosuppression may occur, although this typically occurs following cumulative dosing of fludarabine (administered for 3 consecutive days every 28 days). In this study, patients will receive one 3-day administration of fludarabine. Autoimmune hematologic phenomena, including hemolytic anemia or immune thrombocytopenia may occur following 1 or more cycles of fludarabine. Patients undergoing treatment with fludarabine will be evaluated and monitored closely for cytopenias and/or hemolysis. Nausea and vomiting may occur with fludarabine, and subjects will be offered antiemetic prophylaxis and therapy.

The above describes the risks of cyclophosphamide and fludarabine when given as part of routine care. We expect similar side effects when administered as part of this study.

1.7.1.5 Fludarabine in Combination with Cyclophosphamide

- Common: Lowered neutrophils/granulocytes that may lead to infection. Lowered platelets may lead to an increase in bruising or bleeding, Lowered red blood cells. Lowered lymphocytes may lead to infection, fatigue, nausea, vomiting, time away from work, hair loss, or Herpes zoster infection of the skin.
- Less Likely: Allergic reaction, severe allergic reaction that causes fever, aches and pains in the joints, skin rash, and swollen lymph glands, stuffy or runny nose, sneezing, sore throat, abnormal fast heartbeat, excessive sweating, flushing, itching, rash, swelling of the lips, eyes, tongue, and throat which can be severe, hives, diarrhea, high blood sugar, low blood potassium, dizziness, convulsion or seizure, abdominal pain, pain such as back, joint, and/or muscle pain, headache, wheezing, cough, shortness of breath, inflammation of the lung which may cause difficulty breathing and difficulty getting oxygen, infertility or sterility, or irregular menstrual periods. Some women may not resume their periods, SIADH (syndrome of inappropriate antidiuretic hormone), increased production of tears associated with the administration of cyclophosphamide, metallic taste, cystitis and hematuria.
- Rare But Serious: hemolytic anemia. Changes in vision or changes in degree of alertness both of which can be severe or fatal, rash which may become severe, potentially life-threatening condition affecting less than 10% of the skin in which cell death causes the outer skin layer to separate from the middle layer, life-threatening condition affecting greater than 30% of the skin

in which cell death causes the outer layer of skin to separate from the middle layer, severe lung dysfunction resulting in the ability to breathe which can be life-threatening, allergic reactions to blood transfusions, Tumor Lysis Syndrome - a rapid decline in the number of tumor cells that can lead to kidney failure and/or chemical imbalances that may have a serious effect on other organs such as heart. If this were to occur, subjects will receive close monitoring and blood tests, as well as appropriate medical treatment. It is possible that secondary malignancies including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) may also be experienced.

1.7.2 Mesothelin Expression

Mesothelin IHC testing was required as part of eligibility screening for subjects enrolled prior to the implementation of Protocol Amendment V9. The risk of a failed mesothelin immunohistochemistry test (false-positive) resulting in huCART-meso infusion in a patient with no mesothelin expression on his/her tumor is the same as the risks listed above, with the exception of TLS and CRS which are not expected due to the lack of tumor antigen expression.

Mesothelin IHC testing was subsequently eliminated as an eligibility parameter with Protocol Amendment V9. Eliminating IHC testing as an enrollment activity is based on the heterogeneity of mesothelin expression on accessible tumors, and the limited, and likely non-representative, amount of tissue being analyzed by IHC. This heterogeneity may bias diagnostic conclusions based on mesothelin-negative IHC results, since other areas of the tumor may in fact express mesothelin at study-qualifying levels. Therefore, IHC analysis is being removed in favor of the more sensitive, specific, and reliable GeoMx Digital Spatial Profiling analysis for both the presence of mesothelin on tumor tissue as well as CAR T cell localization to tumor. Furthermore, since a primary objective of the study is to evaluate the safety of huCART-meso cells, removing IHC testing neither impacts the safety endpoint nor adds additional risk to subjects. However, by eliminating IHC testing as an enrollment activity, it is anticipated to improve subject eligibility. Finally, the risk mitigation strategies employed on the study (e.g.: eliminating the first pass of huCART-meso cells through the lungs by implementing local delivery, inpatient monitoring post-infusion, an acute awareness of complications based on prior experience, etc.) are designed to mitigate on-target, off-tumor adverse reactions such as ARDS.

1.7.3 Research Procedures

Additional risks for the participating subjects are related to procedures including the following:

1.7.3.1 Apheresis

Potential risks of the apheresis procedure include nausea, vomiting, fainting or dizziness, seizures, skin rash, hives, flushing, blood loss, and infection. Tingling of the lips, muscle cramping and, very rarely, changes in heart rhythm can occur. These can be prevented or made milder by giving calcium supplements, either by mouth or in the vein, also called intravenous (IV).

Very rarely, (less than 1 in 1,000 procedures), clotting may occur in the apheresis machine or in a patient and is potentially life-threatening. To reduce the risk of clotting, a drug called ACD (acid-citrate-dextrose) will be used. This drug may increase the risk of bleeding and may cause temporary tingling of the lips and limbs, muscle cramping, seizures, or changes in the heart rhythm.

After the apheresis procedure the patient may experience temporary discomfort, including irritation, swelling or bruising at the place where the needle was inserted into the vein to collect the blood. Apheresis can also occasionally cause hives, numbness and tingling, or swelling of the feet and ankles.

1.7.3.2 Blood Draws

Risks associated with blood draws include bruising, swelling, black and blue marks, fainting and/or infection at the site. Rarely, patients may experience anemia from having blood drawn frequently.

1.7.3.3 Tumor Biopsy

Risks may differ depending on the type of biopsy performed; however, common side effects may include: pain, discomfort, soreness, bleeding, and bruising.

Given that the patient populations recruited for this protocol have limited therapeutic options, we believe that the above risks are acceptable.

1.7.4 Potential Benefits

It is possible that the anti-mesothelin CAR T cells will exert an anti-tumor effect; in two preliminary studies (UPCC#17510 and 21211) performed at UPENN testing RNA CART-meso cells, 2 subjects had an objective anti-tumor response out of 14 total subjects. CART 19 cells infused in patients with ALL, CLL, lymphoma and multiple myeloma have been shown to exert sustained clinical responses (Brentjens et al., 2013; Garfall et al., 2015; Grupp et al., 2013b; Kalos et al., 2011; Maude et al., 2014; Porter et al., 2011), (Brentjens et al., 2013). Additionally, safety and efficacy of CAR T cells redirected against BCMA are tested in patients with multiple myeloma at UPENN and the preliminary results indicate that this cellular therapy is tolerable and induce durable responses (unpublished data).

1.8 Justification of Route and Dose Regimen

Route of Administration (ROA).

- Intravenous: Autologous huCART-meso cells will be administered intravenously (IV) as a single dose infusion. This route of administration is the most likely to result in distribution of huCART-meso to all sites of tumor. Intravenous infusion is being evaluated in Cohorts 1-4 and 6. Cohorts 3 and 4 were permanently closed with Protocol V5 due to toxicity.
- Intrapleural (IP): Cohort 5 will evaluate autologous huCART-meso cells administered intrapleurally (IP) in patients with malignant effusions in the pleural space (i.e. MPM, and ovarian cancer with cytologically confirmed malignant pleural effusion), where the tumor is uniquely accessible and directed administration of therapeutic agents, such as CART cells, is possible. The method of IP instillation for targeted therapeutic delivery has been employed previously at the University of Pennsylvania in patients with malignant mesothelioma (Sterman et al., 2016; Sterman et al., 2005; Sterman et al., 1998) and elsewhere in ovarian cancer patients (Hasenburg et al., 2001). In these studies, no toxicities were associated directly with the IP route of administration.

  Note: As of protocol Amendment 10, recruitment of NSCLC patients into Cohort 5 is suspended due to limited enrollment of this disease population to date. There are also no plans to further develop this investigational product in this disease population.
- Intraperitoneal (i.p.): Cohort 7 will evaluate autologous huCART-meso cells administered via intraperitoneal instillation. This cohort will target subjects with ovarian cancer and peritoneal mesothelioma. Local administration will allow for delivery of the huCART-meso cells directly to the tumor, minimizing their peripheral distribution, reducing the potential for off-tumor, on-target toxicity, minimizing the first-pass effect in the lungs, and maximizing the number of CAR T cells that extravasate into the tumor microenvironment. There, it is anticipated that local intraperitoneal administration may increase the overall therapeutic index of this investigational product.

- The method of localized instillation for targeted therapeutic delivery has been employed previously at the University of Pennsylvania in patients with malignant mesothelioma who had pleural administration of agent (Sterman et al., 2016; Sterman et al., 2005; Sterman et al., 1998) and elsewhere in ovarian cancer patients (Hasenburg et al., 2001). In one patient with pancreatic adenocarcinoma with peritoneal carcinomatosis and no other treatment options available, anti-mesothelin CAR T cells were safely delivered by intraperitoneal administration on a compassionate-use basis at UPenn with the expectation that delivery of T cells via an i.p. route of administration may provide significant clinical benefit (UPCC#21211; IRB#814373).

- Repeat IV Administration (Cohorts 6 and 7): In an effort to further balance both toxicity and efficacy, repeat administration of huCART-meso cells will be performed via IV infusion after the initial dose (approximately every 21-42 days, as per the physician-investigator's clinical discretion). We hypothesize that additional IV infusions may maintain the therapeutic index of previously administered cells in the tumor microenvironment, which may have become exhausted or hypofunctional. There is strong pre-clinical data supporting this hypothesis in a mouse model of pancreatic cancer (Stromnes et al., 2015), and we anticipate that repeat infusions may bolster the anti-tumor potential of huCART-meso cells in this setting.

Dose Rationale for huCART-meso Product. The decision for a starting dose for biologics is guided based on 1) FDA Guidance for Industry: S6 Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals; 2) Joint taskforce report from the BioIndustry Association (BIA) and the Association of the British Pharmaceutical Industry (ABPI); 3) the available literature on phase I trials to date conducted with CAR T cells, and 4) accumulating experience on phase I trials targeting mesothelin using the CAR technology.

For biologics, the goal is to start at or below the minimum anticipated biological effect level (MABEL) and the conventional dose escalation for cell based therapies is half log10 increments. Since toxicity usually arises from exaggerated pharmacological effects with biologics, sometimes combined with a narrow therapeutic range and/or a steep dose-response relationship from the no observed adverse effect level (NOAEL) to toxicity, the pharmacologically active dose is often a more sensitive indicator of potential toxicity for biotechnology-derived pharmaceuticals. There are various ways of estimating MABEL. One way is to model the occupancy of the target with different doses; another is to test effects on human tissues ex vivo (readily applied if the target is carried by the human lymphocyte in peripheral blood); the third is to use data from a carefully qualified animal model. Finally, a literature review of the effects of other relevant molecules is important.

In an *in vivo* study using the NOD/SCID/γc-/- mouse tumor xenograft performed by our group at UPenn with a similar CAR of different specificity, the pharmacologically effective dose (PED) of the CAR T cells was $1x10^7$ CAR T cells/mouse. At this dose, some of the mice developed xenogeneic graft versus host disease. The maximum tolerated dose (MTD) in the mouse model was $50x10^7$cells/mouse. The NOAEL was ~$1x10^6$ CAR T cells per mouse. The NOD/SCID/γc-/- is the best available model for evaluating preclinical efficacy of CAR T cells for mesothelin-expressing tumors; however, the mouse tumor xenograft model is a non-relevant species for determination of some aspects of toxicology for this particular CAR T cell, because the anti-mesothelin scFv SS1 component does not bind to normal mouse mesothelial tissue, and therefore, the mouse will under-predict toxicity. However, the lack of an immune response to the CAR T cells in the mouse tumor model will tend to over-predict other effects, because humans will make responses that limit the persistence of vector- modified T cells(Kershaw et al., 2006; Lamers et al., 2006).

If mice are considered 2000-fold smaller than humans, the estimated values of NOAEL and PED are $2x10^9$ cells and $2x10^{10}$ cells, respectively. These estimated values suggest that 10^9 meso-CAR T cells per subject might be safe.

In the clinical studies of meso RNA CAR T cells opened at UPENN, flat doses of 10^8 and 10^9 total cells/infusion for multiple infusions have been administered to 14 subjects with either pleural mesothelioma or pancreatic cancer (NCT01355965, NCT01897415). In all cases, the efficacy of electroporation and CAR expression was over 85% cells on a flow based assay. In several cases, mild cytokine release syndromes have been observed upon multiple exposures to the higher dose T cell product. One subject developed an anaphylactic reaction after the 3rd infusion due to development of HAMA; however, 101 infusions have been given to date to 14 subjects and no other SAE related to investigational product has been reported suggesting that doses up to 10^9 cells are safe.

The follow up study at UPENN has been the one testing the safety of lentivirally transduced autologous T cells targeting mesothelin (CART-meso cells) using the same CAR construct as the RNA based studies, namely one using the mouse SS1 derived scFv (NCT02159716). Our group treated 15 subjects (mesothelioma, serous ovarian cancer and pancreatic cancer) with one infusion of either $3x10^7$ or $3x10^8$ CART-meso cells/m^2 with or without lymphodepleting chemotherapy. The safety profile of this study has been favorable. The majority of the related AEs are Grades 1-2 (79.5%). The majority of Grade 3-4 related AEs were for one pancreatic cancer patient (20 of 24, 83.3%) and they met the DLT criteria. The study was completed with infusion of 15 subjects without any other DLT.

In the reported literature, CAR T cells have been tested at up to $5x10^{10}$ cells (Kershaw et al., 2006). However, considering all the information presented herein, we have chosen to start our study with a low dose of 1-$3x10^7$ huCART-meso cells/m^2 per subject, with an acceptable dose range to allow for small variations in manufacturing and transduction. As part of this study, 6 subjects have been safely treated at dose level of 1-$3x10^7$ huCART-meso cells/m^2 with and without lymphodepleting chemotherapy (3 subjects in Cohort 1; 3 subjects in Cohort 2) and no DLTs were identified. However, when the dose was escalated to 1-$3x10^8$ huCART-meso cells/m^2 (Cohort 3), unacceptable toxicity was experienced, thus 1-$3x10^7$ huCART-meso cells/m^2 was established as the MTD. This dose level will be further explored using local delivery methods as well as through additional IV infusions (given approximately 21-42 days apart).

The minimum acceptable dose for infusion will continue to be $1x10^6$ huCART-meso cells/m^2.

2 STUDY OBJECTIVES

2.1 Primary Objectives

Determine the safety and feasibility of intravenous administration and local delivery of lentiviral transduced huCART-meso cells, with and without lymphodepleting chemotherapy in the target population.

2.2 Secondary Objectives

Clinical Objectives:

- Assess the clinical anti-tumor effect by standard criteria (RECIST [modified RECIST for mesothelioma] and immune-related response criteria [where feasible]) for each tumor type.
- Assess progression-free survival (PFS) and overall survival (OS).

Correlative Objectives:

- Evaluate peripheral engraftment and persistence huCART-meso cells in peripheral blood and body fluid.
- Determine the bioactivity of huCART-meso cells in peripheral blood and body fluid.
- Evaluate the development of anti-CART immune responses favoring rejection of huCART-meso cells (where scientifically relevant).
- Evaluate the development of secondary cellular and humoral anti-tumor responses as a consequence of epitope spreading.
- Where tumor material or body fluids can be obtained:
    a. Measure trafficking of huCART-meso
    b. Evaluate mesothelin expression on tumor cells to assess for antigen-escape.
    c. Evaluate genetic editing (if feasible)
    d. Analyze tumor microenvironment and cell interactions (if feasible)

3 STUDY DESIGN

3.1 General Design

This protocol will test the safety of 2 dose level of huCART-meso cells administered intravenously alone or after lymphodepleting chemotherapy. huCART-meso cells have been permanently modified to be directed to mesothelin protein with an anti-mesothelin CAR fused to the signaling domains of 4-1BB and TCRζ. The study population includes patients with mesothelin-expressing cancers.

The original protocol included a 3+3 dose escalation design. However, as of protocol amendment 5, the maximum tolerated dose has been established (1-3x10^7 huCART-meso cells/m^2) and Cohorts 3 and 4 have been permanently closed due to unacceptable toxicity. Moving forward, the MTD will be further evaluated utilizing different routes of administration, including local delivery methods (intrapleural and intraperitoneal) as well as repeat administration via IV infusion.

Once subjects are determined to be eligible and the monitoring visit for eligibility is completed, subjects will be scheduled for apheresis and huCART-meso cell manufacturing. If patients have an apheresis product collected prior to enrollment into this study, this apheresis product may be used for CAR T cell manufacturing. In this instance, the subject will not need to undergo another apheresis, and CAR T manufacturing may be initiated immediately after the monitoring visit for eligibility has been completed.

All subjects will be assigned a cohort for the interventional portion of the protocol (see below). Subjects will be followed for safety and research assessments as detailed in the Schedule of Events (**Appendices 1-3**).

Adverse events will be collected and evaluated during the protocol specified adverse event reporting period outlined in **Section 9.1**. While on study, subjects will be reassessed on an ongoing basis for evidence of acute and cumulative toxicity. At the time a subject progresses and/or initiates another therapy, the subject will be encouraged to enroll in the separate long-term follow-up study that is requested of all subjects who have received therapy with cells that have been gene-modified with an integrating vector, such as huCART-meso cells.

3.2 Cohort Assignment

This is a Phase I study evaluating the safety and feasibility of both intravenous administration and local delivery of lentiviral transduced huCART-meso cells with and without lymphodepleting chemotherapy.

Dose Escalation Cohorts:

- Cohort 1 (N = 3-6): will receive a single dose of 1-3x10^7 huCART-meso cells/m^2 on day 0 without any conditioning chemotherapeutic regimen.
- Cohort 2 (N = 3-6): will receive a single dose of 1-3x10^7 huCART-meso cells/m^2 on day 0, following a flat dose of 1 gram/m^2 of cyclophosphamide administered 2-4 days prior to huCART-meso cells (day -4 to day -2).
- ~~Cohort 3 subjects (N=3-6): will receive a single dose of 1-3x10^8 /m^2 lentiviral transduced huCART-meso cells on day 0 without any conditioning chemotherapeutic regimen~~. **Cohort 3 permanently closed with Protocol V5.**
- ~~Cohort 4 subjects (N=3-6) will receive a single dose of 1-3x10^8 /m^2 lentiviral transduced huCART-meso cells on day 0, following a flat dose of 1 gram/m^2 of cyclophosphamide administered 2-4 days prior to huCART-meso cells (day -4 to day -2).~~ **Cohort 4 permanently closed with Protocol V5.**

Historical Cohort Advancement Plan:
Subjects will be enrolled serially. Infusions will be staggered to allow assessment of DLTs for cohort progression, expansion, or dose de-escalation. For example, the 2nd and 3rd subjects in each cohort may be infused and followed in parallel but only after the 1st subject in that cohort completes the day 28 visit without DLT.

If 0 DLTs occur in the 2nd and 3rd subjects, the study may advance to the next cohort. If there is 1 DLT in the 2nd or 3rd subject the cohort will be expanded to treat up to 6 subjects. If a total of 2 DLTs occur in the 2nd and 3rd subjects, further enrollment in that cohort will be discontinued, and additional subjects will be enrolled into the dose de-escalation cohort. Alternatively, the 1st and 2nd subjects in each cohort may be infused and followed in parallel. If there are 0 DLTs in the 1st and 2nd subjects, a 3rd subject would be dosed at the same regimen/cohort. If there is 1 DLT in the 1st or 2nd subjects, the cohort would be expanded to enroll up to 6 subjects. If a total of 2 DLTs occur in the 1st and 2nd subjects, further enrollment in that cohort will be discontinued, and additional subjects will be enrolled into a dose de-escalation cohort.

The clinical PI and Medical Director will perform official DLT assessments and make formal determinations regarding cohort advancement (where applicable). Please refer to **Section 5.4** for the definition of dose-limiting toxicity as well as additional information on cohort progression.

The Maximum Tolerated Dose (MTD) is defined as the dose at which 0-1 DLT occurs in 6 evaluable subjects tested within the dose range of this study. As of protocol amendment V5, the maximum tolerated dose has been established as 1-3x10^7 huCART-meso cells/m^2.

Subsequent enrollment will occur in the dose expansion cohorts (Cohorts 5-7) at the MTD only. Enrollment in these cohorts may occur in parallel and will include up to 6 subjects/cohort. All subjects will be continually evaluated for dose-limiting toxicities (DLT). In the event of 2 DLTs in a specific cohort, additional enrollment and treatment activity within that cohort will be paused to allow for further investigation.

Dose Expansion Cohorts:

- Cohort 5 (N = up to 6): will receive a single dose of 1-3x10^7 huCART-meso cells/m^2 on day 0 by intrapleural infusion (IP) through an indwelling pleural catheter without any conditioning chemotherapeutic regimen. The safety of this dose level has been established by Cohorts 1 and 2.
- Cohort 6 (N = up to 6): will receive a dose of 1-3x10^7 huCART-meso cells/m^2 via IV infusion on Day 0, following a flat dose of 1 gram/m^2 of cyclophosphamide administered 2-4 days prior to huCART-meso cells (~Day -4 to -2). This initial infusion may be followed by up to two additional IV infusions of huCART-meso cells at the same dose level, given approximately 21-42 days apart, if the subject meets eligibility to receive additional infusions (See **Section 6.8.2**). Cyclophosphamide will not be repeated prior to subsequent doses of huCART-meso cells. Cohort 6 was activated with Protocol V6. Enrollment into Cohort 6 will occur in parallel with Cohort 5.
- Cohort 7 (N = up to 6): will receive a single dose of 1-3x10^7 huCART-meso cells/m^2 via intraperitoneal (i.p.) administration, following lymphodepleting chemotherapy with cyclophosphamide 300 mg/m^2/day and fludarabine 30 mg/m^2/day given over 3 days by intravenous infusion. Lymphodepleting chemotherapy will be scheduled such that the last day of chemotherapy is 3 days (+/- 1 day) prior to the infusion of huCART-meso cells. This initial i.p. infusion may be followed by up to two additional infusions of huCART-meso cells via intravenous (IV) administration at the same dose level, given between 21-42 days apart. The subject must meet eligibility to receive additional infusions (See **Section 6.9.2**). Lymphodepleting chemotherapy will not be repeated prior to additional infusions of huCART-meso cells. Infusion #1 for the first three subjects in Cohort 7 will be staggered by at least 21 days to allow for the assessment of DLTs. Enrollment into Cohort 7 will occur in parallel with Cohort 5 and Cohort 6.

If the harvest of the huCART-meso product is unsuccessful (does not meet release criteria) or if the target dose is not reached, subjects will have the option to undergo a second leukapheresis for a second manufacturing process. Subjects have the option to receive a lower than target dose, if the huCART-meso product meets CVPF release criteria, and the subject cannot or is not willing to perform a second apheresis, or if the second manufacturing process fails. If a subject receives a lower dose, they will be considered evaluable for safety, but will not be evaluable for response and will not be included in DLT evaluations (i.e. will be replaced in that cohort), but will follow all of the study visits as described in the Schedule of Events **(Appendices 1-3).** The minimum acceptable dose for infusion is 1x10^6 huCART-meso/m^2.

3.3 Study Endpoint Analysis

Please refer to **Sections 8.4 and 8.5** for a complete description of primary and secondary endpoint analysis. Please refer to **Section 7.2.2** for correlative endpoint analysis.

4 SUBJECT SELECTION AND WITHDRAWAL

4.1 Inclusion Criteria

1. Histologically confirmed cancer (one of the following):
    a. Cohorts 1-4 and 6 patients:
    Note: Cohorts 3 and 4 permanently closed with Protocol V5.
        i. Metastatic or recurrent lung adenocarcinoma.

ii. Persistent or recurrent serous epithelial ovarian cancer or primary peritoneal carcinoma or fallopian tube carcinoma
iii. Malignant pleural and peritoneal mesothelioma (histologically confirmed epithelial)

b. Cohort 5 patients:
i. ~~Metastatic or recurrent lung adenocarcinoma with cytologically or pathologically confirmed malignant pleural effusion.~~ **RECRUITMENT OF NON-SMALL CELL LUNG CANCER PATIENTS INTO COHORT 5 SUSPENDED WITH PROTOCOL VERSION 10**
ii. Persistent or recurrent serous epithelial ovarian cancer or primary peritoneal carcinoma or fallopian tube carcinoma with cytologically or pathologically confirmed malignant pleural effusion
iii. Malignant pleural and peritoneal mesothelioma (histologically confirmed epithelial) with documented pleural effusion

c. Cohort 7 patients:
i. Persistent or recurrent serous epithelial ovarian cancer or primary peritoneal carcinoma or fallopian tube carcinoma
ii. Malignant peritoneal mesothelioma (histologically confirmed epithelial)

2. ~~Confirmation of tumor mesothelin expression (≥50% of tumor cells)~~ **RETIRED WITH PROTOCOL VERSION 9.**
3. Failure of at least one prior standard of care chemotherapy for advanced stage disease. ~~Prior therapies against PD-1 or PDL-1 are permissible if > 4 weeks from enrollment.~~ **ALLOWANCE FOR PRIOR PD-1 or PDL-1 THERAPIES REMOVED WITH PROTOCOL VERSION 5.**
4. Patients must have measurable disease as defined by RECIST 1.1 criteria or modified RECIST criteria (mesothelioma only).
5. Patients with asymptomatic CNS metastases that have been treated (and are off steroids for the treatment of CNS disease) are allowed. They must meet the following at the time of enrollment:
   a. No concurrent treatment for the CNS disease
   b. No progression of CNS metastasis on MRI at screening scans
   c. No evidence of leptomeningeal disease or cord compression
6. Patients $\geq$ 18 years of age.
7. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
8. Satisfactory organ and bone marrow function as defined by the following:
   i. Absolute neutrophil count $\geq$ 1,000/µl
   ii. Platelets $\geq$75,000/µl
   iii. Hemoglobin $\geq$ 8 g/dL
   iv. Direct Bilirubin $\leq$ 2.0 mg/dl; unless secondary to bile duct obstruction by tumor or Gilbert's syndrome (≤ 3.0 mg/dl).
   v. Creatinine $\leq$ 1.5x the institutional normal upper limit
   vi. Albumin ≥2
   vii. Serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) $\leq$ 5x the institutional normal upper limit
   viii. Cardiac ejection fraction of $\geq$40% as measured by resting echocardiogram, with no clinically significant pericardial effusion.
9. Blood coagulation parameters: PT such that international normalized ratio (INR) is ≤ 1.5 and a PTT $\leq$ 1.2 time the upper limit of normal unless the patient is therapeutically anti-coagulated for history of cancer-related thrombosis and has stable coagulation parameters.
10. Provides written informed consent.

11. Subjects of reproductive potential must agree to use acceptable birth control methods, as described in protocol **Section 4.3**.

4.2 Exclusion Criteria

1. Sarcomatoid and biphasic mesothelioma
2. Known leptomeningeal carcinomatosis or spinal cord compression. Screening for this is not required unless suspicious symptoms.
3. Patients with symptomatic CNS metastases are excluded.
4. ~~Participation in a therapeutic investigational study within 4 weeks prior to eligibility confirmation by physician-investigator, or anticipated treatment with another investigational product while on study. This refers to non-commercially approved investigational drugs different than those used in this protocol.~~ **RETIRED WITH PROTOCOL VERSION 7.**
5. Active invasive cancer other than the one of the three cancers in this study. Patients with active non-invasive cancers (such as non-melanoma skin cancer, superficial cervical and bladder and prostate cancer with PSA level < 1.0) are not excluded.
6. HIV infection
7. Active hepatitis B or hepatitis C infection
8. Active autoimmune disease (including but not limited to: systemic lupus erythematosus, Sjogren's syndrome, rheumatoid arthritis, psoriasis, multiple sclerosis, inflammatory bowel disease, etc.) requiring immunosuppressive therapy within 4 weeks prior to eligibility confirmation by physician-investigator, with the exception of thyroid replacement.
9. Patients with ongoing or active infection.
10. Dependence on systemic steroids or immunosuppressant medications. Please refer to **Section 5.6** for complete details.
11. Patients requiring supplemental oxygen therapy.
12. Prior therapy with lentiviral gene modified cells.
13. History of allergy or hypersensitivity to study product excipients (human serum albumin, DMSO, and Dextran 40)
14. Any clinically significant pericardial effusion, Class II-IV cardiovascular disability according to the New York Heart Association Classification (see **Appendix 4)** or other cardiovascular condition that would preclude assessment of mesothelin induced pericarditis or that may worsen as a result of toxicities expected for this study. This determination will be made by a cardiologist if cardiac issues are suspected.
15. Any clinically significant pleural or peritoneal effusion that cannot be drained with standard approaches. An indwelling drainage device placed prior to eligibility confirmation by physician-investigator is acceptable.
16. Pregnant or breastfeeding women.
17. ~~Interleukin-15 (IL15) > 100 pg/ml~~ **RETIRED WITH PROTOCOL VERSION 9.**
18. ~~Treatment with a PD-1 or PD-L1 inhibitor, including but not limited to nivolumab, pembrolizumab, atezolizumab, and/or durvalumab, within 2 months prior to eligibility confirmation by a physician-investigator.~~ **RETIRED WITH PROTOCOL VERSION 11**
19. Patients with significant lung disease as follows:
    1) Patients with radiographic evidence of greater than lobar lymphangitic pulmonary involvement, greater than lobar bronchial wall thickening suggestive of peribronchial lymphatic disease extension, and/or evidence of extensive bilateral parenchymal metastatic burden.
    2) Patients with radiographic and/or clinical evidence of active radiation pneumonitis.

3) Patients with radiographic evidence of underlying interstitial lung disease, including evidence of unresolved drug toxicity from any agent (e.g. chemotherapy, targeted agents, amiodarone, nitrofurantoin, etc.)

Please refer to the Concomitant Therapy Section 5.6 for windows related to apheresis and huCART-meso infusion.

4.3 Reproductive Risks

Female subjects of reproductive potential (women who have reached menarche or women who have not been post-menopausal for at least 24 consecutive months, i.e., who have had menses within the preceding 24 months, or have not undergone a sterilization procedure [hysterectomy or bilateral oophorectomy]) must have a negative serum or urine pregnancy test performed at screening as per the Schedule of Events in **Appendices 1-3.**

Due to the unknown risks of huCART-meso cells with respect to pregnancy, as well as risk associated with lymphodepleting chemotherapy, it is recommended that all subjects of reproductive potential use at least one medically acceptable form of contraception during the study and for at least 1 year after their last infusion of huCART-meso cells. Investigators shall counsel subjects on the importance of pregnancy prevention and the implications of an unexpected pregnancy. It is possible the huCART-meso cells may also affect male/female fertility. It is also recommended that Investigators discuss options for fertility preservation, if not already discussed as part of their routine cancer care.

Acceptable birth control includes the following methods:

- Abstinence
- Condoms (male or female) with or without a spermicidal agent
- Diaphragm or cervical cap with spermicide
- Intrauterine device (IUD)
- Hormonal-based contraception

Subjects who are not of reproductive potential (women who have been post-menopausal for at least 24 consecutive months or have undergone hysterectomy, salpingotomy, and/or bilateral oophorectomy or men who have documented azoospermia) do not require use of contraception. Acceptable documentation of sterilization, azoospermia, or menopause is required and may take the form of written or oral attestation by clinician or clinician's staff of one of the following:

- Physician report/letter
- Operative report or other source documentation in the subject record (a laboratory report of azoospermia is required to document successful vasectomy)
- Discharge summary
- Laboratory report of azoospermia
- Follicle stimulating hormone measurement elevated into the menopausal range

4.4 Subject Recruitment

Potential participants are identified through the clinical practices of the investigators or sub-investigators and through referrals from outside hospitals and physicians. This study will be posted on ClinicalTrials.gov and publicized via University of Pennsylvania and Abramson Cancer Center websites/press releases. No direct-to-patient advertising will be performed.

4.5 Subject Withdrawal / Discontinuation

4.5.1 Reasons for Subject Discontinuation

Subjects who enroll but do not receive huCART-meso cells will be prematurely discontinued from the study, will not be followed, and will be replaced in the study. Reasons for premature discontinuation prior to receipt of huCART-meso cells may include, but are not limited to, the following:

1. The subject is lost to follow-up.
2. The principal investigator judges that the subject, prior to huCART-meso infusion, is too ill to continue.
3. Pregnancy is documented prior to huCART-meso infusion. If pregnancy occurs after the subject has received huCART-meso cells, they will be kept active in the study for safety and pregnancy follow-up and outcome. No subsequent huCART-meso infusions will be administered.
4. Voluntary withdrawal: a subject may remove himself/herself from the study at any time.
5. Significant and rapid progression of malignancy, requiring alternative medical, radiation or surgical intervention, prior to huCART-meso infusion.
6. Development of a medical condition that precludes CAR T cell infusion.
7. Technical difficulties are encountered in the T cell genetic modification and expansion procedure that precludes the generation of clinical cell doses that meet all Quality Control release criteria as specified by FDA.
8. Termination of the study.

Reasons for discontinuation of subjects after receipt of huCART-meso cells include the following.

1. Voluntary withdrawal: a subject may remove himself/herself from the study at any time.
2. Completion of protocol required follow-up
3. Death
4. Termination of the study

The reasons for discontinuation must be recorded appropriately.

4.5.2 Data Collection and Follow-up

Follow-up data collection after receipt of gene-modified cell therapy clinical trials is specified by the FDA.

Subjects who complete the 2 year follow-up as part of this protocol or discontinue participation early for any reason (per **Section 4.4.1** above), will be offered participation in a long-term follow-up protocol to further evaluate long term adverse events related to the study product for up to 15 years after study treatment. Once subjects are enrolled on the long-term follow-up protocol, all follow-up data collection under this protocol will be discontinued.

In the event that a subject cannot return to the study site for follow-up visits because of subject preference or geographical concerns, the subject's primary care physician and/or local oncologist will be asked to provide information from the subject's medical record to the study team at protocol defined time points (including the results of any routine care examinations and/or laboratory assessments), and assist in the collection of protocol required blood samples (if applicable), which will be sent to the University of Pennsylvania for protocol-required analysis. The subject and local provider will also be contacted via the Penn patient portal called MyPennMedicine (MPM), telephone and/or email/mail by a member of the study team to assess any potential toxicity.

In numerous previous cell therapy trials at the University of Pennsylvania, loss of follow-up is estimated to occur in less than 5% of cases. Every effort will be made to contact subjects who appear to be lost to follow-up in order to at least obtain survival data. In the event a subject fails to complete the follow-up requirements, documentation of all attempts to contact the subject includes at least 3 telephone contacts (on different days and at different times of the day), and a certified letter.

4.5.3 Replacement of Subjects

All subjects who do not receive huCART-meso cells will be replaced. Subjects have the option to receive a lower than target dose if the huCART-meso product meets CVPF release criteria and the subject cannot or is not willing to perform a second apheresis, or if the second manufacturing process fails. If a subject receives a lower dose, they will be considered evaluable for safety, but will not be evaluable for response and will not be considered for DLT evaluations and cohort advancement decisions, and they will be replaced in that cohort. However, these subjects will still be followed for overall safety and included in the analysis of secondary and exploratory endpoints in accordance with the Schedule of Events **(Appendices 1-3).**

5 INVESTIGATIONAL PRODUCTS

5.1 Description

5.1.1 huCART-meso cells

huCART-meso cells are autologous T cells that have been engineered to express an extracellular single chain variable fragment (scFv) with mesothelin specificity. The T cells express an intracellular signaling molecule comprised of the TCR ζ chain, and 4-1BB. The huCART-meso cells are cryopreserved in infusible cryomedia (**Section 5.3.1.2**). Each bag will contain a dose of CART positive cells corresponding to their assigned dose level as per cohort assignment. The total cell dose is dependent on the transduction efficiency; the volume of cells is dependent on the total cell dose with a minimum of 10 mL per bag. The minimum acceptable dose for infusion is $1x10^6$ huCART-meso cells/m^2.

5.1.2 Cyclophosphamide

Cyclophosphamide (Cytoxan®) is considered investigational therapy for subjects participating in targeted Cohorts (**Section 6.4**). Cyclophosphamide is an alkylating agent with antineoplastic activity. The main goal of using cyclophosphamide is to achieve lymphodepletion that may enhance engraftment of adoptive T cells, while minimizing complications from neutropenia.

Cohorts 2 and 6:
Cyclophosphamide will be administered as a flat dose of 1 gram/m^2 2-4 days prior to the huCART-meso infusion. In Cohort 6, cyclophosphamide will only be administered prior to the first huCART-meso infusion. The proposed regimen has been used widely in other studies and has been tolerated well. The ultimate criterion by which to assess the efficacy of the proposed regimen to facilitate T cell engraftment is the lymphocyte count 1-3 weeks after adoptive transfer.

Cohort 7:
Cyclophosphamide will be administered as 300 mg/m^2/day over 3 days so that the last day of chemotherapy falls 3 days (±1 day) prior to the first huCART-meso infusion (Day 0). The proposed regimen has been used widely in other studies and has been tolerated well.

5.1.3 Fludarabine

Fludarabine is considered investigational therapy for subjects participating in Cohort 7 only. Fludarabine is a purine analog that inhibits DNA synthesis through interference with DNA polymerase and ribonucleotide reductase enzymes. Fludarabine is widely used in the treatment of lymphocytic hematologic malignancies and possesses potent immunosuppressive activity. It is excreted principally in the urine as an active metabolite. The main goal of using fludarabine (in combination with cyclophosphamide) is to achieve lymphodepletion that may enhance engraftment of adoptive T cells, while minimizing complications from myelosuppression.

Cohort 7:
Fludarabine will be administered as 30 mg/m^2/day over 3 days so that the last day of chemotherapy falls 3 days (±1 day) prior to the first huCART-meso infusion (Day 0). The proposed regimen has been used widely in other studies and has been tolerated well. Fludarabine doses will be rounded down to the nearest 50 mg vial size, if the rounded dose does not differ by > 10% of the original dose prescribed.

5.2 Treatment Regimen

The treatment regimen received will be dependent on cohort assignment as follows:

- Cohort 1 (N = 3-6): will receive a single dose of 1-3x10^7 huCART-meso cells/m^2 on day 0 without any conditioning chemotherapeutic regimen.
- Cohort 2 (N = 3-6): will receive a single dose of 1-3x10^7 huCART-meso cells/m^2 on day 0, following a flat dose of 1 gram/m^2 of cyclophosphamide administered 2-4 days prior to huCART-meso cells (day -4 to day -2).
- ~~Cohort 3 subjects (N=3-6) will receive a single dose of 1-3x10^8 /m^2 lentiviral transduced huCART-meso cells on day 0 without any conditioning chemotherapeutic regimen.~~ **Cohort 3 permanently closed with Protocol V5.**
- ~~Cohort 4 subjects (N=3-6) will receive a single dose of 1-3x10^8 /m^2 lentiviral transduced huCART-meso cells on day 0, following a flat dose of 1 gram/m^2 of cyclophosphamide administered 2-4 days prior to huCART-meso cells (day -4 to day -2).~~ **Cohort 4 permanently closed with Protocol V5.**
- Cohort 5 (N = up to 6): will receive a single dose of 1-3x10^7 huCART-meso cells/m^2 on day 0 by intrapleural infusion (IP) through an indwelling pleural catheter without any conditioning chemotherapeutic regimen. The safety of this dose level has been established by Cohorts 1 and 2.
- Cohort 6 (N = up to 6): will receive a dose of 1-3x10^7 huCART-meso cells/m^2 via IV infusion on Day 0, following a flat dose of 1 gram/m^2 of cyclophosphamide administered 2-4 days prior to huCART-meso cells (~Day -4 to -2). This initial infusion may be followed by up to two additional IV infusions of huCART-meso cells at the same dose level, given approximately 21-42 days apart, if the subject meets eligibility to receive additional infusions (See **Section 6.8.2**). Cyclophosphamide will not be repeated prior to subsequent doses of huCART-meso cells. Cohort 6 was activated with Protocol V6. Enrollment into Cohort 6 will occur in parallel with Cohort 5.

- Cohort 7 (N = up to 6): will receive a single dose of 1-3x10^7 huCART-meso cells/m^2 via intraperitoneal (i.p.) administration, following lymphodepleting chemotherapy with cyclophosphamide 300 mg/m^2/day and fludarabine 30 mg/m^2/day given over 3 days by intravenous infusion. Lymphodepleting chemotherapy will be scheduled such that the last day of chemotherapy is 3 days (+/- 1 day) prior to the infusion of huCART-meso cells. This initial i.p. infusion may be followed by up to two additional infusions of huCART-meso cells via intravenous (IV) administration at the same dose level, given between 21-42 days apart. The subject must meet eligibility to receive additional infusions (See **Section 6.9.2**). Lymphodepleting chemotherapy will not be repeated prior to additional infusions of huCART-meso cells. Infusion #1 for the first three subjects in Cohort 7 will be staggered by at least 21 days to allow for the assessment of DLTs. Enrollment into Cohort 7 will occur in parallel with Cohort 5 and Cohort 6.

Lymphodepleting chemotherapy will be administered according to standard institutional procedures in the outpatient setting as per cohort assignment above. All subjects will receive huCART-meso cells on an inpatient basis at the Hospital of the University of Pennsylvania. Subjects will remain as inpatients for observation for a minimum of 48 hours after each huCART-meso infusion. The subject may then be discharged in accordance with hospital policy after this observation period is complete and the subject is medically stable. Clinic visits, hematology, and other safety laboratory tests will be performed according to the Schedule of Events (**Appendices 1-3**). Additional assessments may be performed as necessary to evaluate specific adverse events until they resolve to baseline or CTCAE Grade ≤ 1.

5.3 Preparation and Administration of Investigational Product(s)

5.3.1 huCART-meso cells

In addition to the language below, please refer to the Investigational Product Handling Manual for further details on product transport, thaw, and labeling.

5.3.1.1 Manufacturing of huCART-meso

huCART-meso cell product manufacturing will be performed by the Clinical Cell and Vaccine Production (CVPF) at the University of Pennsylvania, using the patients' apheresis product. The weight obtained prior to the apheresis procedure will be used for dosing and will not be modified as a result of changes in the subject's weight.

Each huCART-meso cell dose will be cryopreserved in infusion bag(s) containing cryomedia. The volume of each dose will be dependent on the total cell number and transduction efficiency. The minimum volume will be 10mL per infusion bag. The cells will be stored in the CVPF until ready for product release/administration

5.3.1.2 Release and Preparation of huCART-meso

When ready for administration, the huCART-meso cell product will be dispensed to the clinical team for administration per CVPF standard operating procedures and in accordance with the Investigational Product Handling Manual.

Packaging and Labeling

The investigational product will be affixed with a label containing information regarding the dose, the method of manipulation, the vector, and the following statements: "FOR AUTOLOGOUS USE ONLY" and

"Caution: New Drug – Limited by Federal Law to Investigational Use". In addition, the label will include at least two unique identifiers, including the subject's study identification number. Prior to each infusion, two individuals will independently verify that the product is correctly matched to the subject.

Receipt of huCART-meso Cells/Cell Thawing

The cryopreserved huCART-meso cells will be transported in dry ice to the subject's bedside. The cells will be thawed at the bedside using a water bath maintained between 36°C to 38°C. There should be no frozen clumps left in the container at the time of infusion. If the CAR T cell product appears to have a damaged or leaking bag, or otherwise appears to be compromised, it should not be infused and should be returned to the CVPF as specified below.

Return or Destruction of Investigational Product

huCART-meso cells may need to be returned to the CVPF for a variety of reasons, including but not limited to: 1) Mislabeled product; 2) Condition of patient prohibits infusion/injection, and 3) Subject refuses infusion. Any unused product will be returned to CVPF. Final disposition of the investigational product is to be documented.

5.3.1.3 Administration of huCART-meso

Subjects in Cohorts 5-7 will receive huCART-meso cells on an inpatient basis at the Hospital of the University of Pennsylvania. Please refer to **Section 6.5** for criteria that subject must meet in order to proceed with huCART-meso infusion.

Side effects following T cell infusions may include transient fever, chills, rigors, myalgias/arthralgias, headache, fatigue, and/or nausea. Subjects will be pre-medicated with acetaminophen 650 mg by mouth and diphenhydramine hydrochloride by mouth or IV prior to huCART-meso cell infusion. If Benadryl is contraindicated, an H2-blocker, such as ranitidine, will be administered. These medications may be repeated every six hours as needed. A course of non-steroidal anti-inflammatory medication may be prescribed if the subject continues to have fever not relieved by acetaminophen. It is recommended that subjects do not receive systemic corticosteroids such as hydrocortisone, prednisone, prednisolone (Solu-Medrol), or dexamethasone (Decadron). If corticosteroids are required for an acute infusional reaction, an initial dose of hydrocortisone 100 mg is recommended.

huCART-meso infusions in Cohorts 5-7 will take place on an inpatient basis at the Hospital of the University of Pennsylvania using precautions for immunosuppressed patients. Prior to the infusion, two independent individuals will verify the information on the label of each bag in the presence of the subject and confirm that the information correctly matches the participant. The investigational CAR T cell products should be infused into the subject while cold, within 30 minutes after they are thawed. There should be no frozen clumps left in the bag prior to infusion.

5.3.1.3.1 For huCART-meso cell IV administration

T cell infusions will be performed by a licensed Registered Nurse at the Hospital of the University of Pennsylvania. The transduced T cells will be infused at a rate of 10-20 ml/minute into an intravenous catheter, either through a peripheral vein (preferred) or central vein. A macrodrip intravenous tubing will be used to infuse the CART cells by gravity (i.e. no infusion pump). The macrodrip intravenous tubing will be connected to a "Y" adapter with one end of the adapter spiked to the CART cell product bag and the other to a normal saline solution bag. **A leukoreduction filter must not be used for the infusion of the T cell product**.

5.3.1.3.2 huCART-meso cell intrapleural administration

IP administration will be performed by a licensed provider at the Hospital of the University of Pennsylvania. Subjects without an existing intra-pleural catheter must have one placed for IP administration. The timing of placement and subsequent removal of the intra-pleural catheter will be per clinical discretion. Pleural fluid may be collected for research purposes at the time of catheter placement.

Prior to huCART-meso cell administration, the pleural cavity will be maximally drained. After drainage is complete, the transduced T cells will be instilled through the intrapleural catheter into the affected pleural space. The cells are infused by gravity at a rate of 10-20 ml/minute. After huCART-meso cell infusion is complete, the tube is flushed with 20ml of sterile saline and clamped. The end of the tube is swabbed with alcohol swabs, a new valve cap is placed and the site is re-dressed.

5.3.1.3.3 huCART-meso intraperitoneal (i.p.) instillation:

Some subjects may already have a catheter in place; however, in the event a catheter must be placed for Intraperitoneal (i.p.) Instillation, placement of the catheter will be performed by Interventional Radiology (IR) or other appropriately licensed provider. The subject will have an intraperitoneal catheter placed by IR or appropriately licensed provider according to standard hospital procedure, prior to their first planned huCART-meso infusion. Ideally, the catheter will be placed on the day of the i.p. infusion. Location (IR or bedside) of the paracentesis procedure will depend on the ascites volume (moderate/large amount of ascites, i.e. a 3 cm pocket, is required for bedside placement). If ascites is present at the time of catheter placement, the peritoneal cavity will be maximally drained (if clinically appropriate). Following catheter placement and appropriate routine care monitoring, the subject will be transported back to the inpatient floor for continued care and receipt of huCART-meso cells.

The T cell infusion will be performed by a licensed provider at the Hospital of the University of Pennsylvania. The transduced T cells will be instilled through the indwelling peritoneal catheter into the affected peritoneal space at an infusion rate of 10-20 mL/min.

Following the infusion, the subject should lie in the lateral decubitus position on the opposite side of the catheter for 15 minutes, if feasible. The intraperitoneal catheter will be removed prior to subject discharge per clinical discretion. Timing of placement and subsequent removal of the intraperitoneal catheter will be per clinical discretion.

5.3.1.3.4 Safety Monitoring

Emergency medical equipment (i.e. a crash cart) must be available for an emergency situation during the infusion in the event that the subject has an allergic response, or severe hypotensive crisis, or any other reaction to the infusion.

Vital signs (temperature, respiration rate, heart rate, blood pressure, and oxygen saturation by pulse oximetry) will be measured within 10 minutes prior and within 15 minutes after the infusion. Thereafter, vital signs will be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the infusion, and then every hour (+/- 10 minutes) for the next two hours until these signs are satisfactory and stable. If vital signs are not satisfactory and stable 3 hours after the huCART-meso infusion, vital signs will continue to be monitored as clinically indicated until stable.

Subjects in Cohorts 5-7 will remain inpatient for observation for a minimum of 48 hours after each huCART-meso infusion. Please refer to **Sections 6.6** and **6.8.2** for additional information. The subject may

then be discharged in accordance with hospital policy after this observation period is complete and the subject is medically stable.

5.3.2 Cyclophosphamide (Applicable Cohorts)

5.3.2.1 Receipt of Cyclophosphamide

Cyclophosphamide will be purchased through the clinical site pharmacy for research purposes.

5.3.2.2 Storage

Cyclophosphamide will be a stored according to the manufacturer instructions.

5.3.2.3 Premedication for Cyclophosphamide

It is anticipated that subjects receiving cyclophosphamide may experience nausea and vomiting as a side effect of the treatment. Anti-emetic prophylaxis premedication for nausea (including corticosteroids) can be administered prior to infusion of chemotherapy according to the institutional standards. Choice of specific agent will be left to the discretion of the investigator, and may include corticosteroids as appropriate. For more details, see **Sections 5.6** and **6.4.**

5.3.2.4 Administration

For Cohort 2 and Cohort 6 subjects: Cyclophosphamide will be administered as a flat dose of 1 gram/m^2 by IV infusion 2-4 days prior to the first huCART-meso cell infusion (**Section 6.4**).

For Cohort 7 subjects: Cyclophosphamide will be administered as 300 mg/m^2/day over 3 days by IV infusion so that the last day of chemotherapy falls 3 days (±1 day) prior to the first huCART-meso cell infusion (**Section 6.4**).

Appropriate institutional procedures for proper handling and disposal of antineoplastic drugs should be followed.

5.3.2.5 Cyclophosphamide Toxicity Management Considerations

Potential risks of cyclophosphamide administration and their management are described in **Section 1.7** and **6.4**.

5.3.3 Fludarabine (Cohort 7)

5.3.3.1 Receipt of Fludarabine

Fludarabine will be purchased through the clinical site pharmacy for research purposes.

5.3.3.2 Storage

Fludarabine will be a stored according to the manufacturer instructions.

5.3.3.3 Premedication for Fludarabine

It is anticipated that subjects receiving fludarabine may experience nausea and vomiting as a side effect of the treatment. Anti-emetic prophylaxis premedication for nausea (including corticosteroids) can be administered prior to infusion of chemotherapy according to the institutional standards. Choice of specific

agent will be left to the discretion of the investigator, and may include corticosteroids as appropriate. For more details, see **Sections 5.6** and **6.4.**

5.3.3.4 Administration

Fludarabine will be administered as 30 mg/m^2/day over 3 days so that the last day of chemotherapy falls 3 days (±1 day) prior to the first huCART-meso infusion (Day 0). Fludarabine doses will be rounded down to the nearest 50 mg vial size, if the rounded dose does not differ by > 10% of the original dose prescribed. Appropriate institutional procedures for proper handling and disposal of antineoplastic drugs should be followed.

5.3.3.5 Fludarabine Toxicity Management Considerations

Potential risks of fludarabine administration and their management are described in **Section 1.7** and **6.4**.

5.4 Dose-Limiting Toxicity (DLT) and Cohort Progression

Dose limiting toxicity is defined as either hematologic or non-hematologic toxicity (NCI Common Terminology Criteria for Adverse Events (CTCAE v4.03) which develops following dosing, is new (not a pre-existing condition ongoing at the time of infusion, regardless of grading), at least possibly related to T cells, and non-manageable to Grade 1-2 within 7 days of onset. The duration of the DLT monitoring period will be dependent on cohort assignment as follows:

- Cohorts 1-5: Through the Day 28 safety follow-up visit.
- Cohorts 6-7: Through the Day 21 safety follow-up visit.

The following events will be considered DLTs if determined to be related to huCART-meso cell therapy and the event meets all other criteria in the above definition:

- Grade 3 or higher non-hematological toxicity except; with the following exceptions:
  - Grade 3 electrolyte abnormalities
  - Nausea
  - Vomiting
  - Diarrhea
  - Fatigue
- Grade 3 or higher hematologic toxicity; except those expected following lymphodepleting chemotherapy administration (e.g. thrombocytopenia, lymphopenia, neutropenia, anemia, etc).

Historical Cohort Advancement Plan:
Subjects will be enrolled serially. Infusions will be staggered to allow assessment of DLTs for cohort progression, expansion, or dose de-escalation. For example, the 2nd and 3rd subjects in each cohort may be infused and followed in parallel but only after the 1st subject in that cohort completes the day 28 visit without DLT.

If 0 DLTs in 2nd and 3rd subjects, the study may advance to the next cohort. If there is 1 DLT in the 2nd or 3rd subject the cohort will be expanded to treat up to 6 subjects. If a total of 2 DLTs occur in the 2nd and 3rd subjects, further enrollment in that cohort will be discontinued, and additional subjects will be enrolled into the dose de-escalation cohort. Alternatively, the 1st and 2nd subjects in each cohort may be infused and followed in parallel. If there is 0 DLTs in the 1st and 2nd subjects, a 3rd subject would be dosed at the same regimen/cohort. If there is 1 DLT in the 1st or 2nd subjects, the cohort would be expanded to enroll up to 6 subjects. If a total of 2 DLTs occur in the 1st and 2nd subjects, further enrollment in that cohort will be discontinued, and additional subjects will be enrolled into the dose de-escalation cohort. The study

will be stopped if 2 DLTs occur at the de-escalated dose level (either cohort with or without cyclophosphamide).

The clinical PI and Medical Director will perform official DLT assessments and make formal determinations regarding cohort advancement (where applicable). Please refer to **Section 5.4** for the definition of dose-limiting toxicity as well as additional information on cohort progression.

As of Protocol Amendment V5, Cohort 3 and 4 have been permanently closed due to unacceptable toxicity, and the maximum tolerated dose has been established as 1-3x10^7 huCART-meso cells/m^2.

Subsequent enrollment will occur in the dose expansion cohorts (Cohorts 5-7) at the MTD only. Enrollment in these cohorts may occur in parallel and will include up to 6 subjects/cohort. All subjects will be continually evaluated for dose-limiting toxicities (DLT). In the event of 2 DLTs in a specific cohort, additional enrollment and treatment activity within that cohort will be paused to allow for further investigation.

5.5 Subject Compliance Monitoring

Adherence to scheduled follow-up visits is important in order to assess the primary safety endpoint associated with this study. During the process of informed consent, each patient will be clearly informed of the study schedule, associated procedures, and the requirement for follow-up. Patients that are not able to commit to the study schedule will not be enrolled in the study.

5.6 Prior and Concomitant Therapy

All prescription and nonprescription medication, vitamins, devices, and herbal and nutritional supplements, taken by the subject during the 30 days prior to consent will be recorded. Concomitant medications will continue to be collected at every visit following consent and until the subject has completed primary follow-up or has been discontinued from participation in the study. Any additions, deletions, or changes of these medications will be documented. All prior oncology therapies for their targeted disease will be collected in the appropriate eCRF.

Concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are allowed throughout the subject's participation. Subjects may also continue to receive standard therapies and/or undergo routine care procedures for the management of their disease prior to receipt of study treatment and while their product is being manufactured. However, the following guidelines must be adhered to during the study:

- Apheresis:
    - Steroids or other immunosuppressant drugs should NOT be used within 5 days prior to the apheresis procedure. Patients may be on a stable low dose of steroids (≤10mg daily equivalent of prednisone). Topical and inhaled steroids are permitted at any time as per clinical discretion.
    - Concurrent cancer treatment is not allowed within 1 week prior to the apheresis procedure. Medication intended solely for supportive care (e.g. analgesics, antiemetics, antidiarrheals, antidepressants or bisphosphonates, etc.) may be used at the investigator's discretion. Palliative focal radiation therapy for symptom management is allowed.
    - Subjects must not have received an investigational product within 2 weeks prior to apheresis collection.

- Lymphodepleting Chemotherapy (Applicable Cohorts):
    - Prophylactic antibiotics are recommended post lymphodepleting chemotherapy and prior to CAR T cell administration per institutional standards and practices for cellular therapies.
    - Steroid or other immunosuppressant drugs:
        - Corticosteroids treatment as anti-emetic prophylaxis on the day of lymphodepleting chemotherapy administration is allowed per institutional guidance.
        - Topical and inhaled steroids are permitted at any time as per clinical discretion.
- huCART-meso Cells:
    - Alternative cancer treatment is not allowed within 2 weeks prior to huCART-meso cell infusion. Medication intended solely for supportive care (e.g. analgesics, antiemetics, antidiarrheals, antidepressants or bisphosphonates, etc.) may be used at the investigator's discretion. Palliative focal radiation therapy for symptom management is allowed.
    - Subjects must not have received an investigational product within 4 weeks or 5 half-lives (whichever is shorter) of the first huCART-meso cell infusion.
    - GM-CSF should be avoided due to potential to worsen CRS symptoms. Therapeutic use in patients with serious neutropenic complications such as tissue infection, sepsis syndrome, fungal infection, etc. may be considered at the investigator's discretion, consistent with American Society of Clinical Oncology guidelines. The use of erythropoietin is also permitted at the discretion of the treating physician. Prophylactic use of granulocyte colony-stimulating factor (G-CSF, filgrastim) may be used if medically indicated, particularly in a patient who is experiencing recurrent difficulties with neutropenia, however the effects of G-CSF on product manufacturing are unknown.
    - Steroid or other immunosuppressant drugs:
        - Should NOT be used within 24 hours prior to, or following, the huCART-meso cell infusion(s) unless under life-threatening circumstances or at the physician-investigator's discretion for CRS Management. Patients may continue on a stable low dose of steroids (≤10mg daily equivalent of prednisone).
        - Therapeutic doses of steroids must be stopped >48 hours prior to huCART-meso infusion(s). However, physiological replacement doses of steroids are allowed (6-12 mg/m^2/day hydrocortisone or equivalent).
        - Topical and inhaled steroids are permitted at any time as per clinical discretion.
- Prophylaxis/Toxicity Management:
    - Subjects with severe signs and symptoms attributable to cytokine release syndrome (i.e. CRS) should be managed with administration of tocilizumab or other anti-cytokine directed therapies (Refer to **Section 9.4.2** and to the current version of the huCART-meso Investigator's Brochure for complete details).
- Primary Follow-up:
    - huCART-meso infusions may also be discontinued at any time in favor of resuming standard of care treatment at the physician-investigator's discretion.
    - Data from all additional treatment received, as well as the subject's response to this treatment, will be collected/reported for research purposes.

6 STUDY PROCEDURES

Overview

The study consists of 1) a screening/enrollment phase, 2) apheresis and T cell manufacturing, 3) pre-infusion safety check, 4) an intervention phase consisting of huCART-meso cell administration alone or following study-mandated chemotherapy, and 5) safety follow-up visits. The Schedule of Events is included in **Appendices 1-3**.

6.1 Screening

Informed consent must be obtained before the subject can undergo any research-related procedures. Results obtained in routine clinical care prior to informed consent may also be used for screening purposes as long as they are obtained within the protocol required windows.

Screening procedures include:

- Complete medical history
- Physical examination including vital signs, height, and weight
- Review of concomitant medications and prior therapies (including timing of previous checkpoint inhibitors if given)
- ECOG performance status
- Leukapheresis screening - Subjects can be given the opportunity to undergo temporary central venous access for apheresis if peripheral access cannot be obtained.
- Complete blood count and differential
- Chemistry panel - including sodium, potassium, chloride, CO2, blood urea nitrogen, creatinine, glucose, phosphate, total protein, albumin, calcium, alkaline phosphatase, total bilirubin, ALT, AST, uric acid, LDH
- Coagulation Factors - PT, PTT, fibrinogen, INR, d-dimer
- Viral serologies: HIV, Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody, Hepatitis B core antibody, and Hepatitis C antibody. If the HCV antibody is positive, a screening HCV RNA by any RT-PCR or bDNA assay must be performed. Eligibility will be determined based on the screening value. The test is not required if documentation of a negative result of a HCV RNA test performed within 60 days prior to screening is provided.
- Autoantibody panel: ANA
- Urinalysis
- 12 lead Electrocardiogram (EKG)
- Resting echocardiogram (ECHO)
- Urine β HCG pregnancy (women of childbearing potential)
- MRI Brain - For suspected brain metastasis (performed within 8 weeks of eligibility confirmation by a physician-investigator)
- Tumor assessments with computed tomography performed with or without contrast within 8 weeks of eligibility confirmation by a physician-investigator. The types of scans performed will be per clinical discretion based on the patient population and disease status.

In the event that the time between the screening visit and the infusion of huCART-meso cells exceeds the 8 week Screening/Enrollment Window the following will be repeated: Physical Examination, Performance Status Assessment, Complete Blood Count with differential and Platelet Count, Chemistry Panel, Pregnancy test, HIV and Hepatitis B/C tests, and ECHO scan.

Monitoring Visit for Eligibility

Assignment of subject numbers will occur at initial consent, will be in sequential order (i.e. 02916-01, 02916-02, etc.), and no numbers will be omitted. Subject numbers will be used on all study documentation. Once assigned, the subject number must not be reused for any other subject and it must not be changed, even if the subject is rescreened.

At the time a subject consents to participate in this study, a Consent Notification Form should be completed. Once required screening tests have been completed and the subject has been determined eligible by the physician-investigator, provide the documents listed below to:

Sponsor Protocol Monitor and Sponsor Project Manager
Center for Cellular Immunotherapies (CCI)

Documents required:

- Completed Eligibility Form
- Redacted copy of signed patient informed consent and HIPAA Authorization
- Redacted source documentation to confirm enrollment/eligibility (including patient past medical history, laboratory, radiological reports, physical exam, concomitant medications and any other source documentation to support that the patient meets eligibility criteria and has completed all required screening assessments).

Upon informed consent completion and receipt of screening and eligibility documentation, the Sponsor Protocol Monitor will review and provide documentation that the monitoring visit for eligibility has been completed. This documentation must be received prior to apheresis and cell product manufacturing. If T cells have already been collected during a previous apheresis, it is not necessary to repeat the apheresis procedure.

6.2 Leukapheresis

A large volume apheresis procedure will be carried out at the University of Pennsylvania Apheresis Center according to standard clinical procedures (~4-6 weeks prior to infusion of the huCART-meso cells). PBMC are obtained for CAR T cells during this procedure. From a single leukapheresis, the intention is to harvest at least 5 x 10^9 white blood cells to manufacture huCART-meso cells. The huCART-meso cell product is expected to be ready for release approximately 4 weeks later. If the harvest of cells or the T cells manufacture is unsuccessful, patients will have the option to undergo a second leukapheresis for a second manufacturing process. Subjects have the option to receive a lower than target dose if the huCART-meso product meets CVPF release criteria, and the subject cannot or is not willing to perform a second apheresis, or if the second manufacturing process fails. If a subject has previously had an adequate apheresis collection banked according to current Good Manufacturing Practices at the Clinical Cell and Vaccine Production Facility, these cells may be used as the source of cells for huCART-meso cell manufacturing.

Recommended criteria for apheresis product acceptance to initiate processing for clinical manufacturing to meet the dosing requirements includes the following specifications: It is recommended that the patient have an absolute lymphocyte count (ALC) ≥500/µl, prior to undergoing apheresis. If the patient's ALC is <500/µl, it is recommended that a lymphocyte subset analysis (CD3, CD4, CD8 counts) be performed to confirm that the patient has an absolute CD3 count of ≥150/µl. If the

absolute CD3 count is <150/µl, it is recommended that the leukapheresis procedure be delayed until their ALC is ≥500/µl or absolute CD3 count is ≥150/µl.

The apheresis product is transported to the CVPF for processing. A portion of the peripheral blood leukocytes will be saved for the TCSL laboratory at the University of Pennsylvania and used for baseline immunoassessment research assays and biobanking, and the other portion used for huCART-meso cell manufacturing in CVPF. Baseline blood leukocytes for FDA look-back requirements will also be obtained and cryopreserved.

Historical Apheresis Sample

Cryopreserved historical apheresis products collected from the patient prior to study entry are usable for huCART-meso manufacturing if the sample was collected at an appropriately certified apheresis center and if the product meets adequate mononuclear cell yields. If a historical apheresis product is not available, an apheresis procedure (as described above) will be performed for cell procurement after study eligibility has been confirmed.

6.3 Pre-infusion safety visit (Week -2 to -1)

Subjects will undergo the following evaluations within 14 days prior to their 1st study infusion (unless otherwise specified below). Please refer to the Schedule of Events in **Appendices 1-3**.

- Review of current medical conditions
- Physical examination- including vital sign measurements, ECOG performance status, and review of concomitant medications. Vital sign measurements include weight, temperature, heart rate, blood pressure, and oxygen saturation by pulse oximetry.
- Complete blood count and differential
- Chemistry panel- including sodium, potassium, chloride, CO2, blood urea nitrogen, creatinine, glucose, phosphate, total protein, albumin, calcium, alkaline phosphatase, total bilirubin, ALT, AST, uric acid, LDH
- Coagulation Factors- PT, PTT, fibrinogen, d-dimer
- Serum pregnancy test (females of childbearing potential only)
- Baseline ferritin, triglycerides, haptoglobin and CRP
- EKG
- Subjects must undergo a Respiratory Virus Panel (RVP, inclusive of SARS-CoV-2) within 10 days prior to the first planned huCART-meso infusion and prior to receipt of lymphodepleting chemotherapy. If the subject is positive for influenza, Tamiflu® or equivalent, should be administered per package insert. The subject must complete treatment prior to receiving lymphodepleting chemotherapy (if applicable) and huCART-meso cells. The test does not need to be repeated prior to these infusion(s), however if influenza sign and symptoms are present, the infusion(s) should be delayed until the subject is asymptomatic. If the subject is positive for another virus on the RVP, the infusion(s) will be delayed for at least 7 days to be sure clinical symptoms of a viral infection do not develop, or longer if clinically appropriate for the respective pathogen. If clinical symptoms develop, the infusion(s) will be delayed until resolution of these symptoms.
- Clinical Tumor Markers: CA125.
- Radiographic Imaging: Baseline scans should be obtained within 28 days prior to infusion of huCART-meso cells and after any cancer therapy administered between collection and huCART-

meso infusion. In applicable cohorts, baseline scans should also be obtained prior to lymphodepleting chemotherapy administration.

- A baseline tumor biopsy will be performed prior to huCART-meso infusion (and administration of lymphodepleting chemotherapy if applicable), if the subject has an accessible tumor. The baseline biopsy may occur any time prior to infusion between Day -14 to Day -1. This baseline biopsy sample will be used to evaluate the level of mesothelin expression and if feasible, other immune markers at baseline. If a subject does not have an accessible tumor or a biopsy is not felt to be clinically appropriate, an archived tumor tissue specimen may be obtained for research purposes.
- Research blood sample collection- please refer to the Schedule of Evaluations (**Appendices 1-3**) for complete details.

6.4 Lymphodepleting Chemotherapy Administration

Lymphodepleting chemotherapy will be administered prior to the huCART-meso cell infusion for subjects participating in targeted cohorts only:

- **Cohorts 2 and 6:** a single flat dose of 1 gram/m^2 cyclophosphamide by intravenous infusion given 2-4 days prior to the huCART-meso infusion. In Cohort 6, cyclophosphamide will only be administered prior to the first huCART-meso infusion. Note: Cohort 4 (which also included Cyclophosphamide administration) was permanently closed with Protocol V5.
- **Cohort 7:** cyclophosphamide 300 mg/m^2/day and fludarabine 30 mg/m^2/day given over 3 days by intravenous infusion. Lymphodepleting chemotherapy will be scheduled such that the last day of chemotherapy is 3 days (+/- 1 day) prior to the 1st infusion of huCART-meso cells. Fludarabine doses will be rounded down to the nearest 50 mg vial size, if the rounded dose does not differ by > 10% of the original dose prescribed.

The main goal of using lymphodepleting chemotherapy is to achieve lymphodepletion that may enhance engraftment of adoptive T cells, while minimizing complications from neutropenia. The proposed regimen has been used widely in other studies and has been tolerated well. At the discretion of the investigator, some subjects may require hospitalization for administration of lymphodepleting chemotherapy. In this event, the hospitalization will not be recorded as an SAE.

Prior to lymphodepleting chemotherapy administration, subjects will undergo tests/procedures performed according to the Schedule of Events in **Appendices 1-3**. A CBC with differential and comprehensive chemistry panel must be performed within 48 hours prior to initiating lymphodepleting chemotherapy. If already performed within this window, these laboratory tests do not need to be repeated on the day of lymphodepleting chemotherapy. Tests/procedures do not need to be repeated prior to subsequent lymphodepleting chemotherapy infusions unless clinically indicated.

Subjects who receive lymphodepleting chemotherapy, but in whom the huCART-meso infusion is subsequently delayed >2 weeks after the first day of lymphodepleting chemotherapy, may receive a second cycle of lymphodepleting chemotherapy prior to huCART-meso infusion at the clinical investigator's discretion.

<u>The following criteria must be met in order to proceed with lymphodepleting chemotherapy administration</u>:

1) Subjects should not have received systemic chemotherapy within 2 weeks prior to the planned huCART-meso infusion. If systemic chemotherapy was administered, then this procedure will be re-scheduled.

2) Subjects must meet all concomitant therapy guidelines as outlined in **Section 5.6**. This includes restrictions on checkpoint inhibitor therapies while on study, and receipt of prior anti-PD-1 or PDL1 therapy within 4 months of the planned huCART-meso infusion.
3) Subjects who have received chemotherapy or other immunotherapies > 2 weeks and < 6 weeks of the planned huCART-meso cell infusion, must have recovered from all toxicities, including myelosuppression.
4) Subjects should not experience a significant change in performance status compared to initial eligibility criteria that would, in the opinion of the treating physician, increase the risk of experimental cell infusion.
5) Subjects must not have laboratory abnormalities after enrollment that, in the opinion of the treating investigator or PI, may impact subject safety or the subjects' ability to receive lymphodepleting chemotherapy or the huCART-meso cells. If this occurs, patients may have their treatment delayed until both the treating investigator and PI determine it is clinically appropriate to proceed.
6) Subjects must undergo a Respiratory Virus Panel (RVP, inclusive of SARS-CoV-2) within 10 days prior to the planned huCART-meso cell infusion and prior to receipt of lymphodepleting chemotherapy. If the subject is positive for influenza, Tamiflu® or equivalent, should be administered per package insert. The subject must complete treatment **prior** to receiving lymphodepleting chemotherapy. The RVP does not need to be repeated prior to infusion; however, if influenza signs and symptoms are present, the infusions should be delayed until the subject is asymptomatic. If the subject is positive for another virus on the RVP, the infusions will be delayed for at least 7 days to be sure clinical symptoms of a viral infection do not develop, or longer if clinically appropriate for the respective pathogen. If clinical symptoms develop, the infusions will be delayed until resolution of these symptoms
7) Subjects should not experience any of the following specific toxicities:
    a. Pulmonary: New requirement for supplemental oxygen or presence of progressive radiographic abnormalities on chest x-ray (chest x-ray is not required at this juncture but should be evaluated if performed for clinical purposes).
    b. Cardiac: New cardiac arrhythmia not controlled with medical management.
    c. Hypotension requiring pressor support.
    d. Active Infection: Positive blood cultures for bacteria, fungus, or virus within 48-hours of lymphodepleting chemotherapy.

Anti-emetic prophylaxis:
It is anticipated that subjects receiving lymphodepleting chemotherapy may experience nausea and vomiting as a side effect of the treatment. Premedication for nausea can be administered prior to infusion of chemotherapy. Choice of specific agent will be left to the discretion of the investigator. Corticosteroid treatment as anti-emetic prophylaxis prior to lymphodepleting chemotherapy administration is allowed per institutional guidance per **Section 5.6**.

Prophylactic antibiotics are recommended post lymphodepleting chemotherapy per institutional standards and practices for cellular therapies.

Potential Side Effects
Chemotherapy is an integral part of this study and therefore subjects will be evaluated for toxicity that may be related to the chemotherapy conditioning regimen. See **Section 1.7**/Risks of cyclophosphamide and fludarabine for complete information.

6.5 huCART-meso product administration (Day 0)

Subjects in Cohorts 5-7 will receive huCART-meso cells on an inpatient basis at the Hospital of the University of Pennsylvania. The subjects will remain inpatient for observation for a minimum of 48 hours after the huCART-meso infusion (Study Visit Days 1 and 2). The subject may then be discharged in accordance with hospital policy after this observation period is complete and the subject is medically stable. The subject may remain hospitalized beyond this protocol-required window if deemed necessary for medical management of serious adverse events.

Prior to the administration of huCART-meso cells, all subjects will undergo tests/procedures in accordance with the Schedule of Events in **Appendices 1-3**. Results of CBC with differential and chemistry panel must be reviewed by a physician-investigator prior to administration of study treatment; otherwise, results of other laboratory testing are not required prior to the huCART-meso infusion except as necessary for investigator to evaluate criteria to proceed with the huCART-meso infusion as described below. A CBC with differential and comprehensive chemistry panel must be performed within 48 hours prior to the huCART-meso infusion (and after receipt of lymphodepleting chemotherapy if applicable). If already performed within this window, these laboratory tests do not need to be repeated on Day 0.

<u>The following criteria must be met in order to proceed with the huCART-meso infusion:</u>

1) Subjects should not have received systemic chemotherapy within 2 weeks prior to the huCART-meso infusion, with the exception of protocol-required lymphodepleting chemotherapy (applicable cohorts). If chemotherapy was administered, then this infusion will need to be re-scheduled.
2) Subjects must meet all concomitant therapy guidelines as outlined in **Section 5.6**.
3) Subjects should not experience a significant change in performance status compared to initial eligibility criteria that would, in the opinion of the treating physician, increase the risk of experimental cell infusion.
4) Subjects must not have laboratory abnormalities after enrollment that, in the opinion of the treating investigator or PI, may impact subject safety or the subjects' ability to receive huCART-meso cells. If this occurs, patients may have their treatment delayed until both the treating investigator and PI determine it is clinically appropriate to proceed.
5) Subjects must undergo a Respiratory Virus Panel (RVP, inclusive of SARS-CoV-2) within 10 days prior to the huCART-meso cell infusion. If the subject is positive for influenza, Tamiflu® or equivalent, should be administered per package insert. The subject must complete treatment **prior** to receiving huCART-meso cells. The RVP does not need to be repeated prior to infusion; however, if influenza signs and symptoms are present, the infusion should be delayed until the subject is asymptomatic. If the subject is positive for another virus on the RVP, the infusion will be delayed for at least 7 days to be sure clinical symptoms of a viral infection do not develop. If clinical symptoms develop, or longer if clinically appropriate for the respective pathogen, the infusion will be delayed until resolution of these symptoms.
6) Subjects should not experience any of the following specific toxicities:
    a. Pulmonary: New requirement for supplemental oxygen or presence of progressive radiographic abnormalities on chest x-ray (chest x-ray is not required at this juncture but should be evaluated if performed for clinical purposes).
    b. Cardiac: New cardiac arrhythmia not controlled with medical management.
    c. Hypotension requiring pressor support.
    d. Active Infection: Positive blood cultures for bacteria, fungus, or virus within 48-hours of T cell infusion.

Please refer to **Section 5.3** [Preparation and Administration of Investigational Product(s)] for complete details.

6.6 Post-Infusion Inpatient Observation (Cohorts 5-7 only)

The subject will remain inpatient for observation for a minimum of 48 hours after the huCART-meso infusion. During post-infusion observation, the subject will undergo vital sign assessments (temperature, respiration rate, heart rate, blood pressure, and oxygen saturation by pulse oximetry) every 4-6 hours (+/- 30 minutes) from the end of the infusion vital sign monitoring period until 48 hours post infusion. Additional monitoring may be performed per clinical discretion. Subjects will also undergo physical examinations, clinical labs, and collection of research samples as per the Schedule of Events (**Appendices 1-3**). Additional assessments will be performed as deemed clinically appropriate. The subject may then be discharged after this observation period is completed when medically stable and in accordance with hospital policy. The subject may remain hospitalized beyond this protocol-required window if deemed necessary for medical management of serious adverse events.

*6.7 Post-infusion Visits (Cohorts 1-5)**

**** Note: Cohorts 3 and 4 are permanently closed as of Protocol V5.***

6.7.1 Safety Follow-up Visits

Subjects will have safety follow-up visits on Days 6 (±1), 9 (±1), 14 (±1), and 21 (±1) post-infusion as per the Schedule of Events in **Appendix 1**.

If the subject's tumor is measurable by CT and accessible by image-guided biopsy, subjects will undergo a tumor biopsy under image guidance at Day 14(±7) to evaluate the impact of therapy on the tumor microenvironment and to assess for the presence of CAR T cells and mesothelin expression. These will be compared to baseline values from fresh biopsies and/or stored surgical tumor specimens, if available. Please refer to the Laboratory Manual for complete details.

In case of unexpected AEs or SAEs, additional samples may be collected for research analysis, focused at evaluating the potential causality with the infused huCART-meso cells see **Section 3.3**/Collection of Research Samples.

6.7.2 Day 28 (+/- 5 days): Safety and Tumor Staging

At this study visit, subjects will undergo tests and procedures in accordance with the Schedule of Events in **Appendix 1.** Tumor response will be assessed as described in **Section 7.1**. A mini-apheresis procedure (~5 L) will also be performed at this visit for research purposes. A 60 ml blood draw may be substituted for the mini-apheresis procedure at the discretion of the treating investigator.

6.7.3 Month 2 (+/- 5 days): Safety

Subjects will return to the center at Month 2 following huCART-meso infusion. At this study visit, subjects will undergo tests and procedures in accordance with the Schedule of Events in **Appendix 1.**

6.7.4 Month 3 (+/- 5 days): Safety and Tumor Staging

Subjects will return to the center at Month 3 following huCART-meso infusion. At this study visit, subjects will undergo tests and procedures in accordance with the Schedule of Events in **Appendix 1**. In addition,

tumor staging (CT imaging as clinically indicated as per patient population and disease status) will be performed. A CT scan within 4 weeks of this visit is acceptable. Tumor response will be assessed as described in **Section 7.1**.

6.7.5 Month 6 (+/- 5 days): Safety and Tumor Staging

Subjects will return to the center at Month 6 following huCART-meso infusion. At this study visit, subjects will undergo tests and procedures in accordance with the Schedule of Events in **Appendix 1**. In addition, tumor staging (CT imaging as clinically indicated as per patient population and disease status) will be performed. A CT scan within 4 weeks of this visit is acceptable. Tumor response will be assessed as described in **Section 7.1**.

6.7.6 Quarterly Evaluations for up to 2 Years Post-Infusion

After month 6, subjects will be evaluated on a quarterly basis until 2 years post infusion. At these study visits, subjects will undergo tests and procedures in accordance with the Schedule of Events in **Appendix 1.**

6.8 Post-Infusion Visits (Cohort 6 only)

6.8.1 Safety Follow-up Visits (Post Infusion #1)

Subjects will have safety follow-up visits on Days 6 (±1), 9 (±1), and 14 (±1) post-infusion as per the Schedule of Events in **Appendix 2**.

6.8.2 Additional huCART-meso Infusion(s)

Cohort 6 subjects may receive up to two additional IV infusions of huCART-meso cells at the same dose level, if determined clinically appropriate by the physician-investigator. Each additional infusion may be given approximately 21 and 42 days after the Day 0 infusion, allowing for a +3 week window as needed. Therefore, huCART-meso Infusion #2 is targeted for Day 21 (+3 weeks) and huCART-meso Infusion #3 is targeted for Day 42 (+ 3 weeks). Additional huCART-meso infusions performed outside of this window require approval from the Principal Investigator and Sponsor Medical Director. The subject must be evaluated for the below criteria prior to each additional infusion. Subjects will <u>not</u> receive lymphodepleting chemotherapy prior to additional huCART-meso infusions.

<u>The following criteria must be met in order to proceed with additional infusions of huCART-meso cells:</u>

1) Subjects must have manufactured huCART-meso cell product available which meets the minimum acceptable dose for infusion ($1x10^6$ huCART-meso cells/m^2).
2) Subjects should not experience a significant change in performance or clinical status that would, in the opinion of the treating physician-investigator or PI, increase the risk of experimental cell infusion.
3) Subjects experiencing laboratory abnormalities that, in the opinion of the physician-investigator or PI, may impact subject safety or the subjects' ability to receive huCART-meso cells, may have their infusion delayed until determined to be clinically appropriate to proceed with the CAR T cell infusion. If this occurs, patients may have their treatment delayed until both the treating investigator and PI determine it is clinically appropriate to proceed.
4) Subjects should not be experiencing signs/symptoms of an active infection.

5) Subjects should not be experiencing any ongoing treatment-related grade > 3 events, that in the opinion of the treating physician-investigator and PI are anticipated to be exacerbated by additional infusions of huCART-meso cells.
6) Subjects should not be experiencing signs/symptoms of cytokine release syndrome (CRS) or other severe CAR T cell related toxicities.
7) Subjects must undergo a Respiratory Virus Panel (RVP, inclusive of SARS-CoV-2) within 10 days prior to each huCART-meso cell infusion. If the subject is positive for influenza, Tamiflu® or equivalent, should be administered per package insert. The subject must complete treatment **prior** to receiving huCART-meso cells. The RVP does not need to be repeated prior to infusion; however, if influenza signs and symptoms are present, the infusion should be delayed until the subject is asymptomatic. If the subject is positive for another virus on the RVP, the infusion will be delayed for at least 7 days to be sure clinical symptoms of a viral infection do not develop, or longer if clinically appropriate for the respective pathogen. If clinical symptoms develop, the infusion will be delayed until resolution of these symptoms.

If the additional huCART-meso infusions are delayed by greater than 7 days or not performed for any reason, the additional infusion study visits [at Day 21 and Day 42] will still be performed as scheduled, however they will be repurposed as a safety follow-up visit only. These safety follow-up visits will be identified as the actual study visit from Day 0 (i.e. Day 26), and should be performed within +7 days of the planned study timepoint. If the additional huCART-meso infusions are then subsequently performed within the protocol allowable window (+3 weeks), the actual infusion day will be calculated as the actual study visit day from Day 0 (i.e. Day 35) and the study visit assessments required for an infusion visit will be repeated prior to the infusion as per the Schedule of Events (**Appendix 2**)**.**

The subject will remain inpatient for observation for a minimum of 48 hours after each huCART-meso infusion. During post-infusion observation, the subject will undergo vital sign assessments (temperature, respiration rate, heart rate, blood pressure, and oxygen saturation by pulse oximetry) every 4-6 hours (+/- 30 minutes) from the end of the infusion vital sign monitoring period until 48 hours post infusion. Additional monitoring may be performed per clinical discretion. Subjects will also undergo physical examinations, clinical labs, and collection of research samples as per the Schedule of Events (**Appendix 2**).

Additional assessments will be performed as deemed clinically appropriate. Should evidence of cytokine release syndrome or respiratory symptoms appear, the subject will be supported as noted in **Section 9.4.2**, and may require a more prolonged admission. The subject may then be discharged after this observation period is complete and the subject is medically stable. The subject may remain hospitalized beyond this protocol-required window if deemed necessary for medical management of serious adverse events.

6.8.3 Post-Infusion Safety Follow-up (Infusions #2 and #3)

As described above, the additional huCART-meso infusions may be performed within a +3 week window from the targeted infusion days i.e. [Day 21 (+3 weeks) and Day 42 (+3 weeks)]. Given the allowable window for these additional infusions, the timing of the post-infusion safety follow-up visits will be dependent on the actual timing of the additional huCART-meso infusions as follows.

- The timing of the post-infusion safety follow-up visits will be calculated as the actual study day they occurred post Infusion #2 and Infusion #3 (i.e. **+** 6 days post Infusion #2,+7 days post Infusion #2, **+**13 days post Infusion #3, etc.). This should be recorded as the actual study day, even if an

assessment falls within the allowable visit window (i.e. Day +6 visit occurs on Day +7, record as Day +7). This guidance applies to the timing of study procedures (i.e. imaging, etc) as well as study visits.

- In addition, the actual study visit day calculated from Day 0 (the initial huCART-meso infusion) must also be captured (i.e. Day 30, Day 31, etc).
- Example:
  - Infusion # + Study Visit Day Post Additional Infusion/Study Visit Day from Day 0
  - Infusion #2 Day +6/Day 28
- Both time points must be reported for tests/procedures performed during post-infusion safety follow-up visits for Infusion #2 and Infusion #3 only.

In addition to the required inpatient observation Day +1 and +2 post each additional huCART-meso infusion, subjects will have safety follow-up visits on Day +6 (±1), +9 (±1), and +14 (±1) post each infusion as per the Schedule of Events in **Appendix 2**. An additional safety follow-up visit will be performed Day +21 (±1) after the last huCART-meso infusion. If an infusion is omitted, these post-infusion safety follow-up visits will not be required. If an infusion is delayed, these additional safety follow-up visits will be shifted to align with the timing of the infusion (as indicated above). Please refer to the Cohort 6 Schedule of Events in **Appendix 2** for complete details.

Day +14 (±7) Post the huCART-meso Infusion #2:

- Tumor Biopsy:
  - If the subject's tumor is measurable by CT and accessible by image-guided biopsy, subjects will undergo a tumor biopsy under image guidance to evaluate the impact of therapy on the tumor microenvironment and to assess for the presence of CAR T cells and mesothelin expression. These will be compared to baseline values from fresh biopsies or stored surgical tumor specimens, if available. Please refer to the Laboratory Manual for complete details.
- Tumor Imaging
- Tumor Biomarkers

If huCART-meso Infusion #2 is not performed for any reason, the post-infusion biopsy/tumor markers/ imaging will be performed at the time the decision is made by the physician-investigator **not** to administer additional infusions. Tumor imaging may also be performed at any time as per clinical discretion.

Day +21 (±7) Post the 3rd huCART-meso Infusion (or last huCART-meso infusion if all three infusions are not received for any reason):

- Apheresis – A mini-apheresis procedure (~5 L) will also be performed at this visit for research purposes. A 60 ml blood draw may be substituted for the mini-apheresis procedure at the discretion of the treating investigator.

In case of unexpected AEs or SAEs, additional samples may also be collected for research analysis, focused at evaluating the potential causality with the infused huCART-meso cells see **Section 3.3**/Collection of Research Samples.

6.8.4 Month 3 (+/- 5 days): Safety and Tumor Staging

The timing of this study visit will be calculated from the initial huCART-meso infusion (Day 0). At this study visit, subjects will undergo tests and procedures in accordance with the Schedule of Events in **Appendix**

2. In addition, tumor staging (CT imaging as clinically indicated as per patient population and disease status) will be performed. A CT scan within 4 weeks of this visit is acceptable. Tumor response will be assessed as described in **Section 7.1**.

6.8.5 Month 6 (+/- 5 days): Safety and Tumor Staging

The timing of this study visit will be calculated from the initial huCART-meso infusion (Day 0). At this study visit, subjects will undergo tests and procedures in accordance with the Schedule of Events in **Appendix 2**. In addition, tumor staging (CT imaging as clinically indicated as per patient population and disease status) will be performed. A CT scan within 4 weeks of this visit is acceptable. Tumor response will be assessed as described in **Section 7.1**.

6.8.6 Quarterly Evaluations for up to 2 Years Post-Infusion

After month 6, subjects will be evaluated on a quarterly basis until 2 years post infusion or until the subjects' progress and/or initiate another cancer-related therapy. The timing of these study visits will be calculated from the initial huCART-meso infusion (Day 0). At these study visits, subjects will undergo tests and procedures in accordance with the Schedule of Events in **Appendix 2**.

6.9 Post-Infusion Visits (Cohort 7)

6.9.1 Safety Follow-up Visits (Post Infusion #1)

Subjects will have safety follow-up visits on Days 6 (±1), 9 (±1), and 14 (±1) post-infusion as per the Schedule of Events in **Appendix 3**.

If the subject's tumor is measurable by CT and accessible by image-guided biopsy, subjects will undergo a tumor biopsy at Day 14 (+/- 7 days) after the 1st huCART-meso infusion (Day 0). This biopsy sample will be used to evaluate the impact of therapy on the tumor microenvironment and to assess for the presence of CAR T cells and mesothelin expression. These samples will be compared to baseline values from fresh biopsies or stored surgical tumor specimens, if available. Please refer to the Laboratory Manual for complete details.

6.9.2 Additional huCART-meso Infusion(s)

Cohort 7 subjects may receive up to two additional infusions of huCART-meso cells via intravenous (IV) administration at the same dose level, if determined clinically appropriate by the physician-investigator. Each additional infusion may be given approximately 21 and 42 days after the Day 0 infusion, allowing for a +3 week window as needed. Therefore, huCART-meso Infusion #2 is targeted for Day 21 (+3 weeks) and huCART-meso Infusion #3 is targeted for Day 42 (+ 3 weeks). Additional huCART-meso infusions performed outside of this window require approval from the Principal Investigator and Sponsor Medical Director. The subject must be evaluated for the below criteria prior to each additional infusion. Subjects will <u>not</u> receive lymphodepleting chemotherapy prior to additional huCART-meso infusions.

<u>The following criteria must be met in order to proceed with additional infusions of huCART-meso cells:</u>

1) Subjects must have manufactured huCART-meso cell product available which meets the minimum acceptable dose for infusion ($1x10^6$ huCART-meso cells/m^2).
2) Subjects should not experience a significant change in performance or clinical status that would, in the opinion of the treating physician-investigator or PI, increase the risk of experimental cell infusion.

3) Subjects experiencing laboratory abnormalities that, in the opinion of the physician-investigator or PI, may impact subject safety or the subjects' ability to receive huCART-meso cells, may have their infusion delayed until determined to be clinically appropriate to proceed with the CAR T cell infusion. If this occurs, patients may have their treatment delayed until both the treating investigator and PI determine it is clinically appropriate to proceed.
4) Subjects should not be experiencing signs/symptoms of an active infection.
5) Subjects should not be experiencing any ongoing treatment-related grade > 3 events, that in the opinion of the treating physician-investigator and PI are anticipated to be exacerbated by additional infusions of huCART-meso cells.
6) Subjects should not be experiencing signs/symptoms of cytokine release syndrome (CRS) or other severe CAR T cell related toxicities.
7) Subjects must undergo a Respiratory Virus Panel (RVP, inclusive of SARS-CoV-2) within 10 days prior to each huCART-meso cell infusion. If the subject is positive for influenza, Tamiflu® or equivalent, should be administered per package insert. The subject must complete treatment **prior** to receiving huCART-meso cells. The RVP does not need to be repeated prior to infusion; however, if influenza signs and symptoms are present, the infusion should be delayed until the subject is asymptomatic. If the subject is positive for another virus on the RVP, the infusion will be delayed for at least 7 days to be sure clinical symptoms of a viral infection do not develop, or longer if clinically appropriate for the respective pathogen. If clinical symptoms develop, the infusion will be delayed until resolution of these symptoms.

If the additional huCART-meso infusions are delayed by greater than 7 days or not performed for any reason, the additional infusion study visits [at Day 21 and Day 42] will still be performed as scheduled, however they will be repurposed as a safety follow-up visit only. These safety follow-up visits will be identified as the actual study visit from Day 0 (i.e. Day 26), and should be performed within +7 days of the planned study timepoint. If the additional huCART-meso infusions are then subsequently performed within the protocol allowable window (+3 weeks), the actual infusion day will be calculated as the actual study visit day from Day 0 (i.e. Day 35) and the study visit assessments required for an infusion visit will be repeated prior to the infusion as per the Schedule of Events **(Appendix 3).**

The subject will remain inpatient for observation for a minimum of 48 hours after each huCART-meso infusion. During post-infusion observation, the subject will undergo vital sign assessments (temperature, respiration rate, heart rate, blood pressure, and oxygen saturation by pulse oximetry) every 4-6 hours (+/- 30 minutes) from the end of the infusion vital sign monitoring period until 48 hours post infusion. Additional monitoring may be performed per clinical discretion. Subjects will also undergo physical examinations, clinical labs, and collection of research samples as per the Schedule of Events (**Appendix 3**).

Additional assessments will be performed as deemed clinically appropriate. Should evidence of cytokine release syndrome or respiratory symptoms appear, the subject will be supported as noted in **Section 9.4.2**, and may require a more prolonged admission. The subject may then be discharged after this observation period is complete and the subject is medically stable. The subject may remain hospitalized beyond this protocol-required window if deemed necessary for medical management of serious adverse events.

6.9.3 Post-Infusion Safety Follow-up (Infusions #2 and #3)

As described above, the additional huCART-meso infusions may be performed within a +3 week window from the targeted infusion days i.e. [Day 21 (+3 weeks) and Day 42 (+3 weeks)]. Given the allowable window for these additional infusions, the timing of the post-infusion safety follow-up visits will be dependent on the actual timing of the additional huCART-meso infusions as follows.

- The timing of the post-infusion safety follow-up visits will be calculated as the actual study day they occurred post Infusion #2 and Infusion #3 (i.e. **+** 6 days post Infusion #2, +7 days post Infusion #2, **+**13 days post Infusion #3, etc.). This should be recorded as the actual study day, even if an assessment falls within the allowable visit window (i.e. Day +6 visit occurs on Day +7, record as Day +7). This guidance applies to the timing of study procedures (i.e. imaging, etc) as well as study visits.
- In addition, the actual study visit day calculated from Day 0 (the initial huCART-meso infusion) must also be captured (i.e. Day 30, Day 31, etc).
- Example:
  - Infusion # + Study Visit Day Post Additional Infusion/Study Visit Day from Day 0
  - Infusion #2 Day +6/Day 28
- Both timepoints must be reported for tests/procedures performed during post-infusion safety follow-up visits for Infusion #2 and Infusion #3 only.

In addition to the required inpatient observation Day +1 and +2 post each additional huCART-meso infusion, subjects will have safety follow-up visits on Day +6 (±1), +9 (±1), and +14 (±1) post each infusion as per the Schedule of Events in **Appendix 3**. If an infusion is omitted, these post-infusion safety follow-up visits will not be required. If an infusion is delayed, these additional safety follow-up visits will be shifted to align with the timing of the infusion (as indicated above). Please refer to the Cohort 7 Schedule of Events in **Appendix 3** for complete details.

Day +14 (±7) Post the huCART-meso Infusion #2:

- Tumor Imaging
- Tumor Biomarkers
- 60mL blood draw- for research purposes

If huCART-meso Infusion #2 is not performed for any reason, the post-infusion tumor imaging will be performed at the time the decision is made by the physician-investigator **not** to administer additional infusions. Tumor imaging may also be performed at any time as per clinical discretion.

Day +14 (±7) Post the 3rd huCART-meso Infusion:

- 60mL blood draw- for research purposes

If a subject does not receive either huCART-meso Infusion #2 or Infusion #3, the subject will undergo this 60mL blood draw at the Day 42 (+7) Safety Follow-up Visit, which still must be performed in the absence of Infusion #3.

In case of unexpected AEs or SAEs, additional samples may also be collected for research analysis, focused at evaluating the potential causality with the infused huCART-meso cells see **Section 3.3**/Collection of Research Samples.

6.9.4 Month 3 (+/- 5 days): Safety and Tumor Staging

The timing of this study visit will be calculated from the initial huCART-meso infusion (Day 0). At this study visit, subjects will undergo tests and procedures in accordance with the Schedule of Events in **Appendix 3**. In addition, tumor staging (CT imaging as clinically indicated as per patient population and disease status) will be performed. A CT scan within 4 weeks of this visit is acceptable. Tumor response will be assessed as described in **Section 7.1**.

6.9.5 Month 6 (+/- 5 days): Safety and Tumor Staging

The timing of this study visit will be calculated from the initial huCART-meso infusion (Day 0). At this study visit, subjects will undergo tests and procedures in accordance with the Schedule of Events in **Appendix 3**. In addition, tumor staging (CT imaging as clinically indicated as per patient population and disease status) will be performed. A CT scan within 4 weeks of this visit is acceptable. Tumor response will be assessed as described in **Section 7.1**.

6.9.6 Quarterly Evaluations for up to 2 Years Post-Infusion

After month 6, subjects will be evaluated on a quarterly basis until 2 years post infusion. The timing of these study visits will be calculated from the initial huCART-meso infusion (Day 0). At these study visits, subjects will undergo tests and procedures in accordance with the Schedule of Events in **Appendix 3**.

6.10 Long-term Follow-up Protocol

All infused subjects who complete or prematurely discontinue participation in this study (as defined in **Section 4.5.1**), will be asked to participate in a separate 15-year long-term follow-up destination protocol. This long-term follow-up protocol includes evaluations performed for up to 15 years on all subjects as recommended by the FDA for protocols utilizing integrating viral vectors.

7 ASSESSMENTS

7.1 Disease Assessments

All subjects who receive the target dose of huCART-meso cells infusion will be considered evaluable for response [including progression-free survival (PFS) and overall survival (OS)]. All subjects with radiologically confirmed measurable disease (per RECIST 1.1) at baseline will also be considered evaluable for overall radiologic response (ORR).

Radiographic Imaging

Participants will undergo radiographic imaging by CT/MRI with or without contrast. Baseline scans should be obtained within 28 days prior to infusion of huCART-meso cells and after any cancer therapy administered between collection and huCART-meso infusion. In applicable cohorts, baseline scans should also be obtained prior to administration of lymphodepleting chemotherapy.

Post infusion imaging will be performed as described in the Schedule of Events (**Appendices 1-3**).

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

Radiographic Response

Radiographic responses will be measured using two imaging assessment approaches: 1) Response Evaluation Criteria in Solid Tumors (RECIST 1.1) and 2) immune related response criteria (irRC) when feasible. Note: irRC will not be evaluated in mesothelioma subjects.

For RECIST 1.1 evaluation, the determination of antitumor efficacy will be based on objective tumor assessments made according to the system of unidimensional evaluation. Mesothelioma will be assessed using the modified RECIST criteria. The same method and technique should be used to characterize each identified and reported lesion at baseline, during the study treatment period, and during follow-up. Imaging-based evaluation rather than clinical examination is the required technique when either could be used to assess the antitumor effect of the treatment. Computed Tomography (CT) scan is the preferred method for the tumor assessment. Tumor assessment scans may be performed with or without contrast. Because immune therapy can cause an initial tumor volume increase due to inflammation prior to subsequent tumor shrinkage, an exploratory analysis of response status will also be conducted using the recently developed immune related response criteria (irRC)(Wolchok et al., 2009). Novel to the irRC is the measurement of overall tumor burden as a metric of disease progression, compared to the limitation of using only baseline lesion measurements according to RECIST 1.1. According the irRC, new lesions do not constitute disease progression if net tumor burden including new lesions is stable or decreases. The irRC also permit disease progression at a subsequent time point after first detection. This accounts for the period required for activated T-cells to infiltrate the tumor, which may cause initial tumor volume increase due to inflammation but can subsequently translate into tumor shrinkage. The irRC also classify durable stable disease as clinical activity. Thus, this protocol will explore two distinct imaging criteria for determination of disease outcome in response to treatment with huCART-meso cells.

Tumor biomarkers

Tumor biomarkers as appropriate for each malignancy will also be measured. For ovarian cancer patients, CA125 will be measured. There are no clinical tumor markers routinely evaluated in mesothelioma patients.

Mesothelin is the receptor for CA125, and the interaction between mesothelin and CA125 is thought to play a role in tumor progression (details in **Section 1.7**). Therefore, binding of mesothelin by huCART-meso cells may directly affect the level of circulating CA125 irrespective of disease activity. Therefore, tumor biomarker assessment alone is not sufficient to determine response or progression in this study.

7.2 Research Correlative Studies Assessment

7.2.1 Collection of Research Samples

Research samples (including peripheral blood, fluid, and tumor tissue) will be obtained at defined time points to monitor for measures of safety and T cell persistence. This includes an image-guided minimally invasive biopsy pre-infusion and post-infusion for research purposes. Please refer to the Schedule of Events **(Appendices 1-3)** for required time points.

In case of unexpected AEs or SAEs, additional samples may also be collected for research analysis, focused at evaluating the potential causality with the infused huCART-meso cells. Additional blood work for research evaluation may also be requested at any time at the investigators' discretion, and is especially encouraged whenever there is a clinical concern for a potential toxicity related to huCART-meso cells, but also for patients with an unusual efficacy; additional studies may identify critical biomarkers of potency

which may inform future trial designs. The additional samples collected for research will not exceed 3 tablespoons of blood twice in a week, and one procedure for collecting tissue/lymph node samples in a month.

Tissue samples (e.g. fluids, tissue biopsy) that are obtained as part of standard of care procedures for clinical indications will also be sent for research analysis, wherever feasible. Fluid samples that would otherwise be discarded may also be diverted for research purposes.

7.2.2 Planned Correlative Studies

The planned correlative studies are as follows:

1. Determine the persistence of huCART-meso cells in peripheral blood.
   Peripheral blood mononuclear cells' DNA will be evaluated by Q-PCR using a validated assay that detects a fragment unique to the CAR sequence. PBMC may also be used for a flow cytometry based assay for determination of huCART-meso cells. These analyses will be performed in bulk on each patient on peripheral blood, tumor derived material where available, or other tissues/fluids (ascites, pleural fluid, etc.). CAR T cell vector sequences will be performed at pre- and all post-infusion study visits as outlined in the Schedule of Events **(Appendices 1-3).** Additional testing may also be performed at unscheduled time points at the discretion of the Sponsor. This analysis will continue until any 2 sequential tests are negative documenting loss of CAR T cells. Additionally, flow cytometry may be used to detect cells expressing the anti-mesothelin CAR construct, if feasible.
2. Determine the bioactivity of huCART-meso cells in peripheral blood.
   Serum or fluid (e.g. ascites) will be analyzed using Luminex and ELISA Technologies to determine the presence of a panel of cytokines/chemokines/immune factors. Measurements will be performed in bulk to maximize utilization of immune assay kits. In addition, bioactivity of the manufactured and infused huCART-meso cells may be assessed by functional flow cytometry via detection of cytokine production and cytotoxic activity of the cells stimulated with a mesothelin-expressing cell line.
3. Evaluate the development of immune responses favoring rejection of huCART-meso cells (where scientifically relevant). Development of HACA antibody responses are expected to result in elimination of huCART-meso cells. Cellular immune responses directed to the modified T cells may also develop and be measured. Correlate the occurrence of antibody responses with loss of engraftment of CAR T cells.
4. Evaluate the development of secondary anti-tumor responses as a consequence of huCART-meso cells. Tumor cell death mediated by huCART-meso cells may expose the immune system to new antigens, leading to new immune responses directed to the tumor. The development of new antibodies and cellular immune responses that develop to established tumor cell lines may be assessed and displayed as appropriate to each assay. This will be determined using Protoarray or other appropriate technologies, or via next-generation sequencing of T cell receptor and immunoglobulin rearrangements in the tumor at baseline and post-infusion.
5. Where tumor material or body fluids can be obtained:
   a. Measure trafficking of huCART-meso cells;
   b. Evaluate mesothelin expression on tumor cells to assess for antigen-escape.
   c. Evaluate genetic editing (if feasible)
   d. Analyze tumor microenvironment and cell interactions (if feasible)- Immune correlates such as local immune activation (using qRT-PCR); the breath and hierarchy of the B and T cell repertoire via deep sequencing and compare with baseline specimens.

8 STATISTICAL PLAN

8.1 General Design Issues

This is a single-center phase I study in cancer patients to evaluate the safety and feasibility of autologous T cells engineered to express a CAR that targets mesothelin.

8.2 Sample Size Determination

This study will enroll up to 27 evaluable subjects depending on the occurrence of DLT. Evaluable subjects are defined as those subjects who receive the target dose of huCART-meso cells as per their cohort assignment.

8.3 Subject Population(s) for Analysis

- The **Screened Set** comprises all subjects who are consented to participate in this study.
- The **Enrolled Set** comprises all subjects who are determined eligible to participate in the study (excluding screen failure).
- The **Safety Evaluable Set** comprises all subjects who receive the target dose of huCART-meso cells per their cohort assignment. The Safety Set will be used for primary safety and feasibility analysis, official DLT evaluations, and cohort advancement decisions.
- The **Efficacy Evaluable Set** comprises all subjects who receive the huCART-meso cells at their cohort assigned dose level and who have at least one post-infusion disease assessment available. Efficacy evaluable subjects also include those with disease progression or death prior to completion of study safety visits. The Efficacy Evaluable Set will be used for the secondary efficacy endpoint analysis.
- The **Efficacy Non-Evaluable Set** comprises subjects who receive huCART-meso at lower than intended dose or do not have at least one post-infusion disease assessment available. These subjects will be evaluable for safety, but will not be evaluable for response. These subjects will also not be included in official DLT evaluations or cohort advancement decisions, thus will be replaced in that cohort.
- The **Full Analysis Set (FAS)** comprises all subjects who received the huCART-meso cells. This set includes both evaluable and efficacy non-evaluable subjects and will be used for the primary safety and feasibility, and secondary correlative endpoints or other exploratory analyses.

A second population of subjects in the **Enrolled Set** includes subjects eligible for participation in the study but who do not receive huCART-meso cells. Reasons subjects do not receive cell infusions are likely to include 1) ineffective transduction of autologous T cells or an inability to manufacture an infusible dose; 2) rapid progression, clinical deterioration, and/or death between the time of enrollment and infusion; and 3) subject withdrawal. Subjects who do not receive therapy will be replaced as detailed in **Section 4.4.3**.

The number of subjects in the **Enrolled Set** versus the number of subjects in the **Safety Evaluable Set** will be described and is a measure of the feasibility of this therapy.

8.4 Analysis of Primary Endpoints

The primary safety endpoint will be evaluated using the Safety Evaluable Set, to determine the safety of huCART-meso cells based on the type, frequency, severity, and attribution of AE/SAEs and the occurrence of DLTs. DLT and adverse events will be collected and evaluated for all subjects during the protocol specified AE reporting period. AEs will be graded for severity using the National Cancer Institute (NCI) – Common Terminology Criteria (v4.03). All adverse events will be described using descriptive statistics and exact 90% confidence intervals will be produced for adverse event rates, both overall and within major categories. Results will be tabulated and summarized, both overall and by individual cohorts.

Feasibility endpoints will be evaluated using the Enrolled Set as follows:

- Study feasibility will be summarized as the proportion of subjects who receive huCART-meso cells out of the number of subjects enrolled, as well as the proportion of subjects who cannot receive all planned doses of huCART-meso cells.
- Manufacturing feasibility will be evaluated by:
    - The proportion of huCART-meso products that meet release criteria as measured by CAR expression and T cell numbers, out of the number of subjects enrolled with products manufactured; and
    - The proportion of huCART-meso products that meet the targeted dose, out of the number of subjects enrolled with products manufactured. huCART-meso products that fail to meet the intended dose are considered dose failures.

8.5 Analysis of Secondary Endpoints

For the preliminary efficacy endpoints, overall response rate (ORR), progression-free survival (PFS), and overall survival (OS) will be evaluated as follows:

- Objective response rate (ORR): will be computed as proportions with exact 90% confidence interval. Overall response rate (ORR) is defined as the proportion of subjects in the efficacy-evaluable set with radiologically confirmed measurable disease at baseline, who achieved partial response (PR) or better after infusion as determined by RECIST 1.1. Missing or non-evaluable time points will not be included.
- Progression-free survival (PFS): Progression-free survival (PFS) is defined as the time from the date of the infusion to the date of first documented disease progression (per RECIST 1.1) or death (from any cause), or censored at the date of the last adequate assessment. PFS will be described using Kaplan-Meier curves.
- Overall Survival (OS): Overall survival is defined as the time from the date of the infusion to the date of death from any cause. If a subject is not known to have died at the date of analysis cut-off, OS will be censored at the last date of contact. OS will be estimated using Kaplan-Meier curves. Median survival times and survival probabilities at selected time points (e.g., 6-, 12-months) will be presented.

8.6 Analysis of Correlative Endpoints

Please refer to **Section 7.2.2** for complete details.

9 SAFETY AND ADVERSE EVENTS

9.1 Definitions

9.1.1 Adverse Event

An ***adverse event (AE)*** is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Intercurrent illnesses or injuries should be regarded as adverse events.

9.1.2 Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- leads to a persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly or birth defect
- an important medical event

Hospitalizations that meet the following criteria should not be reported as serious adverse events:

- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, such as preplanned study visits and preplanned hospitalizations for study procedures or treatment administration
- Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
- Social reasons and respite care in the absence of any deterioration in the patient's general condition

Note: Treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event.

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

9.1.3 Unexpected adverse events

An adverse event is considered unexpected if the event, and/or severity of the event (grade), is not consistent with the risk information described in the investigator brochure and/or protocol.

9.1.4 Related adverse events

An adverse event is considered related to participation in the research if there is a reasonable possibility that an event was caused by an investigational product, intervention, or research-required procedures. For the purposes of this study, "reasonable possibility" means there is evidence to suggest a causal relationship. The relationship of the event to the study will be classified as possibly related, probably related, and definitely related.

- **Possibly Related**: There is some evidence to suggest a causal relationship; however, other factors may have contributed to the event.
- **Probably Related:** There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.
- **Definitely Related:** There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.

9.1.5 Unanticipated Adverse Device Effect (UADE)

(Note: Device refers to the Mesothelin Immunohistochemistry Test required for screening in subjects enrolled prior to Protocol Amendment V9 only) is any serious adverse effect on health or safety, or any life threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

9.1.6 Protocol-defined Adverse Event

If/when alternative treatment is initiated post-huCART-meso administration, adverse event data collection/reporting requirements will be reduced. **As of the start date of any new treatment, only protocol-defined adverse events (PDAEs) will be collected and reported. Any events ongoing at the time alternative treatment is initiated must continue to be followed through resolution.**

Protocol-defined adverse events that are also determined to be serious, as defined in **Section 9.1.2** above, will be considered protocol-defined serious adverse events (PDSAEs) and will require expedited reporting to the sponsor per **Section 9.3**.

PDAEs are defined as follows:

1) New incidence or exacerbation of a pre-existing neurologic disorder
2) New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder
3) New incidence of hematologic disorder
4) New malignancy (T cell & non T cell)
5) Any adverse event or condition the investigator believes may have a reasonable relationship to CAR T cell therapy (including unexpected illnesses or hospitalizations in this patient population)

The following correlative laboratory results will also constitute a protocol-defined adverse event, however these events will be identified by the Sponsor and subsequently reported to the PI/site and FDA:

1) Positive RCL test result
2) Vector insertion site sequencing result with a mono- or oligoclonal vector integration pattern or in a location near a known human oncogene.

9.1.7 Adverse Event Reporting Period

Collection of adverse events will begin at the time of initiation of study treatment (lymphodepleting chemotherapy or huCART-meso cell infusion depending on cohort assignment) and continue until the subject is off-study as follows:

- For the Time Period Beginning with the Initiation of Study Treatment and Until Alternative Therapy for their Disease is Administered:
  - All adverse events must be reported. The subject's complete baseline medical history will be collected prior to the initiation of study treatment.
- At the Time Alternative Therapy for their Disease is Initiated:
  - Subjects will only be followed for protocol-defined adverse events (PDAEs). Any events ongoing at the time alternative treatment is initiated must continue to be followed through resolution.
  - PDAE reporting will continue until the end of study (EOS).

9.1.8 Preexisting Condition/General Physical Examination Findings

A preexisting condition is one that is present prior to the start of the Adverse Event Reporting Period. All clinically significant abnormalities should be recorded as a preexisting condition on the medical history eCRF. During the course of the study, a preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens. Preexisting conditions that improve should also be recorded appropriately.

9.1.9 Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if determined to be clinically significant by the physician-investigator. Repeat testing to confirm the abnormality may be performed as per clinical discretion.

Laboratory abnormalities that meet the criteria for Adverse Events should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory or test result corresponds to a sign/symptom of an already reported adverse event, it is not necessary to separately record the lab/test result as an additional event. Laboratory abnormalities that do not meet the definition of an adverse event, should not be reported as adverse events. A Grade 3 or 4 event (severe) as per CTCAE does not automatically indicate a SAE unless it meets the definition of serious defined above and/or as per investigator's discretion. Whenever possible, a diagnosis, rather than a symptom should be provided (i.e. anemia instead of low hemoglobin).

9.2 Recording of Adverse Events

Safety will be assessed by monitoring and recording potential adverse effects of the treatment using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, life-threatening, and death, corresponding to Grades 1-5, will be used whenever possible. Specialized grading systems have also been developed to more appropriately capture events of Cytokine Release Syndrome (**Table 9.2-1**) and CAR Neurotoxicity (**Table 9.2-2**).

Subjects will be monitored through interval medical history evaluations, physical examinations, and clinical laboratory assessments as per the Schedule of Evaluations (**Appendices 1-3**). Adverse events will

be collected on an ongoing basis throughout the subject's participation; using testing/examinations, non-directive questioning (e.g. review of systems), subject self-reporting, etc.

Information on all adverse events should be recorded in the source documentation. All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis. To the extent possible, adverse events should be recorded as a diagnosis and symptoms used to make the diagnosis recorded within the diagnosis event. Do not list symptoms or abnormal diagnostic results separately if a diagnosis can be assigned. The safety team may require events be reported separately if they occur as SAEs (or in the context of a SAE) even if they can also be considered a constituent of another AE such as CRS.

All adverse events occurring during the adverse event reporting period (defined in **Section 9.1.7** above) must be recorded. If there are no adverse events identified during a study visit occurring after the AE reporting period commences, physician-investigator confirmation of the absence of adverse should be documented.

As much as possible, each adverse event should be evaluated to determine:

1. Severity/Grade (CTCAE Grade 1-5)
2. Duration
3. Its relationship to the study treatment (as defined in **Section 9.1.4**)
4. Expectedness to study treatment (as defined in **Section 9.1.3**)
5. Action taken with respect to the investigational treatment
6. Whether medication or therapy was administered
7. Whether it is serious, as defined in **Section 9.1.2**

Physician-investigator assessment of whether an adverse event is serious (as defined by **Section 9.1.2**) must occur within 24 hours from the date of knowledge of the adverse event in order to meet SAE reporting requirements described in **Section 9.3**. Additional assessment of non-serious adverse events, including grade and relationship to study treatment, should occur within 7 days from the date of knowledge of the adverse event or from the date of the study visit where the absence of adverse events was confirmed. Accelerated timelines for adverse event assessments and reporting may be requested in the event of emergent safety concerns and/or to address time-sensitive requests from the DSMB/FDA.

All adverse events should be treated appropriately. If a concomitant medication or non-drug therapy is given, this action should be recorded. Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and evaluated for any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

Progression of malignancy, documented appropriately in the medical records, should not be reported as a serious adverse event. Adverse events that occur concurrently with the progression of malignancy but that are not related to disease progression (i.e. deep vein thrombosis or hemoptysis) will be reported as an adverse event as described above. Progression of malignancy resulting in death should be reported as a serious adverse event.

Serious adverse events that are still ongoing at the end of the adverse event reporting period must be followed to determine the final outcome. Any serious adverse event that occurs after the adverse event reporting period and is considered to be possibly related to the study treatment or study participation, should be recorded and reported.

9.2.1 Cytokine Release Syndrome Grading System

For the purposes of reporting and grading on clinical trials using CAR T-cells, we will use the ASTCT Consensus Grading Criteria for CRS Toxicity (Table 9.2-1). The start date of CRS is a retrospective assessment of the date of onset of persistent fevers and/or myalgia consistent with CRS and not explained by other events (i.e. sepsis). For the purposes of defining the CRS start date, a fever is defined as a temperature of 100.4°F/38°C.

CRS will be considered ongoing until all signs and symptoms leading to the diagnosis of CRS have resolved, or there are alternative causes for these events (i.e. sepsis, etc). Therefore, the CRS stop date is defined as the date when the subject meets all of the following criteria. The stop date would be considered the date the last criteria was successfully met.

- The subject is afebrile for 24 hours; or an alternative cause of fever has been identified.
- The subject is durably off vasopressors (defined as the time pressors are off, but must be off for a minimum of 24 hours); or the use of vasopressors is attributable to another cause.
- The subject is no longer requiring oxygen (defined as the time oxygen and mechanical ventilation have been discontinued, but must be off for a minimum of 24 hours); or the use of oxygen and/or mechanical ventilation is attributable to another cause.

Table 9.2-1. ASTCT CRS Consensus Grading Criteria (Lee et al. 2019)#

CRS parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever[1]	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C
With either				
Hypotension	None	Not requiring vasopressors	Requiring one vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
And/Or[2]				
Hypoxia	None	Requiring low-flow nasal cannula[3] or blow-by	Requiring high-flow nasal cannula, facemask, non-rebreather mask, or Venturi mask	Requiring positive pressure (e.g. CPAP, BiPAP, intubation and mechanical ventilation)

CPAP = Continuous positive airway pressure; BiPAP= Bilevel positive airway pressure

Organ toxicities associated with CRS may be graded according to CTCAE v4.03 but they do not influence CRS grading.

[1] Fever is defined as temperature ≥38°C not attributable to any other cause. In subjects who have CRS then receive antipyretics or anti-cytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

[2] CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a subject with temperature of 39.5°C, hypotension requiring one vasopressor and hypoxia requiring low-flow nasal cannula is classified as having Grade 3 CRS.

[3] Low-flow nasal cannula is defined as oxygen delivered at ≤6 liters/minute. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at > 6 liters/minute.

9.2.2 ASTCT Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) Consensus Grading (Lee et al. 2019)

Neurotoxicity has been observed with CAR T cell products. Since the myriad manifestations of CAR-related neurotoxicity may not fall cleanly under a single CTCAE category, this study will utilize the ASTCT ICANS (Immune Effector Cell-Associated Neurotoxicity Syndrome) consensus grading system in Table 9.2-2 to categorize qualifying adverse events that are: a) judged by the investigator to constitute neurotoxicity related to CAR T cells; and b) which can be evaluated using the Neurotoxicity Domains described in Table 9.2-2 below. All other events will be graded in accordance with CTCAE V4.03 (e.g. headache, tremors/hallucinations that do not affect the subject's ICE Score, etc.). Please see Table 9.2-2 Footnote #3 for additional guidance.

If a non-qualifying event becomes a qualifying event, it would be followed by both the original CTCAE term, as well as the new CAR Neurotoxicity event term, as these events are evaluated differently and may have different start/stop dates. In addition, specific component neurotoxicity adverse events (seizures, cerebral edema and papilledema) occurring as part of a CAR Neurotoxicity event also must be reported separately and graded in accordance with CTCAE V4.03.

For qualifying events defined above, the Toxicity Term will be entered as "CAR Neurotoxicity" in accordance with the corresponding grades below. Constituent adverse events of neurotoxicity will contribute to an evaluation of the overall neurotoxicity grade as described in Table 9.2-2. The ICANS grade is determined by the most severe event (ICE score, level of consciousness, seizure, motor findings, raised intracranial pressure (ICP)/cerebral edema) not attributable to any other cause. For example, a patient with an ICE score of 3 who has a generalized seizure is classified as having Grade 3 ICANS.

Table 9.2-2. ASTCT Neurotoxicity Consensus Grading Criteria (ICANS)

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE Score[1,3]	7-9	3-6	0-2	0 (patient is unarousable and unable to perform ICE)
Depressed level of consciousness[2]	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma
Seizure	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly; or non-convulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (>5 min); or Repetitive clinical or electrical seizures without return to baseline in between.
Motor findings[3]	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
Elevated Intracranial pressure (ICP) / Cerebral edema	N/A	N/A	Focal/local edema on neuroimaging[4]	Diffuse cerebral edema on neuroimaging; Decerebrate or decorticate posturing; or Cranial nerve VI palsy; or Papilledema; or Cushing's triad

[1] A patient with an ICE score of 0 may be classified as having Grade 3 ICANS if the patient is awake with global aphasia. But a patient with an ICE score of 0 may be classified as having Grade 4 ICANS if the patient is unarousable. Please refer to **Appendix 5** for ICE scoring criteria.
[2] Depressed level of consciousness should be attributable to no other cause (e.g. no sedating medication)
[3] Tremors, myoclonus, hallucinations, and/or other similar events that are associated with immune effector cell therapies, but which do not affect the overall ICE Score (e.g. ability to write a standard sentence, follow commands, etc), do not alone constitute ICANS. Therefore, these events will be graded according to CTCAE v4.03 and will not be reported as CAR Neurotoxicity.
[4] Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE V4.03.

9.3 Reporting of Serious Adverse Events

Every SAE, PDSAE, and UADE, **regardless of suspected causality**, occurring during the adverse event reporting period defined in **Section 9.1.7** must be reported to the sponsor within 24 hours of learning of its occurrence. The original SAE notification may take place by email to meet the 24-hour reporting window.

Within 3 business days of initial knowledge of the event, the investigator must submit a complete SAE form to the Sponsor along with any other diagnostic information that will assist the understanding of the event. The Investigator will keep a copy of this SAE Form on file at the study site.

New or follow-up information on SAEs/PDSAEs should be promptly reported as updates become available.

At a minimum follow-up SAE Forms should be submitted:

- Within 1 week of ICU admission or any life-threatening event
- Within 2 weeks of hospital discharge

Follow-up information should be submitted as an amendment to the initial SAE form, and should include both the follow-up number and report date. The follow-up information should describe whether the event has resolved or continues, if there are any changes in assessment, if and how it was treated, and whether the patient continued or withdrew from study participation.

Report serious adverse events by email to:

Attention: Clinical Safety Manager or designee
Center for Cellular Immunotherapies (CCI)
University of Pennsylvania

At the time of the initial report, the following information should be provided:

- Study identifier
- Subject number
- A description of the event
- Date of onset
- Current subject status
- Whether study treatment was discontinued
- The reason the event is classified as serious
- Investigator assessment of the association between the event and study treatment
- Expectedness relative to investigational product(s)

9.3.1 Investigator Reporting: Local Regulatory Review Committees

Report events to local regulatory review committees per institutional policy.

9.3.2 Pregnancies

To ensure subject safety, each pregnancy occurring while the subject is on study treatment must be reported to protocol sponsor within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. If a pregnancy occurs on study, this will be reported as an SAE using an SAE Report Form.

Pregnancy outcomes must be collected for the female partners of any males who took study treatment in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

9.4 Toxicity Management, Stopping Rules and Study Termination

It is expected that AEs may occur frequently in this population based on the underlying advanced cancer and that these can be SAEs. Therefore, there is no specific occurrence of SAEs that define a stopping rule, but the review of SAEs will form the basis for potential early stopping of the study. Only unexpected SAEs that are related to the huCART-meso cells would define a stopping rule. The review of these adverse events, and any decision to prematurely stop subject enrollment, will be determined by the Sponsor and/or DSMB.

In addition to the above, premature termination of the clinical trial may also occur because of a regulatory authority decision, change in opinion of the IRB, determination that there are problems in the cell product generation, as a result of safety concern, or at the discretion of the PI. Additionally, recruitment may be stopped for reasons of particularly low recruitment, protocol violations, or inadequate data recording.

9.4.1 Criteria for stopping or pausing the study

The study will be paused and reviewed for potential stopping rules if:

- Any death within 30 days of the 1st huCART-meso infusion of any subject.
- Any Grade 4 respiratory adverse event. This will require an immediate temporary hold followed by an urgent DSMB meeting.
- The study meets the DLT safety signal for Grade 3 AEs described in **Section 5.4**.
    - Dose Escalation Cohorts (Cohorts 1-4): In the event of 2 DLTs at a dose level, the enrollment into that cohort will stop and the study will not advance to the next cohort. If 2 DLTs occur in Cohort 1, we will dose de-escalate by 10-fold and enroll up to 6 subjects to be infused with huCART-meso cells only. If 2 DLTs occur at the de-escalated dose, the study will be stopped.

- Dose Expansion Cohorts (Cohorts 5-7): All subjects will be routinely evaluated for dose-limiting toxicities (DLT). In the event of 2 DLTs in a specific cohort, additional enrollment and treatment activity within that cohort will be paused to allow for further investigation.

If the study accrual/treatment activity is paused in accordance with the above, a formal assessment of the event(s) will be generated by the Sponsor and submitted to the DSMB for review/acknowledgement. The outcome of DSMB review will be shared with the PI for submission to local regulatory review committees as required per institutional policy. If all parties are in agreement as to the event resolution, then the pause will be lifted. The sponsor will also notify the FDA as appropriate.

The study will be stopped in the event of any of the following:

- Any subject develops uncontrolled T cell proliferation that does not respond to management.
- Grade 5 respiratory adverse event.
- The PI, Sponsor, Medical Director, DSMB, or any independent review board or regulatory body decides for any reason that subject safety may be compromised by continuing the study.
- The Sponsor decides to discontinue the development of the intervention used in this study.

9.4.2 General Toxicity Management Considerations

Please refer to the current version of the huCART-meso Investigator's Brochure for complete information.

Replication-competent lentivirus (RCL)

RCL may in theory be generated during the CAR T cell manufacturing phase or subsequently after introduction of vector transduced cells into the subject. However, an RCL resulting from the production phase is highly unlikely since elements are incorporated in the design of the vector system that minimize vector recombination and generation of RCL. Furthermore, the vector used to transduce the product undergoes sensitive assays to confirm that the vector is RCL negative before it can be released for use in product manufacture. Though theoretical, development of RCL could pose a risk to both the subject and their close contact(s), and therefore, samples will be archived during the course of the trial. In the event of a suspected RCL, measures to detect RCL will be followed per the recent FDA guidance, *Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up*.

Clonality and insertional oncogenesis

Monitoring for T cell clonal outgrowth will be performed by qPCR analysis for CAR-expressing cells and/or flow cytometric analysis (if feasible), and by CBC count. If during long-term follow-up > 1% of the subject's sample is positive for vector sequences by qPCR, then the subject will be asked to return for a confirmatory blood test prior to the next study visit. If > 1% of the subject's sample is positive upon the receipt of the confirmatory qPCR result, then the genomic vector integration sites will be determined. Identified vector integration sites will be evaluated using bioinformatics approaches to determine if the integration events are in regions with known relationships to human cancers (i.e. near oncogenes).

If integration site analysis reveals mono- or oligoclonality and/or integration at or near an oncogenic locus, a monitoring plan, including follow-up molecular analyses, will be developed in collaboration between the PI and Regulatory Sponsor that is specific for the health care risks that are anticipated given the nature of the integration site and vector target cell type.

Infusion reaction
Acetaminophen and an antihistamine may be repeated every 6 hours as needed. It is recommended that subjects not receive corticosteroids at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on CAR T cells.

Febrile reaction
In the event of febrile reaction, an evaluation for infection should be initiated, and subjects managed appropriately with antibiotics, fluids and other supportive care as medically indicated and determined by the treating physician. In the event that the subject develops sepsis or systemic bacteremia following CAR T cell infusion, appropriate cultures and medical management should be initiated. If a contaminated CAR T cell product is suspected, the product can be retested for sterility using archived samples that are stored in the CVPF. Consideration of a cytokine release syndrome (see below) should be given.

Cytokine Release Syndrome (CRS) / Macrophage Activation Syndrome (MAS)
Selective tocilizumab therapy has been utilized (described below) to manage CRS/MAS toxicity without precluding CAR T cell expansion in subjects. Steroids or other immunosuppressant drugs should NOT be used as pre-medication for CAR T cell therapy but may be considered in the management of CRS.

The moderate to severe cases of CRS observed required intervention with single dose tocilizumab with or without high dose corticosteroids, between 2 and 9 days after T cell infusion to date. This resulted in rapid reversal of the high persistent fevers and hemodynamic instability associated with CRS in most but not all subjects.

Given the clinical improvement of subjects after treatment with anti-cytokine therapy, subjects with moderate to severe cytokine toxicities should be managed with administration of tocilizumab.

Tocilizumab should be used as a single, weight-based dose of 8 mg/kg at the time of hemodynamic instability. This management approach is designed to avoid life-threatening toxicities, while attempting to allow the CAR T cells to establish a proliferative phase that appears to correlate with clinical efficacy. Thus, the timing of the tocilizumab should be individualized, in close consultation with the Principal Investigator and/or expert consultants for the trial. Steroids have not always been effective in this setting and may not be necessary given the rapid response to tocilizumab. Because steroids are thought to interfere with CAR T cell function and efficacy, if used, they should be rapidly tapered.

Upon developing the prodrome of persistent fevers following CAR T cell infusion, subjects should then be followed closely. Infection and tumor lysis syndrome work up should be immediately undertaken. The pharmacy should be notified of the potential need for tocilizumab. Subject management in an intensive care unit may be required and the timing is dependent upon local institutional practice. In addition to supportive care, tocilizumab may be administered in cases of moderate to severe CRS, especially if the subject exhibits any of the following:

- Hemodynamic instability despite intravenous fluid challenges and moderate stable vasopressor support
- Worsening respiratory distress, including pulmonary infiltrates, increasing oxygen requirement including high-flow O_2, and/or need for mechanical ventilation.
- Any other signs or symptoms of rapid deterioration despite medical management

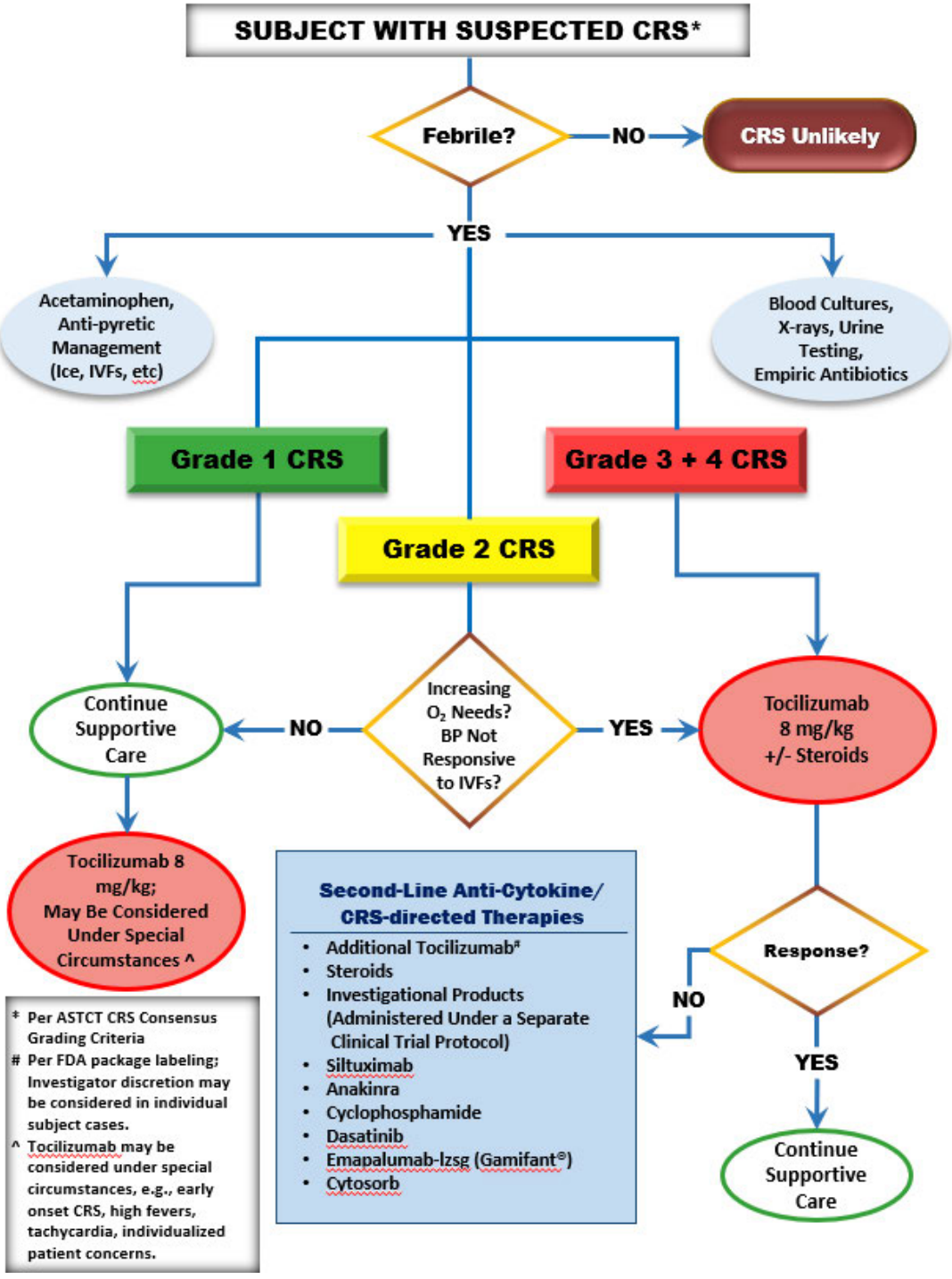
The recommended dosing for tocilizumab is 8 mg/kg (maximum individual dose of 800 mg) i.v. every 8 hours for a maximum of four doses; this is the dosing specified in the tocilizumab product label for

management of CRS. Failure of CRS to improve after two tocilizumab doses, however, should also prompt consideration of adjunctive therapies, as described below, in addition to further tocilizumab doses. Not all Grade 4 CRS reactions following CAR T cell infusions have been immediately treated with tocilizumab and decisions are, in part, based upon the rapidity of the syndrome onset and the individual subject's physiologic reserve.

Other anti-cytokine therapies, such as repeat administration of tocilizumab or use of siltuximab or etanercept, may also be considered if the patient does not respond to the initial dose of tocilizumab. If the patient experiences ongoing CRS despite administration of anti-cytokine directed therapies, anti T-cell therapies such as cytoxan, ATG, campath may be considered.

A detailed treatment algorithm has been established with clear criteria for CRS management following CART-19 administration and guidance on when to administer tocilizumab (**Figure 9.4-1**). This approach was designed to avoid life-threatening toxicities, while attempting to allow the CART-19 transduced cells to establish a proliferative phase which appears to correlate with tumor response. The management of CRS is based solely upon clinical parameters. This algorithm may be used to guide CRS management related to huCART-meso treatment; however, changes specific to the huCART-meso should be considered and based on clinical judgment. Specific CRFs have been developed for the capture of CRS elements, severity, management and response to intervention.

Figure 9.4-1: CRS Management Algorithm



Off-Tumor On-Target Toxicity

It is possible that serositis, peritonitis, or pleuropericarditis could occur due to normal mesothelin expression on serosal surfaces. Relevant information on the occurrence of serositis is available from the study done at the NCI testing an anti-mesothelin pseudomonas exotoxin conjugate (SS1P) (Hassan, Bullock, et al. 2007). SS1P was given by an IV bolus infusion to 34 patients. The DLT was Grade 3 pleuritis and was seen in two of two patients treated at a dose of 60 µg/kg and in one of nine patients treated at a dose of 45µg/kg. The DLT of pleuritis was characterized by fever, hypoxia, pleural effusion, and pain. Pleuritis developed after the second dose of SS1P in one patient and after the third dose in two patients. The clinical presentation was pleuritic pain leading to hypoxia due to hypoventilation. Chest x-ray showed unilateral or bilateral pleural effusion. Patients were treated with supplemental oxygen and narcotics for pain relief. Pain and hypoxia resolved within 3 to 9 days. Pleural effusion improved over time and did not require drainage.

Serositis should be considered an on-target toxicity of huCART-meso cells. If the subject has significant clinical symptoms, supportive care and therapy to ablate the huCART-meso cells should be considered.

> Management: In the event of symptomatic pleuritis, pericarditis, or peritonitis following T cells, patient will have an investigation to exclude infectious causes, and then will be treated with anti-inflammatory agents per physician preference. T cell ablating therapies include corticosteroids, chemotherapy such as cyclophosphamide, or immunotherapy such as alemtuzumab may also be considered at the investigators' discretion. In the event of fluid accumulation, these cavities can be quickly and readily accessed in a minimally invasive fashion to remove the fluid as anti-lymphocyte therapy is initiated. Patient will be monitored by clinical exam, laboratory tests, x-ray and EKG for the development of serositis.

Anaphylaxis

According to our experience treating 14 subjects with SS1 CAR mRNA T cells (7 mesothelioma subjects under UPCC# 17510, 6 pancreatic cancer subjects under UPCC# 08212, and one pancreatic cancer subject under UPCC# 21211), anaphylaxis is a rare but possible toxicity created by repeated CAR T cells infusions with long periods of time between infusions (Please refer to the huCART-meso Investigator's Brochure for additional information).

> Management: includes epinephrine, volume resuscitation, and corticosteroids, and other supportive care as needed (oxygen, ventilation, beta2 agonists for bronchoconstriction, etc.). Management is generally similar to that described for cytokine release syndrome (as above), but with the use of epinephrine as the principal therapeutic medication. The dose of epinephrine used depends on the degree of systemic involvement (low dose for hypotension, high dose -1 mg iv repeated every minute as necessary) for PEA arrest, according to ACLS guidelines.

Pulmonary Toxicity

Given experience in this trial and in a related trial of huCART-meso cells in patients with ovarian cancer, mesothelioma, and lung cancer, ALI/ARDS may occur secondary to direct activity of the huCART-meso cells on mesothelin-expressing type I pneumocytes, first pass effect, and/or initiation of an organ-specific immune reaction. Given these adverse events, subjects will be admitted for the huCART-meso infusion and remain inpatient for observation for 48 hours following huCART-meso cell infusion. Should pulmonary toxicity occur, the subject will be managed as below. For any subject who develops respiratory symptoms, including but not limited to dyspnea, cough, and hypoxia, beyond the inpatient observation time point, the subject will be admitted to an inpatient unit for further evaluation and care.

Management: In addition to standard and research laboratory tests being drawn, a cytokine panel will be drawn and processed as quickly as possible to ascertain if an actionable pathway (e.g. IL-6 treatment with tocilizumab) is activated. Should the subject develop increasing respiratory compromise or other signs or symptoms consistent with a cytokine release syndrome, the subject will be transferred to the medical intensive care unit and treated with tocilizumab per protocol. Subsequent doses of tocilizumab will be administered as dictated by the subject response to the initial dose and clinical scenario evolution. Should the subject develop increasing respiratory distress, standard supportive care measures for hypoxia will be initiated, including increasing oxygen delivery as indicated to maintain adequate oxygenation and/or utilizing non-invasive or invasive ventilation support if clinically indicated. Should the subject fail to respond to these supportive measures and tocilizumab dosing as manifest by progressive or refractory hypoxemic respiratory failure, consideration to lympholytic cyclophosphamide doses to abrogate all huCART-meso activity would be made in consultation with the critical care team.

Neurologic Toxicity

Guidelines for management of neurologic toxicity are described in **Table 9.4-1**. The mechanism underlying neurotoxicity after CAR T cell administration is poorly understood. Anecdotal evidence suggests that anti-IL6 therapies, which are effective for CRS, do not reverse neurotoxicity. Neurotoxicity can be asynchronous with cytokine release syndrome, often reaching its maximum intensity later than CRS or even arising after resolution of CRS. This has led to the appearance in some cases that treatment of CRS with anti-IL6 agents worsens or precipitates neurotoxicity; a causative relationship between anti-IL6 therapy and neurotoxicity has not been conclusively established, however, and severe neurotoxicity has been observed in the absence of CRS and in cases where no anti-IL6 therapy has been used. The following principles guide the recommended management of neurotoxicity:

- Most instances of neurologic toxicity are self-limited, but life-threatening complications such as cerebral edema develop in some cases. Corticosteroid therapy aimed at dampening CAR T cell proliferation and activity is therefore recommended for moderate (Grade 2-3) neurotoxicity with the objective of preventing development of life-threatening, Grade 4 manifestations. The efficacy of corticosteroids is unproven, however, and corticosteroids may adversely affect CAR T cell efficacy. The recommended dose of corticosteroids is therefore stratified based on perceived risk of developing life-threatening complications; low/moderate doses are recommended for Grade 2, moderate doses for Grade 3, and high-doses for Grade 4. In addition, it is recommended that cytotoxic chemotherapy be considered in Grade 4 cases based on a report that cerebral edema improved promptly after cyclophosphamide chemotherapy in one case(Garfall et al. 2016).
- Consultation with a neurologist is helpful for properly documenting and tracking neurologic abnormalities, evaluating for other potential etiologies of neurologic abnormalities, and managing neurologic emergencies such as seizure or elevated intracranial pressure.
- Coagulopathy and thrombocytopenia should be aggressively managed. Both coagulopathy and thrombocytopenia often develop with CRS. Intracranial hemorrhage has been observed in conjunction with severe neurotoxicity. Intracranial hemorrhage may provide a portal of entry for CAR T cells and systemic cytokines to affect the CNS or may simply confound interpretation of neurologic abnormalities. In addition, a recent report suggests that thrombocytopenia is an independent risk factor for CAR-related neurotoxicity, and platelets may serve as a source of mediators that stabilize the endothelium and counteract destabilizing effects of cytokines elaborated during CAR T cell proliferation(Gust et al. 2017).

– CRS should be managed concurrently with neurotoxicity according to guidelines enumerated above. It is difficult to distinguish delirium secondary to CRS from mild/early CAR-related neurotoxicity (and these phenomena may not be distinct pathophysiologically). In general, for subjects with Grade ≥2 neurologic toxicity in conjunction with CRS requiring tocilizumab, it is recommended that corticosteroids be administered together with or before tocilizumab to dampen a potentially exacerbating effect of tocilizumab on neurotoxicity.

Management:

Table 9.4-1: Neurotoxicity management

Neurologic Event Grade	Toxicity Management Guidelines - If subject experiencing concurrent CRS, follow CRS management guidelines in parallel.
Grade 1	• Consider non-sedating antiseizure medicines (e.g. levetiracetam) for seizure prophylaxis.
Grade 2	• Consider neurology consultation • Perform head imaging, preferably MRI; consider lumbar puncture and/or funduscopic exam. • Consider dexamethasone 8-40 mg/day in divided doses • Consider non-sedating antiseizure medicines (e.g. levetiracetam) for seizure prophylaxis • Administer platelet transfusion if platelet count <30000/µl; monitor for coagulopathy- if fibrinogen < 150 mg/dl give cryoprecipitate
Grade 3	• Consider neurology consultation • Perform head imaging, preferably MRI; consider lumbar puncture and/or funduscopic exam. • Administer dexamethasone 10mg every 6 hours. Continue dexamethasone use until the event is Grade 1 or less, then taper over 3 days • Consider non-sedating antiseizure medicines (e.g. levetiracetam) for seizure prophylaxis • Administer platelet transfusion if platelet count <30000/µl; monitor for coagulopathy- if fibrinogen < 150 mg/dl give cryoprecipitate
Grade 4	• Consider neurology consultation • Perform head imaging, preferably MRI; consider lumbar puncture and/or funduscopic exam. • Administer methylprednisone 1000 mg intravenous per day for a total of 3 days then taper as indicated. Consider a 50% decrease every 3 days. • Consider non-sedating antiseizure medicines (e.g. levetiracetam) for seizure prophylaxis • Administer platelet transfusion if platelet count <30000/µl; monitor for coagulopathy- if fibrinogen < 150 mg/dl give cryoprecipitate • Consider cytotoxic chemotherapy (e.g., cyclophosphamide 1.5 g/m^2)

9.5 Protocol Exceptions and Deviations

Exception

A one time, **intentional** action or process that departs from the IRB-approved study protocol, intended for **one** occurrence. If the action disrupts the study progress, such that the study design or outcome (endpoints) may be compromised, or the action compromises the safety and welfare of study subjects, **advance** documented approval from the Regulatory Sponsor and local regulatory review committees per institutional guidelines is required. Approval from the Regulatory Sponsor must be received prior to submission to local regulatory review committees for approval.

Deviation
A one time, **unintentional** action or process that departs from the IRB-approved study protocol, involving one incident and **identified retrospectively**, after the event occurred. If the impact on the protocol disrupts the study design, may affect the outcome (endpoints) or compromises the safety and welfare of the subjects, the deviation must be reported to the Regulatory Sponsor within 10 business days of PI knowledge and to local regulatory review committees per institutional guidelines. Acknowledgement from the Regulatory Sponsor must be received prior to submission to local regulatory review committees.

Other deviations should be appropriately documented per site policies/procedures (such as a subject missing a visit is not an issue unless a critical/important treatment or procedure was missed and must have been done at that specific time).

Include the following information on the Sponsor supplied exception/deviation form: protocol number, subject study number, comprehensive description of the exception/deviation from the protocol, rationale, and corrective and preventative action plan (deviations only). Ensure all completed exception/deviation forms are signed by the Principal Investigator (or physician sub-investigator) and submitted to the Sponsor Project Manager for review.

Attention: Sponsor Project Manager
Center for Cellular Immunotherapies (CCI)
University of Pennsylvania

Once approval of the exception request or acknowledgement of the deviation has been granted by the Regulatory Sponsor, the exception or deviation will be submitted to all applicable committees for review and approval/acknowledgement per institutional guidelines.

9.6 Data and Safety Monitoring Board

An Independent Data and Safety Monitoring Board (DSMB) comprised of at least four individuals including physicians with experience in oncology and/or gene transfer therapy will be assembled, and will work under a charter specifically developed for safety oversight of this study. The DSMB will provide guidance/advice to the Sponsor. The DSMB will evaluate patient-subject safety as specified in the DSMB Charter.

The DSMB will review safety data at the following time points:

- At the end of Cohort 2 (after all subjects in the Cohort have completed their Day 28 follow-up visit) to allow opening of Cohort 3 and 5 in parallel
- At the time safety data is available for the first two subjects treated with huCART-meso after May 18, 2018.
- Approximately every 6 months.

- If necessary, additional meetings of the DSMB may be held if safety issues arise in between scheduled meetings.

It is envisioned that the DSMB may make four types of recommendations, namely:

- No safety or efficacy issues, ethical to continue the study as planned
- Serious safety concerns precluding further study treatment, regardless of efficacy
- Overwhelming evidence for futility, recommend stopping the study.
- Recommendation to continue the study but proposing an amendment to the protocol (e.g., incorporate an additional safety assessments)

A sponsor representative will share the outcome of the DSMB meeting with the PI via email, for submission to local regulatory review committees as required per institutional policy.

10 DATA HANDLING AND RECORDKEEPING

10.1 Confidentiality

Information about study subjects obtained as part of this study will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA) of 1996. Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI

In the event that the subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

10.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

The investigator must maintain source documents for each subject in the study, consisting of case and visit notes (hospital or clinical medical records) containing demographic and medical information, laboratory data, and the results of any other tests or assessments. All information recorded on the eCRFs must be traceable to source documents in the subject's file.

Paper based records will be kept in a secure location and only be accessible to personnel involved in the study. Computer-based records or files will only be made available to personnel involved in the study through the use of access privileges and passwords. Whenever feasible, subject identifiers will be redacted from study related records and replaced with study assigned identification numbers.

10.3 Data Management

Data management responsibilities will be governed by CCI Clinical Operations policies/procedures including current Clinical Operations Standard Operating Procedures (SOPs), Guide to Daily Operations (GDOs) and Work Instructions (WI).

A part 11 compliant electronic data capture (EDC) system will be used as the primary data collection tool for the purposes of this study. All data requested on the study-specific CRF must be recorded. Data entry will be performed by clinical site team members who have been delegated this responsibility by the Principal Investigator and who have completed all required protocol and database training. The Principal Investigator is responsible for assuring that the data entered into eCRF is complete, accurate, and that entry and updates are performed in a timely manner.

10.4 Sharing of Study Data

Participants will have access to research related information within their medical record through Penn Medicine's patient portal – called MyPennMedicine (MPM). This includes information from all clinical tests/procedures required as part of their study participation. Results of research testing conducted in non-CLIA certified laboratories will not be included in the participant's medical record or made available to the patient.

10.5 Future Use of Research Data/Specimens

Blood, remaining unmanufactured cells, unused manufactured huCART-meso cells or other samples obtained from the participant will be stored indefinitely and used for future research. Study data and samples may also be shared with other researchers within Penn, or other research institutions, as well as with for-profit pharmaceutical or biotechnology companies. This future research may include genetic testing and/or whole genome sequencing. Data/specimens used for future research will be coded using the unique subject identifier.

There are no plans to tell participants about future testing on their specimens, or share the results of this analysis.

10.6 Records Retention

Essential documents must be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by local regulations or per sponsor agreement. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained. No records will be destroyed without the written consent of the sponsor.

11 STUDY MONITORING, AUDITING, AND INSPECTING

11.1 Study Monitoring Plan

This study will be monitored according to the Sponsor Data and Safety Monitoring Plan.

Interim Monitoring Visits will be conducted during the course of the study. The Monitors will assure that submitted data are accurate and in agreement with source documentation; verify that investigational products are properly stored and accounted for, verify that subjects' consent for study participation has been properly obtained and documented, confirm that research subjects entered into the study meet inclusion and exclusion criteria, and assure that all essential documentation required by Good Clinical Practices (GCP) guidelines are appropriately filed.

At the end of the study, Monitors will conduct a close-out visit and will advise on storage of study records and disposition of unused investigational products.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

11.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and University compliance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance offices.

The Principal Investigator must notify the Sponsor in real-time if an audit/inspection notification is received.

12 ETHICAL CONSIDERATIONS

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations, the revised Common Rule, and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. All study consent forms will be submitted with the protocol for review and approval by the University of

Pennsylvania IRB. The Investigator (according to applicable regulatory requirements), or a person designated by the Investigator, will facilitate the informed consent discussion with the participant, in language and terms they are able to understand. All subjects must have the cognitive ability to provide consent, as determined by the treating physician-investigator.

Written informed consent will be signed/dated by the participant and the person conducting the informed consent discussion. A copy of the signed/dated informed consent will be provided to the participant, and the original consent should be retained for the investigator's research records. Participant consent, using the IRB-approved consent form, must be obtained before that subject undergoes any study-specific procedures.

The protocol is listed under clinicaltrials.gov.

13 STUDY FINANCES

13.1 Funding Source

This study will be funded by the University of Pennsylvania, the NIH, and Tmunity Therapeutics.

13.2 Conflict of Interest

All University of Pennsylvania Investigators will follow the University of Pennsylvania Policy on Conflicts of Interest Related to Research. This requires that any individuals who have a role in the design, conduct, or analysis of this clinical trial disclose all potential conflict of interest as part of their participation in this clinical trial, including changes in their conflict of interest as they occur. Persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial.

13.3 Subject Stipends or Payments

There is no subject stipend/payment for participation in this protocol.

13.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the Sponsor, the FDA, or other government agencies as part of their duties that research subjects are protected.

14 PUBLICATION PLAN

Publication of the results of this trial will be governed by University of Pennsylvania policies, the Center for Cellular Immunotherapies (CCI) Authorship Guidance, and any applicable contractual agreements. Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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16 APPENDIX 1: SCHEDULE OF EVENTS (COHORTS 1-5*)

*** COHORTS 3 AND 4 PERMANENTLY CLOSED WITH PROTOCOL V5.**

Trial Period	~Week -8	~Week -6 to -4	Week -2 to -1	Day -4 To -2[10]	Day 0	Days 1 + 2[33]	Days 6 (±1), 9 (±1), 14 (±1), 21 (±1)	Day 28 (±5)	Month 2, 3, 6[36] (±5 days)	Month 9, 12, 15, 18, 21, 24[36] (±2 weeks)
Visits	**Screening**	**Apheresis**	**Pre Infusion SAFETY**	**Chemo Therapy**	**Infusion**	**Inpatient Observation**	**Follow-up**	**Follow-up**	**Follow-up**	**Follow-up**
CLINICAL ASSESSMENTS										
Informed Consent	X									
Demographics	X									
Diagnosis and Extent of Cancer	X									
Relevant Medical History	X									
Antineoplastic Therapy	X[30]									
Physical Examination	X		X	X	X	X[20]	X[20]	X[20]	X[20]	X[20]
Vital Sign Assessments[1]	X		X	X	X	X[31]	X	X	X	X
Prior and Concomitant Medications	X		X----------							----------X
Performance Status	X		X	X	X	X	X	X	X	X
Adverse Events				X[18]----------						----------X[18]
EKG	X		X					X	X[8]	
Echocardiogram	X									
Pathological confirmation of malignancies	X									
Leukapheresis screening[12]	X									
ICE Score[37]					X	X	X	X		

Trial Period	~Week -8	~Week -6 to -4	Week -2 to -1	Day -4 To -2[10]	Day 0	Days 1 + 2[33]	Days 6 (±1), 9 (±1), 14 (±1), 21 (±1)	Day 28 (±5)	Month 2, 3, 6[36] (±5 days)	Month 9, 12, 15, 18, 21, 24[36] (±2 weeks)
Visits	**Screening**	**Apheresis**	**Pre Infusion SAFETY**	**Chemo Therapy**	**Infusion**	**Inpatient Observation**	**Follow-up**	**Follow-up**	**Follow-up**	**Follow-up**
CLINICAL TESTS[2]										
CBC with differential (4 ml-lavender top)	X	X[32]	X	X	X[16]	X	X	X	X	X
Chemistry (5 ml-gold top)[15]	X		X	X	X[16]	X	X	X	X	X
PT, PTT, fibrinogen, d-dimer (7.5 ml-blue top)	X[19]		X							
Autoantibodies, ANA (1 ml-gold top)	X									
Viral screens: HIV, HCV, HBV (20ml – gold top)[25]	X									
Urinalysis	X								X[14]	
Urine β HCG	X									
Serum pregnancy (5ml-gold top)			X							
Ferritin, CRP, Haptoglobin, Triglycerides, ESR[6] (4ml-gold top; 4ml lavender top)			X		X[22]	X[22]	X[22]	X[22]		
Respiratory Virus Panel (RVP)			X[34]							
Tumor biomarkers[9] CA125 (5ml- gold top)			X					X	X[13]	
RADIOGRAPHIC IMAGING										
Tumor imaging[3]	X[3]		X[24]					X	X[13]	
Brain MRI	X[23]									
INTERVENTIONS										
Apheresis[32]		X[5]						X[27]		
Cyclophosphamide[10]				X						
huCART-meso cell infusion					X					

Trial Period	~Week -8	~Week -6 to -4	Week -2 to -1	Day -4 To -2[10]	Day 0	Days 1 + 2[33]	Days 6 (±1), 9 (±1), 14 (±1), 21 (±1)	Day 28 (±5)	Month 2, 3, 6[36] (±5 days)	Month 9, 12, 15, 18, 21, 24[36] (±2 weeks)
Visits	**Screening**	**Apheresis**	**Pre Infusion SAFETY**	**Chemo Therapy**	**Infusion**	**Inpatient Observation**	**Follow-up**	**Follow-up**	**Follow-up**	**Follow-up**
Tumor Biopsy			X[17]				X[21]			
RESEARCH SPECIMENS [2, 4]										
Pleural fluid[35]					X	X[35]	X	X		
Serum (Red top tube (~6ml))			X		X[7]	X	X	X	X	X
Interleukin-15 (IL-15) [Cohort 5 only]			X							
Peripheral Blood Mononuclear Cells (~ Two 10mL purple top tubes)[11]			X		X[7]	X	X[29]		X	X
q-PCR persistence[28]			X		X	X	X	X[27]	X	X
TOTAL BLOOD DRAWS										
Approximate Milliliters	38.5	4	60.5	9	69	~43/day	Up to 63/visit	88	40/visit	35/visit
Approximate Tablespoons	2.6	0.27	4.1	0.6	4.7	2.9/day	4.3	6	2.7	2.4

1. Includes temperature, heart rate, blood pressure and pulse oximetry. Height and weight collected at screening only. On Day 0 (huCART-meso infusion), vital signs (temperature, respiration rate, heart rate, blood pressure, and oxygen saturation by pulse oximetry) will be measured within 10 minutes prior and within 15 minutes after the infusion. Thereafter, vital signs will be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the infusion, and then every hour (+/- 10 minutes) for the next two hours until these signs are satisfactory and stable. If vital signs are not satisfactory and stable 3 hours after the huCART-meso infusion, vital signs will continue to be monitored as clinically indicated until stable.
2. Additional blood draws and tissue collection may be performed at the discretion of the investigator (for example, to evaluate unexpected clinical events). Moreover, additional tissue and fluid samples obtained as part of standard of care procedures for clinical indications will be sent for research analysis, wherever feasible.
3. Tumor Imaging: CT imaging as clinically indicated as per patient population and disease status. MRI scans may be substituted at the investigator's discretion. Additional scans may be performed as clinically appropriate. Scans may be performed with or without contrast. At the screening visit, a CT performed within 8 weeks of eligibility confirmation by a physician-investigator is acceptable.
4. Research blood samples are collected to evaluate T cell persistence, cytokines, anti-CAR and anti-tumor immune responses.
5. Subjects have the option for a second apheresis in case the T cell product is not successfully manufactured from the first apheresis. Refer to **Section 6.2** for additional details.
6. May be performed post-huCART-meso infusion if CRS is suspected, or as clinically indicated.
7. Blood draw before infusion and ~1hour after T cell infusion

8. EKG at month 6 only
9. Tumor biomarkers as appropriate for each cancer.
10. Cyclophosphamide for Cohorts 2 and 4 subjects only. Cohort 4 permanently closed with protocol V5. Please refer to **Section 6.4** for additional details.
11. RCL VSV-G testing will not be routinely performed as of Protocol V8. Blood samples will be collected and banked at pre-infusion and post-infusion at Months 3, 6, and 12. These samples will be used for future RCL VSV-G testing if indicated.
12. If a subject's veins are not adequate for apheresis procedure, the subject may have a central venous apheresis catheter line placed by interventional radiology. It is recommended that the patient have an absolute lymphocyte count (ALC) ≥500/µl, prior to undergoing apheresis. If the patient's ALC is <500/µl, it is recommended that a lymphocyte subset analysis (CD3, CD4, CD8 counts) be performed to confirm that the patient has an absolute CD3 count of ≥150/µl. If the absolute CD3 count is <150/µl, it is recommended that the leukapheresis procedure be delayed until their ALC is ≥500/µl or absolute CD3 count is ≥150/µl. Up to a 4-week delay may occur; following this, further discussion is needed with the study PI and the CVPF prior to proceeding.
13. Performed at Months 3 and 6. CT scans within 4 weeks of Month 3 and 6 visits are acceptable.
14. Month 2 only
15. Chemistry panel includes sodium, potassium, chloride, CO2, blood urea nitrogen, creatinine, glucose, phosphate, total protein, albumin, calcium, alkaline phosphatase, total bilirubin, ALT, AST, uric acid, LDH.
16. A CBC with differential and comprehensive chemistry panel must be performed within 48 hours prior to the huCART-meso infusion. If already performed within this window, these laboratory tests do not need to be repeated on Day 0.
17. A baseline tumor biopsy will be performed prior to huCART-meso infusion (and cyclophosphamide administration if applicable), if the subject has an accessible tumor. The baseline biopsy may occur any time prior to infusion between Day -14 to Day -1. This baseline biopsy sample will be used to evaluate the level of mesothelin expression (using GeoMx Digital Profiling analysis; for exploratory purposes only) and if feasible, other immune markers at baseline. If a subject does not have an accessible tumor or a biopsy is not felt to be clinically appropriate, an archived tumor tissue specimen may be obtained for research purposes. Please refer to the Tissue Handling Manual for complete details.
18. Collection of adverse events will begin at the time of initiation of study treatment (lymphodepleting chemotherapy or huCART-meso cell infusion depending on cohort assignment). Cohort 5 Participants (Effective Protocol Amendment V11) Only: At the time alternative therapy for their disease is initiated, subjects will only be followed for protocol-defined adverse events (PDAES) as defined in **Section 9.1.6**. Any events ongoing at the time alternative treatment is initiated must continued to be followed through resolution.
19. INR at screening only
20. Physical examination to include a visual examination of the throat. If toxicity is suspected, please consult with a Pulmonology Study Investigator.
21. Tumor biopsy to be performed at Day 14 (±7 days). This biopsy sample will be used to evaluate the level of mesothelin expression (using GeoMx Digital Profiling analysis; for exploratory purposes only) and if feasible, other immune markers. Please refer to the Laboratory Manual for complete details.
22. Only ferritin and CRP required; other labs repeated as clinically indicated.
23. For suspected brain metastasis (performed within 8 weeks of eligibility confirmation by a physician-investigator).
24. Baseline scans should be obtained within 28 days prior to infusion of huCART-meso cells and after any cancer therapy administered between collection and huCART-meso infusion. In applicable cohorts, baseline scans should also be obtained prior to cyclophosphamide administration.
25. Viral serologies: HIV, Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody, Hepatitis B core antibody, and HIV Hepatitis C antibody. If the HCV antibody is positive, a screening HCV RNA by any RT-PCR or bDNA assay must be performed. Eligibility will be determined based on the screening value. The test is not required if documentation of a negative result of a HCV RNA test performed within 60 days prior to screening is provided.
26. Collected at Months 2, 3.

27. A mini-apheresis procedure (~5 L) will also be performed at Day 28 for research purposes. A 60 ml blood draw (6 purple top tubes) may be substituted for the mini-apheresis procedure at the discretion of the treating investigator.
28. Testing for huCART-meso persistence by q-PCR will occur at pre- and all post-infusion study visits. Additional testing may also be performed at unscheduled time points at the discretion of the Sponsor. This analysis will continue until any 2 sequential tests are negative documenting loss of CAR T cells. Please refer to **Section 7.2** for additional details.
29. At the Day 14 study visit, four 10mL purple top tubes will be collected.
30. Prior antineoplastic therapies up through study treatment will be collected. Additional antineoplastic therapy received post study treatment and while on study, will also be collected.
31. During post-infusion observation, the subject will undergo vital sign assessments (temperature, respiration rate, heart rate, blood pressure, and oxygen saturation by pulse oximetry) every 4-6 hours (+/- 30 minutes) from the end of the infusion vital sign monitoring period until 48 hours post infusion. Additional monitoring may be performed per clinical discretion.
32. Performed per Apheresis Unit policy. It is recommended that the patient have an absolute lymphocyte count (ALC) ≥500/µl prior to undergoing apheresis. If the patient's ALC is <500/µl, it is recommended that a lymphocyte subset analysis (CD3, CD4, CD8 counts) be performed to confirm that the patient has an absolute CD3 count of ≥150/µl. If the absolute CD3 count is <150/µl, it is recommended that the leukapheresis procedure be delayed until their ALC is ≥500/µl or absolute CD3 count is ≥150/µl. Up to a 4-week delay may occur; following this, further discussion is needed with the study PI and the CVPF prior to proceeding.
33. The subject will remain inpatient for observation for a minimum of 48 hours after the huCART-meso infusion per **Section 6.6**. The subject may then be discharged in accordance with hospital policy after this observation period is complete and the subject is medically stable. The subject may remain hospitalized beyond this protocol-required window if deemed necessary for medical management of serious adverse events.
34. All subjects must undergo a Respiratory Virus Panel (RVP; inclusive of SARS-CoV-2) within 10 days prior to the planned huCART-meso infusion. If the subject is positive for influenza, Tamiflu® or equivalent, should be administered per package insert. The subject must complete treatment prior to receiving lymphodepleting chemotherapy (if applicable) and huCART-meso cells. The test does not need to be repeated prior to these infusion(s); however, if influenza sign and symptoms are present, the infusion(s) should be delayed until the subject is asymptomatic. If the subject is positive for another virus on the RVP, the infusion(s) will be delayed for at least 7 days to be sure clinical symptoms of a viral infection do not develop, or longer if clinically appropriate for the respective pathogen. If clinical symptoms develop, the infusion(s) will be delayed until resolution of these symptoms.
35. Cohort 5 subjects only: Pleural Fluid will be collected on Day 0 prior to instillation of huCART-meso cells and at post-infusion follow-up visits through Day 28 as clinically appropriate. Pleural fluid will not be collected on Day 1 and Day 2 post-infusion unless clinically indicated. If collected as part of routine care at other timepoints, a sample of pleural fluid will be collected for research purposes.
36. Monthly visits are defined as 28 day or 4 week time periods.
37. Cohort 5 Participants (Effective Protocol Amendment V11) Only: The ICE Score may be assessed by investigators or trained study staff (study coordinators or research nurses). The total ICE Score should be assessed on a 10-point scale. Therefore, if any of the assessments are missed, an ICE score cannot be reported. If certain assessments cannot be performed by the subject (e.g., writing sample), this individual assessment will be scored as a zero. Please refer to **Appendix 5** for the ICE scoring criteria.

17 APPENDIX 2: SCHEDULE OF EVENTS (COHORT 6)

Trial Period	~Week -8	~Week -6 to -4	Week -2 to -1	Day -4 to-2[10]	Day 0	Days 1 + 2[33]	Days 6 (±1), 9 (±1), 14 (±1)	Day 21 (+ 3 wks)[37]	Days Post Infusion #2[26]		Day 42 (+3 wks)[37]	Days Post Infusion #3[26]			Month 3, 6 (±5 days)[37]	Month 9, 12, 15, 18, 21, 24 (±2 weeks)[37]
									Days +1 and +2	Day +6, +9, +14 (±1)		Days +1 and +2	Day +6, +9, +14 (±1)	Day +21 (±1)		
Visits	Screening	Apheresis	Pre- Infusion Safety	Chemo-Therapy	Infusion #1	Inpatient Observation	Safety Follow-up	Infusion #2[42]	Inpatient Observation[33]	Safety Follow-up	Infusion #3[42]	Inpatient Observation[33]	Safety Follow-up	Safety Follow-up	Follow-up	Follow-up
CLINICAL ASSESSMENTS																
Informed Consent	X															
Demographics	X															
Diagnosis and Extent of Cancer	X															
Relevant Medical History	X															
Antineoplastic Therapy	X[30]															
Physical Examination	X		X	X	X	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]
Vital Sign Assessments[1]	X		X	X	X	X[31]	X	X	X[31]	X	X	X[31]	X	X	X	X
Prior and Concomitant Medications	X		X----------													----------X
Performance Status	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events				X[18]----------												----------X
EKG	X		X												X[8]	
Echocardiogram	X															

Trial Period	~Week -8	~Week -6 to -4	Week -2 to -1	Day -4 to-2[10]	Day 0	Days 1 + 2[33]	Days 6 (±1), 9 (±1), 14 (±1)	Day 21 (+ 3 wks)[37]	Days Post Infusion #2[26]		Day 42 (+3 wks)[37]	Days Post Infusion #3[26]			Month 3, 6 (±5 days)[37]	Month 9, 12, 15, 18, 21, 24 (±2 weeks)[37]
									Days +1 and +2	Day +6, +9, +14 (±1)		Days +1 and +2	Days +6, +9, +14 (±1)	Day +21 (±1)		
Visits	Screening	Apheresis	Pre- Infusion Safety	Chemo-Therapy	Infusion #1	Inpatient Observation	Safety Follow-up	Infusion #2[42]	Inpatient Observation[33]	Safety Follow-up	Infusion #3[42]	Inpatient Observation[33]	Safety Follow-up	Safety Follow-up	Follow-up	Follow-up
Pathological confirmation of malignancies	X															
Leukapheresis screening[12]	X															
CLINICAL TESTS[2]																
CBC with differential (4 ml-lavender top)	X	X[32]	X	X[39]	X[40]	X	X	X[40]	X	X	X[40]	X	X	X	X	X
Chemistry (5 ml-gold top)[15]	X		X	X[39]	X[40]	X	X	X[40]	X	X	X[40]	X	X	X	X	X
PT, PTT, fibrinogen, d-dimer (7.5 ml-blue top)	X[19]		X													
Autoantibodies, ANA (1 ml-gold top)	X															
Viral screens: HIV, HCV, HBV (20ml – gold top)[25]	X															
Urinalysis	X														X[14]	
Urine β HCG	X															
Serum pregnancy (5ml-gold top)			X													

Trial Period	~Week -8	~Week -6 to -4	Week -2 to -1	Day -4 to-2[10]	Day 0	Days 1 + 2[33]	Days 6 (±1), 9 (±1), 14 (±1)	Day 21 (+ 3 wks)[37]	Days Post Infusion #2[26]		Day 42 (+3 wks)[37]	Days Post Infusion #3[26]			Month 3, 6 (±5 days)[37]	Month 9, 12, 15, 18, 21, 24 (±2 weeks)[37]
									Days +1 and +2	Day +6, +9, +14 (±1)		Days +1 and +2	Day +6, +9, +14 (±1)	Day +21 (±1)		
Visits	Screening	Apheresis	Pre- Infusion Safety	Chemo-Therapy	Infusion #1	Inpatient Observation	Safety Follow-up	Infusion #2[42]	Inpatient Observation[33]	Safety Follow-up	Infusion #3[42]	Inpatient Observation[33]	Safety Follow-up	Safety Follow-up	Follow-up	Follow-up
Ferritin, CRP, Haptoglobin, Triglycerides, ESR[6] (4ml-gold top; 4ml lavender top)			X		X[22]	X[22]	X[22]	X[22]	X[22]	X[22]	X[22]	X[22]	X[22]	X[22]		
Interleukin 15	X															
Respiratory Virus Panel (RVP)			X[34]					X[38]			X[38]					
Tumor biomarkers[9] CA125 (5ml- gold top)			X							X[35]					X[13]	
RADIOGRAPHIC IMAGING																
Tumor imaging[3]	X[3]		X[24]							X[35]					X[13]	
Brain MRI	X[23]															
INTERVENTIONS																
Apheresis[32]		X[5]												X[27]		
Cyclophosphamide				X[10]												
huCART-meso cell infusion					X			X[36]			X[36]					
Tumor Tissue Acquisition/Tumor biopsy	X[16]		X[17]							X[21]						

Trial Period	~Week -8	~Week -6 to -4	Week -2 to -1	Day -4 to-2[10]	Day 0	Days 1 + 2[33]	Days 6 (±1), 9 (±1), 14 (±1)	Day 21 (+ 3 wks)[37]	Days Post Infusion #2[26]		Day 42 (+3 wks)[37]	Days Post Infusion #3[26]			Month 3, 6 (±5 days)[37]	Month 9, 12, 15, 18, 21, 24 (±2 weeks)[37]
									Days +1 and +2	Day +6, +9, +14 (±1)		Days +1 and +2	Day +6, +9, +14 (±1)	Day +21 (±1)		
Visits	**Screening**	**Apheresis**	**Pre- Infusion Safety**	**Chemo-Therapy**	**Infusion #1**	**Inpatient Observation**	**Safety Follow-up**	**Infusion #2[42]**	**Inpatient Observation[33]**	**Safety Follow-up**	**Infusion #3[42]**	**Inpatient Observation[33]**	**Safety Follow-up**	**Safety Follow-up**	**Follow-up**	**Follow-up**
RESEARCH SPECIMENS [2, 4]																
Serum (Red top tube (~6ml)			X		X[7]	X	X	X[7]	X	X	X[7]	X	X	X	X	X
Serum mesothelin related protein (SMRP)			X							X[35]					X[13]	
Peripheral Blood Mononuclear Cells (~ Two 10mL purple top tubes)[11]			X		X[7]	X	X[29]	X[7]	X	X[29]	X[7]	X	X[29]	X[27]	X	X
q-PCR persistence[28]			X		X	X	X	X	X	X	X	X	X	X	X	X
TOTAL BLOOD DRAWS																
Approximate Milliliters	38.5	4	60.5	9	69	43/ day	Up to 63/ visit	69	43/day	Up to 68/ visit	69	43/day	Up to 63/visit	88	40/ visit	35/ visit
Approximate Tablespoons	2.6	.27	4.1	0.6	4.7	2.9/ day	4.3	4.7	2.9	4.6	4.7	2.9	4.3	6	2.7	2.4

1. Includes temperature, heart rate, blood pressure and pulse oximetry. Height and weight collected at screening only. On the day of each huCART-meso infusion, vital signs (temperature, respiration rate, heart rate, blood pressure, and oxygen saturation by pulse oximetry) will be measured within 10 minutes prior and within 15 minutes after the infusion. Thereafter, vital signs will be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the infusion, and then every hour (+/- 10 minutes) for the next two hours until these signs are satisfactory and stable. If vital signs are not satisfactory and stable 3 hours after the huCART-meso infusion, vital signs will continue to be monitored as clinically indicated until stable.

2. Additional blood draws and tissue collection may be performed at the discretion of the investigator (for example, to evaluate unexpected clinical events). Moreover, additional tissue and fluid collections from the standard of care procedures may be used for research studies.
3. Tumor Imaging: CT imaging as clinically indicated as per patient population and disease status. MRI scans may be substituted at the investigator's discretion. Additional scans may be performed as clinically appropriate. Scans may be performed with or without contrast. At the screening visit, a CT performed within 8 weeks of confirmation of eligibility by a physician-investigator is acceptable.
4. Research blood samples are collected to evaluate T cell persistence, cytokines, anti-CAR and anti-tumor immune responses.
5. Subjects have the option for a second apheresis in case the T cell product is not successfully manufactured from the first apheresis. Refer to **Section 6.2** for additional details.
6. May be performed post-huCART-meso infusion if CRS is suspected, or as clinically indicated.
7. Blood draw before infusion and ~1 hour after T cell infusion
8. EKG at month 6 only
9. Tumor biomarkers as appropriate for each cancer.
10. Please refer to **Section 6.4** for additional details.
11. RCL VSV-G testing will not be routinely performed as of Protocol V8. Blood samples will be collected and banked at pre-infusion and post-infusion at Months 3, 6, and 12. These samples will be used for future RCL VSV-G testing if indicated.
12. If a subject's veins are not adequate for apheresis procedure, the subject may have a central venous apheresis catheter line placed by interventional radiology. It is recommended that the patient have an absolute lymphocyte count (ALC) ≥500/µl, prior to undergoing apheresis. If the patient's ALC is <500/µl, it is recommended that a lymphocyte subset analysis (CD3, CD4, CD8 counts) be performed to confirm that the patient has an absolute CD3 count of ≥150/µl. If the absolute CD3 count is <150/µl, it is recommended that the leukapheresis procedure be delayed until their ALC is ≥500/µl or absolute CD3 count is ≥150/µl. Up to a 4-week delay may occur; following this, further discussion is needed with the study PI and the CVPF prior to proceeding.
13. Performed at Months 3 and 6. CT scans within 4 weeks of Month 3 and 6 visits are acceptable.
14. Month 3 only.
15. Chemistry panel includes sodium, potassium, chloride, CO2, blood urea nitrogen, creatinine, glucose, phosphate, total protein, albumin, calcium, alkaline phosphatase, total bilirubin, ALT, AST, uric acid, LDH.
16. Subjects will be asked to sign a separate pre-screening consent form requesting permission to test their tumor for mesothelin expression. Evaluation of mesothelin expression will occur on archived tumor tissue, if the patient has not had an anti-mesothelin directed therapy since the collection of the archived sample. However if archived tumor tissue is not available, a biopsy may be performed. If the mesothelin expression of the subjects' tumor is known prior to signing the pre-screening consent for this study, the results of this testing will need to be confirmed as part of the subjects' participation on this protocol. If a fresh biopsy is performed to evaluate mesothelin expression, samples will also be collected for research analysis. Please refer to the Tissue Handling Manual for additional information. Once mesothelin expression has been confirmed, subjects may proceed to the next step and be presented the main informed consent for the study.
17. A baseline tumor biopsy will be performed prior to huCART-meso infusion (and cyclophosphamide administration if applicable), if the subject has an accessible tumor. The baseline biopsy may occur any time prior to infusion between Day -14 to Day -1. This baseline biopsy sample will be used to evaluate the level of mesothelin expression and if feasible, other immune markers at baseline. If the subject had a fresh biopsy at pre-screening in order to evaluate mesothelin expression, a repeat biopsy at the pre-infusion timepoint is not required.
18. Collection of adverse events will begin at the time of initiation of study treatment (lymphodepleting chemotherapy or huCART-meso cell infusion depending on cohort assignment).

19. INR at screening only
20. Physical examination to include a visual examination of the throat. If toxicity is suspected, please consult with a Pulmonology Study Investigator.
21. Tumor biopsy to be performed at Day +14 (±7 days) after the 2nd huCART-meso infusion. Please refer to the Laboratory Manual for complete details.
22. Only ferritin and CRP required; other labs repeated as clinically indicated.
23. For evaluation of brain metastasis for lung adenocarcinoma patients or suspected brain metastasis in other diseases (performed within 8 weeks of eligibility confirmation by a physician-investigator.
24. Baseline scans should be obtained within 28 days prior to the first infusion of huCART-meso cells and after any cancer therapy administered between collection and huCART-meso infusion. Baseline scans should also be obtained prior to cyclophosphamide administration.
25. Viral serologies: HIV, Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody, Hepatitis B core antibody, and HIV Hepatitis C antibody. If the HCV antibody is positive, a screening HCV RNA by any RT-PCR or bDNA assay must be performed. Eligibility will be determined based on the screening value. The test is not required if documentation of a negative result of a HCV RNA test performed within 60 days prior to screening is provided.
26. Additional infusions may be performed within a +3 week window from the targeted infusion days [Day 21 (+3 weeks) and Day 42 (+3 weeks)]. Given the allowable window for these additional infusions, the timing of post-infusion safety follow-up visits will be dependent on the actual timing of the additional huCART-meso infusions. In summary, the timing of the post-infusion safety follow-up visits will be calculated as the actual study day they occurred post Infusion #2 and Infusion #3 (i.e. **+** 6 days post Infusion #2, +7 days post Infusion #2, **+**14 days post Infusion #3, etc.). This should be recorded as the actual study day, even if an assessment falls within the allowable visit window. In addition, the study day from Day 0 (the initial huCART-meso infusion) must also be captured. Example: Infusion # + Study Visit Day Post Infusion/Study Visit Day from Day 0 = Infusion #2 Day +6/Day 28. Please refer to **Section 6.8.3** for complete details. If an infusion is omitted, these safety follow-up visits will not be required. If an infusion is delayed, these additional safety follow-up visits will be shifted to align with the timing of the infusion.
27. A mini-apheresis procedure (~5 L) will also be performed for research purposes at Day +21 (+/- 7 days) after the 3rd huCART-meso infusion (or the last huCART-meso infusion if all three infusions are not received for any reason). A 60 ml blood draw (6 purple top tubes) may be substituted for the mini-apheresis procedure at the discretion of the treating investigator.
28. Testing for huCART-meso persistence by q-PCR will occur at pre- and all post-infusion study visits. Additional testing may also be performed at unscheduled time points at the discretion of the Sponsor. This analysis will continue until any 2 sequential tests are negative documenting loss of CAR T cells. Please refer to **Section 7.2** for additional details.
29. At the Day +14 study visit post each huCART-meso infusion, four 10mL purple top tubes will be collected.
30. Prior antineoplastic therapies up through study treatment will be collected. Additional antineoplastic therapy received post study treatment and while on study, will also be collected.
31. During post-infusion observation, the subject will undergo vital sign assessments (temperature, respiration rate, heart rate, blood pressure, and oxygen saturation by pulse oximetry) every 4-6 hours (+/- 30 minutes) from the end of the infusion vital sign monitoring period until 48 hours post infusion. Additional monitoring may be performed per clinical discretion.
32. Performed per Apheresis Unit policy. It is recommended that the patient have an absolute lymphocyte count (ALC) ≥500/µl prior to undergoing apheresis. If the patient's ALC is <500/µl, it is recommended that a lymphocyte subset analysis (CD3, CD4, CD8 counts) be performed to confirm that the patient has an absolute CD3 count of ≥150/µl. If the absolute CD3 count is <150/µl, it is recommended that the leukapheresis procedure be delayed until their ALC is ≥500/µl or absolute CD3 count is ≥150/µl. Up to a 4-week delay may occur; following this, further discussion is needed with the study PI and the CVPF prior to proceeding. Additional routine laboratory testing to be performed per Apheresis Unit policies/procedures.

33. The subject will remain inpatient for observation for a minimum of 48 hours after each huCART-meso infusion per **Section 6.6**. The subject may then be discharged in accordance with hospital policy after this observation period is complete and the subject is medically stable. The subject may remain hospitalized beyond this protocol-required window if deemed necessary for medical management of serious adverse events.
34. All subjects must undergo a Respiratory Virus Panel (RVP; inclusive of SARS-CoV-2) within 10 days prior to the first planned huCART-meso infusion and prior to receipt of lymphodepleting chemotherapy. If the subject is positive for influenza, Tamiflu® or equivalent, should be administered per package insert. The subject must complete treatment prior to receiving lymphodepleting chemotherapy (if applicable) and huCART-meso cells. The test does not need to be repeated prior to these infusion(s), however if influenza sign and symptoms are present, the infusion(s) should be delayed until the subject is asymptomatic. If the subject is positive for another virus on the RVP, the infusion(s) will be delayed for at least 7 days to be sure clinical symptoms of a viral infection do not develop. If clinical symptoms develop, the infusion(s) will be delayed until resolution of these symptoms.
35. Performed Day +14 (+/- 7 days) after the huCART-meso Infusion #2. If huCART-meso Infusion #2 is not performed for any reason, the post-infusion biopsy/tumor imaging will be performed at the time the decision is made by the physician-investigator not to administer additional infusions. Tumor imaging may also be performed at any time as per clinical discretion.
36. Cohort 6 subjects may receive up to two additional IV infusions of huCART-meso cells at the same dose level, if determined clinically appropriate by the physician-investigator. Each additional infusion may be given approximately 21 and 42 days after the Day 0 infusion. huCART-meso Infusion #2 is targeted for Day 21 (+3 weeks) and huCART-meso Infusion #3 is targeted for Day 42 (+ 3 weeks). Additional huCART-meso infusions performed outside of this window require approval from the Principal Investigator and Sponsor Medical Director. The subject must be evaluated for the criteria in **Section 6.8.2** prior to each additional infusion. Subjects will not receive lymphodepleting chemotherapy prior to additional huCART-meso infusions.
37. Study visit time point is to be calculated from Day 0 (initial huCART-meso infusion). Monthly visits are defined as 28 day or 4 week time periods.
38. Subjects must undergo a Respiratory Virus Panel (RVP; inclusive of SARS-CoV-2) within 10 days prior to each additional huCART-meso cell infusion. If the subject is positive for influenza, Tamiflu® or equivalent, should be administered per package insert. The subject must complete treatment **prior** to receiving huCART-meso cells. The RVP does not need to be repeated prior to infusion; however, if influenza signs and symptoms are present, the infusion should be delayed until the subject is asymptomatic. If the subject is positive for another virus on the RVP, the infusion will be delayed for at least 7 days to be sure clinical symptoms of a viral infection do not develop. If clinical symptoms develop, the infusion will be delayed until resolution of these symptoms.
39. A CBC with differential and comprehensive chemistry panel must be performed within 48 hours prior to cyclophosphamide administration. If already performed within this window, these laboratory tests do not need to be repeated the day of cyclophosphamide administration.
40. A CBC with differential and comprehensive chemistry panel must be performed within 48 hours prior to the huCART-meso infusion (and after receipt of lymphodepleting chemotherapy if applicable). If already performed within this window, these laboratory tests do not need to be repeated on the day of the huCART-meso infusion.
41. Subjects will first be asked to sign a pre-screening consent to collect information from their medical history and to test their tumor for mesothelin expression. Once mesothelin expression has been confirmed, subjects may be presented the main informed consent for the study.
42. If the additional huCART-meso infusions are delayed by greater than 7 days, or not performed for any reason, the additional infusion study visits [at Day 21 and Day 42] will still be performed as scheduled, however they will be repurposed as a safety follow-up visit only. These safety follow-up visits will be identified as the actual study visit day from Day 0 (i.e. Day 26) and should be performed within +7 days of the planned study timepoint. If the additional huCART-meso infusions are then subsequently performed within the protocol allowable window (+3 weeks), the actual infusion day will be calculated as the actual study visit day from Day 0 (i.e. Day 35) and the study visit assessments required for an infusion visit will be repeated prior to the infusion.

18 APPENDIX 3: SCHEDULE OF EVENTS (COHORT 7)

Trial Period	~Week -8	~Week -6 to -4	Week -2 to -1	Day -5 to -3 (+/-1 d)[10]	Day 0	Days 1 + 2[33]	Days 6 (±1), 9 (±1), 14 (±1)	Day 21 (+ 3 wks)[37]	Days Post Infusion #2[26]		Day 42 (+3 wks)[37]	Days Post Infusion #3[26]		Month 3, 6 (±5 days)[37]	Month 9, 12, 15, 18, 21, 24 (±2 weeks)[37]
									Days +1 and +2	Day +6, +9, +14 (±1)		Days +1 and +2	Days +6, +9, +14 (±1)		
Visits	Screening	Apheresis	Pre- Infusion Safety	Lymphodepleting Chemotherapy	Infusion #1	Inpatient Observation	Safety Follow-up	Infusion #2[29]	Inpatient Observation[33]	Safety Follow-up	Infusion #3[29]	Inpatient Observation[33]	Safety Follow-up	Follow-up	Follow-up
CLINICAL ASSESSMENTS															
Informed Consent	X														
Demographics	X														
Diagnosis and Extent of Cancer	X														
Relevant Medical History	X														
Antineoplastic Therapy	X[30]														
Physical Examination[20]	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Sign Assessments[1]	X		X	X	X	X[31]	X	X	X[31]	X	X	X[31]	X	X	X
Prior and Concomitant Medications	X		X----------												----------X
ECOG Performance Status	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events				X[18]----------											----------X[18]
EKG	X		X											X[8]	
Echocardiogram	X														

Trial Period	~Week -8	~Week -6 to -4	Week -2 to -1	Day -5 to -3 (+/-1 d)[10]	Day 0	Days 1 + 2[33]	Days 6 (±1), 9 (±1), 14 (±1)	Day 21 (+ 3 wks)[37]	Days Post Infusion #2[26]		Day 42 (+3 wks)[37]	Days Post Infusion #3[26]		Month 3, 6 (±5 days)[37]	Month 9, 12, 15, 18, 21, 24 (±2 weeks)[37]
									Days +1 and +2	Day +6, +9, +14 (±1)		Days +1 and +2	Days +6, +9, +14 (±1)		
Visits	Screening	Apheresis	Pre- Infusion Safety	Lymphodepleting Chemotherapy	Infusion #1	Inpatient Observation	Safety Follow-up	Infusion #2[29]	Inpatient Observation[33]	Safety Follow-up	Infusion #3[29]	Inpatient Observation[33]	Safety Follow-up	Follow-up	Follow-up
Pathological confirmation of malignancies	X														
Leukapheresis screening[12]	X														
ICE Score[40]					X	X	X	X	X	X	X	X	X		
CLINICAL TESTS[2]															
CBC with differential (4 ml-lavender top)	X	X[32]	X	X[39]	X[16]	X	X	X[16]	X	X	X[16]	X	X	X	X
Chemistry (5 ml-gold top)[15]	X		X	X[39]	X[16]	X	X	X[16]	X	X	X[16]	X	X	X	X
PT/INR, PTT, fibrinogen, d-dimer (7.5 ml-blue top)	X		X												
Autoantibodies, ANA (1 ml-gold top)	X														
Viral screens: HIV, HCV, HBV (20ml – gold top)[25]	X														
Urinalysis	X													X[14]	
Urine β HCG	X														

Trial Period	~Week -8	~Week -6 to -4	Week -2 to -1	Day -5 to -3 (+/-1 d)[10]	Day 0	Days 1 + 2[33]	Days 6 (±1), 9 (±1), 14 (±1)	Day 21 (+ 3 wks)[37]	Days Post Infusion #2[26]		Day 42 (+3 wks)[37]	Days Post Infusion #3[26]		Month 3, 6 (±5 days)[37]	Month 9, 12, 15, 18, 21, 24 (±2 weeks)[37]
									Days +1 and +2	Day +6, +9, +14 (±1)		Days +1 and +2	Days +6, +9, +14 (±1)		
Visits	**Screening**	**Apheresis**	**Pre- Infusion Safety**	**Lymphodepleting Chemotherapy**	**Infusion #1**	**Inpatient Observation**	**Safety Follow-up**	**Infusion #2[29]**	**Inpatient Observation[33]**	**Safety Follow-up**	**Infusion #3[29]**	**Inpatient Observation[33]**	**Safety Follow-up**	**Follow-up**	**Follow-up**
Serum pregnancy (5ml- gold top)			X												
Ferritin, CRP, Haptoglobin, Triglycerides, ESR[6] (4ml-gold top; 4ml lavender top)			X		X[22]	X[22]	X[22]	X[22]	X[22]	X[22]	X[22]	X[22]	X[22]		
Respiratory Virus Panel (RVP)			X[34]					X[38]			X[38]				
Tumor biomarkers[9] CA125 (5ml- gold top)			X							X[35]				X[13]	
RADIOGRAPHIC IMAGING															
Tumor imaging[3]	X[3]		X[24]							X[35]				X[13]	
Brain MRI	X[23]														
INTERVENTIONS															
Apheresis[32]		X[5]													
Lymphodepleting Chemotherapy				X[10]											
huCART-meso cell infusion[19]					X[19]			X[36]			X[36]				
Tumor Biopsy			X[17]				X[21]								

Trial Period	~Week -8	~Week -6 to -4	Week -2 to -1	Day -5 to -3 (+/-1 d)[10]	Day 0	Days 1 + 2[33]	Days 6 (±1), 9 (±1), 14 (±1)	Day 21 (+ 3 wks)[37]	Days Post Infusion #2[26]		Day 42 (+3 wks)[37]	Days Post Infusion #3[26]		Month 3, 6 (±5 days)[37]	Month 9, 12, 15, 18, 21, 24 (±2 weeks)[37]
									Days +1 and +2	Day +6, +9, +14 (±1)		Days +1 and +2	Days +6, +9, +14 (±1)		
Visits	Screening	Apheresis	Pre- Infusion Safety	Lymphodepleting Chemotherapy	Infusion #1	Inpatient Observation	Safety Follow-up	Infusion #2[29]	Inpatient Observation[33]	Safety Follow-up	Infusion #3[29]	Inpatient Observation[33]	Safety Follow-up	Follow-up	Follow-up
RESEARCH SPECIMENS [2, 4]															
Serum (Red top tube (~6ml)			X		X[7]	X	X	X[7]	X	X	X[7]	X	X	X	X
Interleukin-15 (IL-15)			X												
Peripheral Blood Mononuclear Cells (~ Two 10mL purple top tubes)[11]			X		X[7]	X	X	X[7]	X	X[27]	X[7, 27]	X	X[27]	X	X
q-PCR persistence[28]			X		X	X	X	X	X	X	X	X	X	X	X
TOTAL BLOOD DRAWS															
Approximate Milliliters	38.5	4	60.5	9	69	43/ day	Up to 63/ visit	69	43/day	Up to 68/ visit	69	43/day	Up to 63/visit	40/ visit	35/ visit
Approximate Tablespoons	2.6	.27	4.1	0.6	4.7	2.9/ day	4.3	4.7	2.9	4.6	4.7	2.9	4.3	2.7	2.4

1. Includes temperature, heart rate, blood pressure and pulse oximetry. Height and weight collected at screening only. On the day of each huCART-meso infusion, vital signs (temperature, respiration rate, heart rate, blood pressure, and oxygen saturation by pulse oximetry) will be measured within 10 minutes prior and within 15 minutes after the infusion. Thereafter, vital signs will be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the infusion, and then every hour (+/- 10 minutes) for the next two hours until these signs are satisfactory and stable. If vital signs are not satisfactory and stable 3 hours after the huCART-meso infusion, vital signs will continue to be monitored as clinically indicated until stable.
2. Additional blood draws and tissue collection may be performed at the discretion of the investigator (for example, to evaluate unexpected clinical events). Moreover, additional tissue and fluid samples obtained as part of standard of care procedures for clinical indications will be sent for research analysis, wherever feasible.

3. Tumor Imaging: CT imaging as clinically indicated as per patient population and disease status. MRI scans may be substituted at the investigator's discretion. Additional scans may be performed as clinically appropriate. Scans may be performed with or without contrast. At the screening visit, a CT performed within 8 weeks of confirmation of eligibility by a physician-investigator is acceptable.
4. Research blood samples are collected to evaluate T cell persistence, cytokines, anti-CAR and anti-tumor immune responses.
5. Subjects have the option for a second apheresis in case the T cell product is not successfully manufactured from the first apheresis. Refer to **Section 6.2** for additional details.
6. May be performed post-huCART-meso infusion if CRS is suspected, or as clinically indicated.
7. Blood draw before infusion and ~1 hour after T cell infusion
8. EKG at month 6 only
9. Tumor biomarkers as appropriate for each cancer.
10. Lymphodepleting chemotherapy will consist of cyclophosphamide 300 mg/m^2/day and fludarabine 30 mg/m^2/day given over 3 days by intravenous infusion. Lymphodepleting chemotherapy will be scheduled such that the last day of chemotherapy is 3 days (+/- 1 day) prior to the 1st infusion of huCART-meso cells. Please refer to **Section 6.4** for additional details.
11. RCL VSV-G testing will not be routinely performed as of Protocol V8. Blood samples will be collected and banked at pre-infusion and post-infusion at Months 3, 6, and 12. These samples will be used for future RCL VSV-G testing if indicated.
12. If a subject's veins are not adequate for apheresis procedure, the subject may have a central venous apheresis catheter line placed by interventional radiology.
13. Performed at Months 3 and 6. CT scans within 4 weeks of Month 3 and 6 visits are acceptable.
14. Month 3 only.
15. Chemistry panel includes sodium, potassium, chloride, CO2, blood urea nitrogen, creatinine, glucose, phosphate, total protein, albumin, calcium, alkaline phosphatase, total bilirubin, ALT, AST, uric acid, LDH.
16. A CBC with differential and comprehensive chemistry panel must be performed within 48 hours prior to the huCART-meso infusion (and after receipt of lymphodepleting chemotherapy). If already performed within this window, these laboratory tests do not need to be repeated on the day of the huCART-meso infusion.
17. A baseline tumor biopsy will be performed prior to lymphodepleting chemotherapy and huCART-meso infusion, if the subject has an accessible tumor. The baseline biopsy may occur any time prior to infusion between Day -14 to Day -1. This baseline biopsy sample will be used to evaluate the level of mesothelin expression (using GeoMx Digital Profiling analysis; for exploratory purposes only) and if feasible, other immune markers at baseline. If a subject does not have an accessible tumor or a biopsy is not felt to be clinically appropriate, an archived tumor tissue specimen may be obtained for research purposes. Please refer to the Tissue Handling Manual for complete details.
18. Collection of adverse events will begin at the time of initiation of lymphodepleting chemotherapy. Effective Protocol Amendment V11 Only: At the time alternative therapy for their disease is initiated, subjects will only be followed for protocol-defined adverse events (PDAES) as defined in **Section 9.1.6**. Any events ongoing at the time alternative treatment is initiated must continue to be followed through resolution.
19. Subjects must be evaluated by a physician-investigator for eligibility to receive huCART-meso cells according to criteria in **Section 6.5**. The 1st huCART-meso infusion will occur via intraperitoneal (i.p.) administration. Subsequent infusion will take place via intravenous (IV) administration.
20. Physical examination to include a visual examination of the throat. If toxicity is suspected, please consult with a Pulmonology Study Investigator.
    Tumor biopsy to be performed at Day +14 (±7 days) after the 1st huCART-meso infusion. This biopsy sample will be used to evaluate the level of mesothelin expression (using GeoMx Digital Profiling analysis; for exploratory purposes only) and if feasible, other immune markers.
21. Please refer to **Section 6.9.1** for additional details. Please refer to the Laboratory Manual for complete details.

22. Only ferritin and CRP required; other labs repeated as clinically indicated.
23. For evaluation of suspected brain metastasis (performed within 8 weeks of eligibility confirmation by a physician-investigator).
24. Baseline scans should be obtained within 28 days prior to the first infusion of huCART-meso cells and after any cancer therapy administered between collection and huCART-meso infusion. Baseline scans should also be obtained prior to administration of lymphodepleting chemotherapy.
25. Viral serologies: HIV, Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody, Hepatitis B core antibody, and HIV Hepatitis C antibody. If the HCV antibody is positive, a screening HCV RNA by any RT-PCR or bDNA assay must be performed. Eligibility will be determined based on the screening value. The test is not required if documentation of a negative result of a HCV RNA test performed within 60 days prior to screening is provided.
26. Additional infusions may be performed within a +3 week window from the targeted infusion days [Day 21 (+3 weeks) and Day 42 (+3 weeks)]. Given the allowable window for these additional infusions, the timing of post-infusion safety follow-up visits will be dependent on the actual timing of the additional huCART-meso infusions. In summary, the timing of the post-infusion safety follow-up visits will be calculated as the actual study day they occurred post Infusion #2 and Infusion #3 (i.e. **+** 6 days post Infusion #2, +7 days post Infusion #2, **+**14 days post Infusion #3, etc.). This should be recorded as the actual study day, even if an assessment falls within the allowable visit window. In addition, the study day from Day 0 (the initial huCART-meso infusion) must also be captured. Example: Infusion # + Study Visit Day Post Infusion/Study Visit Day from Day 0 = Infusion #2 Day +6/Day 28. Please refer to **Section 6.9.3** for complete details. If an infusion is omitted, these safety follow-up visits will not be required. If an infusion is delayed, these additional safety follow-up visits will be shifted to align with the timing of the infusion.
27. At the Day +14 study visit post huCART-meso Infusion #2 and Infusion #3, up to 60mL (6 purple top tubes) will be collected. If a subject does not receive either huCART-meso Infusion #2 or Infusion #3, the subject will undergo this 60mL blood draw at the Day 42 (+7) Safety Follow-up Visit.
28. Testing for huCART-meso persistence by q-PCR will occur at pre- and all post-infusion study visits. Additional testing may also be performed at unscheduled time points at the discretion of the Sponsor. This analysis will continue until any 2 sequential tests are negative documenting loss of CAR T cells. Please refer to **Section 7.2** for additional details.
29. If the additional huCART-meso infusions are delayed by greater than 7 days, or not performed for any reason, the additional infusion study visits [at Day 21 and Day 42] will still be performed as scheduled, however they will be repurposed as a safety follow-up visit only. These safety follow-up visits will be identified as the actual study visit day from Day 0 (i.e. Day 26) and should be performed within +7 days of the planned study timepoint. If the additional huCART-meso infusions are then subsequently performed within the protocol allowable window (+3 weeks), the actual infusion day will be calculated as the actual study visit day from Day 0 (i.e. Day 35) and the study visit assessments required for an infusion visit will be repeated prior to the infusion.
30. Prior antineoplastic therapies up through study treatment will be collected. Additional antineoplastic therapy received post study treatment and while on study, will also be collected.
31. During post-infusion observation, the subject will undergo vital sign assessments (temperature, respiration rate, heart rate, blood pressure, and oxygen saturation by pulse oximetry) every 4-6 hours (+/- 30 minutes) from the end of the infusion vital sign monitoring period until 48 hours post infusion. Additional monitoring may be performed per clinical discretion.
32. Performed per Apheresis Unit policy. It is recommended that the patient have an absolute lymphocyte count (ALC) ≥500/µl prior to undergoing apheresis. If the patient's ALC is <500/µl, it is recommended that a lymphocyte subset analysis (CD3, CD4, CD8 counts) be performed to confirm that the patient has an absolute CD3 count of ≥150/µl. If the absolute CD3 count is <150/µl, it is recommended that the leukapheresis procedure be delayed until their ALC is ≥500/µl or absolute CD3 count is ≥150/µl. Up to a 4-week delay may occur; following this, further discussion is needed with the study PI and the CVPF prior to proceeding. Additional routine laboratory testing to be performed per Apheresis Unit policies/procedures.

33. The subject will remain inpatient for observation for a minimum of 48 hours after each huCART-meso infusion per **Section 6.6**. The subject may then be discharged in accordance with hospital policy after this observation period is complete and the subject is medically stable. The subject may remain hospitalized beyond this protocol-required window if deemed necessary for medical management of serious adverse events.
34. All subjects must undergo a Respiratory Virus Panel (RVP; inclusive of SARS-CoV-2) within 10 days prior to the first planned huCART-meso infusion and prior to receipt of lymphodepleting chemotherapy. If the subject is positive for influenza, Tamiflu® or equivalent, should be administered per package insert. The subject must complete treatment prior to receiving lymphodepleting chemotherapy (if applicable) and huCART-meso cells. The test does not need to be repeated prior to these infusion(s), however if influenza sign and symptoms are present, the infusion(s) should be delayed until the subject is asymptomatic. If the subject is positive for another virus on the RVP, the infusion(s) will be delayed for at least 7 days to be sure clinical symptoms of a viral infection do not develop, or longer if clinically appropriate for the respective pathogen. If clinical symptoms develop, the infusion(s) will be delayed until resolution of these symptoms.
35. Performed Day +14 (+/- 7 days) after huCART-meso Infusion #2. If huCART-meso Infusion #2 is not performed for any reason, tumor imaging and tumor markers will be performed at the time the decision is made by the physician-investigator not to administer additional infusions. Tumor imaging may also be performed at any time as per clinical discretion.
36. Cohort 7 subjects may receive up to two additional infusions of huCART-meso cells via IV administration at the same dose level, if determined clinically appropriate by the physician-investigator. Each additional infusion may be given approximately 21 and 42 days after the Day 0 infusion. huCART-meso Infusion #2 is targeted for Day 21 (+3 weeks) and huCART-meso Infusion #3 is targeted for Day 42 (+ 3 weeks). Additional huCART-meso infusions performed outside of this window require approval from the Principal Investigator and Sponsor Medical Director. The subject must be evaluated for the criteria in **Section 6.9.2** prior to each additional infusion. Subjects will not receive lymphodepleting chemotherapy prior to additional huCART-meso infusions.
37. Study visit time point is to be calculated from Day 0 (initial huCART-meso infusion). Monthly visits are defined as 28 day or 4 week time periods.
38. Subjects must undergo a Respiratory Virus Panel (RVP, inclusive of SARS-CoV-2) within 10 days prior to each additional huCART-meso cell infusion. If the subject is positive for influenza, Tamiflu® or equivalent, should be administered per package insert. The subject must complete treatment **prior** to receiving huCART-meso cells. The RVP does not need to be repeated prior to infusion; however, if influenza signs and symptoms are present, the infusion should be delayed until the subject is asymptomatic. If the subject is positive for another virus on the RVP, the infusion will be delayed for at least 7 days to be sure clinical symptoms of a viral infection do not develop, or longer if clinically appropriate for the respective pathogen. If clinical symptoms develop, the infusion will be delayed until resolution of these symptoms.
39. A CBC with differential and comprehensive chemistry panel must be performed within 48 hours prior to the first dose of lymphodepleting chemotherapy, then as clinically necessary during remaining chemotherapy days. If already performed within this window, these laboratory tests do not need to be repeated.
40. Effective Protocol Amendment V11 Only: The ICE Score may be assessed by investigators or trained study staff (study coordinators or research nurses). The total ICE Score should be assessed on a 10-point scale. Therefore, if any of the assessments are missed, an ICE score cannot be reported. If certain assessments cannot be performed by the subject (e.g., writing sample), this individual assessment will be scored as a zero. Please refer to **Appendix 5** for the ICE scoring criteria.

19 APPENDIX 4: NEW YORK HEART ASSOCIATION (NYHA) FUNCTIONAL CLASSIFICATION

Class	Functional Capacity: How a patient with cardiac disease feels during physical activity
I	Patients with cardiac disease but resulting in no limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea or anginal pain.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea or anginal pain.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea or anginal pain.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort increases.

20 APPENDIX 5: IMMUNE EFFECTOR CELL-ASSOCIATED ENCEPHALOPATHY (ICE) SCORE

Category	Test	Scoring
Orientation	Orientation to year, month, city, hospital	4 points total (1 point each)
Naming	Ability to name 3 objects (e.g. point to clock, pen, button)	3 points total (1 point each)
Following Commands	Ability to follow simple commands (e.g. "Show me 2 fingers" or "Close your eyes and stick out your tongue")	1 point
Writing	Ability to write a standard sentence (e.g. "Our national bird is the bald eagle")	1 point
Attention	Ability to count backwards from 100 by 10	1 point