3) Record review for appropriate diagnosis and stage of disease or confirmation of diagnosis by internal pathology review of initial or subsequent biopsy or other pathologic material at UWMC
4) Baseline pulse oximetry and documentation of O_2 saturation on room air
5) Baseline 12-lead EKG
6) Patients 60 years or older: Echocardiogram or MUGA scan within 60 days of enrollment.
7) Baseline imaging with RECIST read (generally CT chest/abdomen/pelvis (and if present affected extremity), MRI brain if history of brain metastasis or neurologic symptoms concerning for brain metastasis, CT neck if prior cervical adenopathy, MRI or PET may be substituted as clinical judgement dictates). Imaging must be repeated if not within 45 days prior to first infusion of FH-MagIC TCR-T

Research samples:

1) PBMC Baseline Samples: A 60 ml blood sample (in EDTA [lavender top] tube) for research should be sent to the Specimen Processing/Research Cell Bank lab at the Fred Hutch for PBMC isolation and cryopreservation. These cells will be used as PBMC baseline samples for comparison to post-treatment samples for all cellular correlative tests, including T cell persistence and immunophenotype by flow cytometry or Q-PCR or TCR-β deep sequencing (e.g., with Adaptive Biotechnologies) assays, assessment of anti-transgene and anti-tumor immune responses. These tests are all optional and may be performed depending on tumor response and T cell persistence.
2) Baseline serum: A 20 ml blood sample (serum separator tube) should be sent to the Specimen Processing/Research Cell Bank lab at the Fred Hutch to be used as serum baseline to assess anti-transgene and anti-tumor immune responses. These tests may be performed depending on tumor response and T cell persistence.
3) Other biopsy or sample: If biopsy or sampling of tissues (i.e., CSF, pleural fluid, etc.) is performed for clinical indications, then additional tissue may be obtained during the same procedure and sent to the Specimen Processing/Research Cell Bank Lab at the Fred Hutch for research studies. Please discuss the planned procedure with the study PI.
4) RCL Testing: A 10 ml (EDTA/lavender top) blood sample should be collected as baseline for RCL testing and sent to the Specimen Processing Lab/ Research Cell Bank Lab at the Fred Hutch.
5) Research RECIST 1.1 read of baseline scans.
6) Biopsy samples: Enrollment (after eligibility and prior to bridging chemo if given and LD) research biopsy samples defined as a core needle (6 needle passes) or punch biopsy (1 x 6 mm or 2 x 4 mm) will be obtained.

10.3 Evaluations prior to leukapheresis

Treatment consent signed and eligibility confirmation must be completed prior to leukapheresis start.

10.4 Evaluations within 5 days before LD if receiving (if no LD planned, then within 2 days before cell infusion)

1) Interval history and physical exam and ECOG performance status
2) Blood draw for laboratory studies:
    a. CBC, differential, platelet count
    b. Renal/Hepatic Function Panel
    c. Magnesium and Lactate Dehydrogenase (LDH)
    d. Research Labs:
        i. A 30 ml blood sample (in EDTA [lavender top] tube) for research should be sent to the Specimen Processing/Research Cell Bank lab
        ii. A 10 ml blood sample (serum separator tube) should be sent to the Specimen Processing/Research Cell Bank lab
3) In order to be eligible for LD/TCR infusion, the patient must meet the following pre-infusion criteria:

- Continue to meet all inclusion/exclusion criteria
    - All assessments do not need to be repeated at this timepoint, but if there are any incidental findings between screening assessments and this timepoint that would make the patient ineligible, they will be excluded from moving forward with LD/cell infusion
- No disease complications from bridging therapy or related to the patient's underlying disease that, in the opinion of the investigator, would make it unsafe to proceed with LD chemotherapy or TCR infusion.
- Adequate respiratory function with oxygen saturation ≥94% on room air and no dyspnea at rest.
- No new cardiac issues, including CHF, MI, arrhythmias, or stroke.
- No evidence of an active infection (e.g., temperature ≥38.3°C, positive blood cultures, requirement for antibiotics) within 14 days before LD chemotherapy.

10.5 Evaluations prior to T cell infusion

On the day of scheduled T cell infusion, the patient should undergo a clinical evaluation and a clinical determination for appropriateness to proceed with treatment.

1) Interval history and physical exam and ECOG performance status
2) Blood draw for laboratory studies:
    a. CBC, differential, platelet count
    b. Renal/Hepatic Function Panel
    c. Magnesium and Lactate Dehydrogenase (LDH)
    d. Uric acid
3) Serum pregnancy test within 72 hours of infusion

10.6 Evaluations following each T cell infusion

The following evaluations will be performed after the T cell infusion(s):

1) Updated History and physical exam 1 day after the T cell infusion and at least weekly for 3 weeks.
2) Laboratory studies:
    a. CBC, differential, platelet count on day 1,3,7,14, 21 and 28
    b. Renal, hepatic function with LDH, Mg on day 1,3,7,14, 21 and 28
    c. Uric acid on day 1,3, and 7
3) If a patient becomes febrile or develop symptoms of cytokine release or tumor lysis between the indicated time points, we may measure serum ferritin, IL-6, CRP, DIC panel, and tumor lysis markers at additional times, as clinically indicated.
4) Tumor biopsy:
    a. Patients will also be asked to undergo a biopsy at time of 12 week evaluation and may also be asked to undergo a tumor biopsy at the time of relapse. The goal of the 12 week biopsy will be to confirm that there is progressive/residual tumor if tumors are stable or growing and to confirm that there is residual tumor if tumors are shrinking because a second T cell infusion is not given if there is no residual viable tumor). The purpose of the repeat biopsy at time of late relapse would be to confirm that the relapsed lesion represents metastatic TNBC, urothelial carcinoma or NSCLC. These 12-week and relapse biopsies will be performed for research purposes.
5) Research Samples:
    a. <u>Tumor biopsy</u>: At approximately 14 days following the first T cell infusion, all patients with a palpable or radiologically accessible tumor will be asked to undergo a punch, excisional or a core biopsy. The biopsy at 2 week time point will be for research. The requirement for a biopsy will be waived for patients who do not have tumor amenable to biopsy.
    b. <u>Research blood samples</u>:
        i. A 20 ml blood sample (serum separator tube) should be sent to the Specimen Processing/Research Cell Bank Lab at Fred Hutch. Blood samples should be obtained on approximately days 1, 3, 7, 14, 21, and 28 during the first month after each T cell infusion, and also at 2, 3, 6, 9, and 12 months after the T cell infusion
        ii. A 60 ml blood sample (in EDTA [lavender top] tube) for research should be sent to the Specimen Processing/Research Cell Bank lab. Blood samples should be obtained on approximately days 1, 3, 7, 14, 21, and 28 during the first month after each T cell infusion, and also at 2, 3, 6, 9, and 12 months after the T cell infusion
        iii. If patients become febrile, develop signs of cytokine release syndrome, or cytokine assessment is clinically appropriate at times other than those indicated, we may collect additional research samples at additional timepoints (optional).

iv. The above research samples will be used in the following ways as described below: for evaluation of serum cytokines, persistence and phenotype of TCR transgenic T cells, evaluation of migration, development, of endogenous anti-tumor immune responses and epitope spreading.

a. Serum storage for measurement of serum cytokine levels

i. Serum cytokine levels will be run at selected time points

b. Evaluation for persistence and phenotype of TCR transgenic T cells

Additional samples may be collected at other times than those indicated, including beyond 12 months, if required for evaluation of persistence of TCR T cells. Conversely, persistence monitoring may be discontinued beyond day 28 after each infusion in patients who do not have detectable transgene-expressing T cells on two consecutive occasions. The cells from these blood samples may also be analyzed by multiparameter flow cytometry for the phenotype of persisting TCR T cells (optional).

c. Evaluation of migration of adoptively transferred TCR T cells

i. Single cell tumor digests will be generated from research biopsy tissue and for selected patients single cell RNA sequencing will be performed (optional).

ii. Multiplex immunohistochemistry will be performed on clinical and/or research biopsies to evaluate T cell localization (optional).

d. Evaluation for development of endogenous anti-tumor immune responses and epitope spreading

We may evaluate whether any cellular or humoral anti-tumor immune responses resulting from activation of endogenous immune cells have occurred. Research samples will be collected; however, performance of research studies is optional and assays may be substituted with alternate assays or omitted as clinical course dictates.

Whole exome sequencing and RNA Seq: For patients in whom endogenous immune responses are detected, more detailed evaluation may be performed to characterize T cell responses by predicting which tumor mutations are likely to be immunogenic and then evaluating peripheral blood T cells for reactivity to these epitopes. To identify mutations and determine the patient's HLA alleles, whole exome sequencing and RNA Seq of baseline tumor as well as whole exome sequencing of normal tissue may be performed (optional).

e. RCL testing

RCL testing, as per FDA guidelines.

v. Leftover materials will be archived for future studies of T cell function. All research assays are optional and may be performed or omitted or replaced with similar studies based on patient's clinical course

10.7 Response Assessment

Participants will have evaluation of tumor burden at baseline (physical exam with tumor measurements, cross-sectional imaging as detailed above, RECIST 1.1 and imRECIST reads of cross-sectional imaging), at approximately 12 weeks after T cell infusion, and then at discretion of treating oncologist (suggested frequency every 3-6 months). *Note*: in patients who receive a second T-cell infusion, repeat baseline imaging should be performed (within 30 days before starting LD chemotherapy or T cell infusion if no LD chemotherapy is given) and again at timepoints outlined above. These assessments have a window of +/- 7 calendar days. Imaging should also be performed if suspicion of progression. Imaging will be performed on a clinical basis with RECIST 1.1/imRECIST reads on a research basis.

Participants will have research biopsy of tumor at baseline, at approximately 12 weeks after first T cell infusion, and if clinically suspected to have progression. Additional research biopsies are planned at 2 weeks post T cell infusion and at 12 weeks after second T cell infusion, and additional research tissue may be requested from clinical biopsies.

10.8 Participant discontinuation of active treatment

A patient will no longer be able to receive active treatment on study (T cells, atezolizumab) for any of the reasons listed below. In this scenario (unless withdrawal of consent or death), a participant will no longer be required to comply with protocol-dictated testing, AE collection, and other timepoints for any of the reasons listed below, with the exception that an attempt will be made to collect samples for RCL and persistence at protocol-dictated timepoints. In this scenario (unless withdrawal of consent or death), the participant will transition to the long term follow up (LTFU) phase of study:

- Progressive disease or development of new metastasis requiring urgent change in treatment following second T cell infusion. Patients can be considered for chemotherapy/radiation for progression between the 1st and second infusions and remain on study if considered appropriate by the PI. Washout between therapies received at time of progression and second lymphodepletion should be either 2 weeks post-radiation or the equivalent of one treatment cycle.
- The participant withdraws consent
- Patient death
- Occurrence of pregnancy
- Participation in another therapeutic trial during the treatment duration of this trial
- Occurrence of an exclusion criterion, which is clinically relevant and affects the subject's safety, if discontinuation is considered necessary by the Investigator or Sponsor
- A patient will no longer be eligible to receive additional therapy if the PI or designee determines that additional T cell infusions are not in the best interest of the patient
- Occurrence of any non-pre-existing grade 3 or higher AEs / repetitive Grade 2 AEDRs that are deemed related to the treatment, except for the exceptions detailed in section 12.

- Cytokine release syndrome requiring pressors for >24 hours or tocilizumab

10.9 Long-term follow-up

Enrolled patients who receive FH-MagIC TCR-T will be asked to participate in long-term follow-up (LTFU) according to guidelines set forth by the FDA's Biologic Response Modifiers Advisory Committee that apply to gene transfer studies. Current recommendations from the FDA suggest a minimum of 15 years of follow-up.

Should the participant develop a late relapse, a 50 ml blood draw and/or leftover/excess biopsy tissue (fresh and/or archival) will be requested for evaluation as to mechanisms of late relapse.

Recommendations will be made for an autopsy to be conducted if the research participant dies.

11.0 ADVERSE EVENT REPORTING

11.1 Adverse Event Definitions

- **Adverse Event**
  An Adverse Event (AE) is any untoward medical occurrence in a clinical investigation subject administered a medicinal product; the event does not necessarily have a causal relationship with study drug administration or usage. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

- **Serious Adverse Event**
  A serious adverse event (SAE) is defined as an untoward medical occurrence that results in any of the following outcomes:
  1. Death.
  2. Life-threatening situation (i.e., with an immediate risk of death from the event as it occurred but not including an event that, had it occurred in a more serious form, might have caused death).
  3. In-patient hospitalization or prolongation of existing hospitalization. Inpatient hospitalization comprises formal admission to a hospital for medical reasons, for any length of time, whether or not hospitalization extends overnight. However, hospital admissions for administration of the study drug, procedures required by the study protocol, or tumor-related diagnostic procedures are not considered serious.
  4. Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
  5. Congenital anomaly/birth defect.
  6. An important medical event that requires intervention to prevent one of the above outcomes.

- **Unexpected Adverse Event**
  An unexpected adverse event is defined as an event that has a nature or severity, or frequency that is not consistent with the investigator brochure, protocol, or consent form. Please see section 11.6 for expected toxicities.

11.2 Monitoring and Recording Adverse Events

Adverse events will be assessed by the investigator or qualified designee and recorded in the CRFs. The investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the adverse event and/or serious adverse event and not described as the individual signs or symptoms. The following information should be recorded:

- Description of the adverse event using concise medical terminology
- Description as to whether or not the adverse event is serious
- The start date (date of adverse event onset)
- The stop date (date of adverse event resolution or return to baseline)
- The severity (grade) of the adverse event
- A description of the potential relatedness of the adverse event to study drug or a study procedure
- Expectedness of the adverse event based on prior observed and documented adverse events
- The outcome of the adverse event

11.3 Grading of the Severity of an Adverse Event

AEs will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. If a CTCAE criterion does not exist, the investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the adverse event. For cytokine release syndrome, the CRS Grading Scale provided in **Appendix E** will be used to grade severity.

11.4 Attribution of Adverse Event

Association or relatedness to the study agent will be assessed by the investigator as follows:

Definite (must have all 4)	• Has a reasonable temporal relationship to the intervention • Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions • Follows a known pattern of response to intervention • Disappears or decreases with reduction in dose or cessation of intervention and recurs with re-exposure
Probable (must have 3)	• Has a reasonable temporal relationship to the intervention • Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions • Follows a known pattern of response to intervention • Disappears or decreases with reduction in dose or cessation of intervention

Possible (must have 2)	• Has a reasonable temporal relationship to the intervention • Could not have readily been produced by the subject's clinical state • Could not readily have been due to environmental or other interventions • Follows a known pattern of response to intervention
Unlikely (must have 2)	• Does not have a temporal relationship to the intervention • Could readily have been produced by the subject's clinical state • Could have been due to environmental or other interventions • Does not follow a known pattern of response to intervention • Does not reappear or worsen with reintroduction of intervention

For general AE assessment, an AE is considered related if it is assessed as definitely, probably, or possibly related; unrelated if it is assessed as unlikely related or unrelated.

For determination of IND safety reporting, AE attribution will be assessed according to the suspected adverse reaction definition described in 21 CFR 312.32 as an AE for which there is a reasonable possibility that the drug caused the adverse event where “reasonable possibility” means there is evidence to suggest a causal relationship between the drug and the AE. The IND Sponsor will report suspected adverse reactions that are both serious and unexpected to the FDA as an IND safety report, in accordance with regulations under 21 CFR 312.32.

11.5 Adverse Event Reporting Period

All adverse events (grades 1-5) will be monitored and recorded in study-specific case report forms (CRFs) throughout the investigative phases of the study: from each T cell infusion through day 14 (for purposes of potential dose modification) and in conjunction with standard follow-up time points during the first year following T cell infusion. The detailed collection of AEs will stop at the time of commencement of new anti-tumor therapy.

AEs with an onset date prior to apheresis will not be recorded, except in the case of clinically significant worsening of the AE during the specified monitoring time frame. A subject withdrawn from the study because of an adverse event must be followed until the clinical outcome from the adverse event is determined.

The following events are *not* identified as AEs in this study:

- Disease progression or relapse.
- Hospitalization to facilitate infusions is not considered an AE. Any AE requiring prolongation of this hospitalization will be recorded and patient to applicable SAE reporting.
- Hospitalization to facilitate monitoring/due to the absence of caregiver/any other reason that is not related to T cells. Medical or surgical procedures in and of themselves, including those that

require hospitalization (e.g., surgery, endoscopy, biopsy procedures) are not considered AEs. However, an event or condition requiring such procedures may be an AE.
- Hospitalization that is otherwise unrelated to adverse events.

11.6 Dose-limiting Toxicities to Guide Dose Modification

Dose-limiting toxicity (DLT) is defined as below and will be used to guide potential dose modification as detailed in **Sections 11.7.4 and 12.2.1**.

DLTs will be assessed for 14 days following the first infusion of the TCR-T cell product. The following events will be considered DLTs if they are attributed as at least possibly related to T cell administration. Grading will be done in accordance with the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 unless otherwise specified.

- Grade ≥ 3 allergic reaction related to the TCR-T cell infusion
- Grade ≥ 3 autoimmune reactions
- Any Grade 3 or 4 non-hematologic event that has not resolved to < grade 3 by day 28 post T cell infusion
- Grade ≥ 3 neurotoxicity of greater than 7 days duration
- Grade ≥ 3 neurotoxicity that does not revert to Grade 1 or baseline within 28 days
- Grade ≥ 3 seizures that do not resolve to < grade 3 within 3 days
- Grade ≥ 4 CRS (using criteria modified from Lee 2014)
- Grade 3 CRS that does not resolve to < grade 3 within 7 days
- Any other toxicity not meeting the above criteria that is deemed by the PI to represent a DLT

11.7 Adverse Event Reporting Requirements

11.7.1 Reporting to IRB

The investigator or designee must report events to the Fred Hutch IRB in accordance with the policies of the IRB.

11.7.2 Reporting to Sponsor

The investigator or designee must report events to the drug or financial sponsor of the study as outlined in the contract.

Classification of an event as serious or non-serious determines the reporting procedures to be followed by the site for reporting the event to the IND Sponsor.

PI to IND Sponsor Reporting Requirements for Adverse Events

Classification	Reporting Time	Reporting Action

Serious Adverse Event (SAE)	Fatal or life-threatening	Within 24 hours of research team awareness	Email notification to Sponsor
	All SAEs	Within 2 business days of research team* awareness	Email notification to Sponsor
Non-serious Adverse Event		Per CRF completion guidelines	Record information on appropriate CRFs

*Research team is defined as the individuals listed on the delegation of authority log. Physicians listed on the study's delegation of authority log as attending physicians with delegated authority to administer informed consent will not be considered part of the research team unless additional responsibilities related to the conduct of the study have been delegated to them by the Principal Investigator.

11.7.3 Institution-Sponsor Reporting Requirements

The sponsor assumes responsibility for IND safety reporting to the FDA and participating investigators, in accordance with regulations under 21 CFR 312.32.

Each serious adverse event report received from the investigator will be evaluated by the Medical Monitor who will assess the seriousness of the event, the expectedness of the event, and the relationship to participation in the study. For regulatory reporting purposes, the IND Sponsor will determine expectedness relating to the investigational product using safety information specified in the Investigator Brochure. An event will be classified as related if either the investigator or the IND Sponsor determines that the event may be related to the study drug.

The IND Sponsor or designee will provide all investigators with a safety letter notifying them of an event that meets FDA IND Safety Reporting criteria. Investigators will be requested to provide written notification of safety report to the Fred Hutch IRB as soon as is practical, consistent with IRB requirements.

11.7.4 Dose Assessment Committee

Because this is a Phase 1 dose finding study of a cellular therapy where both efficacy and toxicity may be observed in non-dose dependent manners, a Dose Assessment Committee (DAC) is established. The DAC charter is available in **Appendix H**. The DAC will work in an advisory capacity to the PI throughout the trial, as needed. The decision to escalate/de-escalate will be made in consultation with the DAC. The PI and IND Sponsor may override the algorithm's allocation of a patient to a particular dose level or schedule.

The IIRC Clinical Operations team will collect, abstract, and present adverse events to the PI and he will attribute. Treatment of patients in the dose-escalation/de-escalation groups will be staggered such that a minimum of a 28-day interval following infusion is required between each set of 3 or 4 patients before escalating to the next dose level, and 14 days between patients within the same dose level. Within 10 business days of Day 28 of at least the prior 3 or 4 patients, data will be presented and reviewed by the DAC.

12.0 STATISTICAL CONSIDERATIONS

12.1 Type of Study

Our study is a phase I/II, non-blinded interventional trial. Our target sample size is 15-18 patients (8-12 per year/1.5-2 years). This enrollment target is chosen for three reasons: it is achievable from an enrollment standpoint (our clinic sees hundreds of metastatic TNBC, urothelial or NSCLC patients potentially eligible per year), it is logistically possible from a funding and cell processing facility standpoint, and it allows >80% power to observe a statistically significant (at one-sided 0.05 level) efficacy signal that is clinically meaningful (see below). Patients will be treated at three potential dose levels (from $1x10^9$up to $5x10^9$ TCR-MagIC transgenic CD3+s).

12.2 Safety Assessment

12.2.1 Guidelines for Dose Modification

The treatment will be considered to have an acceptable safety profile if the observed DLT rate is consistent with a true rate that does not exceed 35%, and this rate will be used as a guide for decisions related to potential dose modification.

The initial dose level 1 patients will be treated and followed for toxicity for 14 days. Subsequent patients within this group will be able to receive their T cell infusion within 28 days of the last patient receiving their first T cell infusion. Per DAC general guidelines but pending their final recommendation, if zero of the first three dose level I patients' experiences treatment-related and unexpected grade ≥3 toxicity, escalation will proceed to the next dose level. If one patient experiences a treatment-related and unexpected grade ≥3 toxicity, 3 additional patients will be recruited at the first dose level. If no additional patients experience treatment-related and unexpected grade ≥3 toxicity, escalation to the next dose level will take place. If 2 or more patients at the first dose level experience treatment-related and unexpected grade ≥3 toxicity, at the point that the two toxicities occur, the trial will be suspended and, after a detailed review of the potential causes of toxicity, the DAC may consider recommending changing the cell dose pending DSMB review. If any of the dose level 1 patients experience treatment related death, the trial will be suspended and, after a detailed review of the potential causes of toxicity, consideration given to changing the cell dose or construct pending DSMB review. If the DSMB deems safe to re-initiate the protocol, de-escalation will take place (Figure 6) for the second cohort.

If at any point any patient experiences grade 3 or higher unexpected treatment-related toxicity, that particular patient will no longer receive T cell infusions, atezolizumab or other study related active therapies. They will continue to be followed for response and outcome.

By determination of the DAC and attributed to cellular therapy, evidence of excessive toxicity will be an observed proportion of toxicities for which the associated lower 80% confidence limit exceeds 40%. Operationally, this limit will be met if any of the following proportions is observed: 2/2, 3/3-4, 4/5-6, 5/7-8, 6/9-10, 7/11-12, 8/13-14, 9/15. Under these rules, if the true probability of toxicity is 20%, the probability of suspension is approximately 0.06. If the true probability of toxicity is 60%, the probability of suspension is approximately 0.84 (based on 5,000 simulations).

12.3 Efficacy Assessment

12.3.1 Definition of response

Lesions will be separately tracked but response determined in totality. As indicated, patient must have at least one trackable lesion by response evaluation criteria in solid tumors (RECIST) 1.1. Response will be a defined as best overall response by RECIST 1.1 of complete or partial response.

12.3.2 Definition of success and power of success

Responses to alternative therapies in this population of patients with advanced TNBC/urothelial carcinoma/NSCLC resistant to PD-1/PD-L1 axis blockade are rare, and we therefore will use a 5% response rate as the fixed benchmark upon which we hope to improve with the proposed treatment. If the true response rate with TCR-transduced cells is 25%, 15 patients yields 84% power to detect a statistically significantly improved response rate (one-sided .05) over the fixed rate of 5%. Three or more responses among 16 pts would occur with probability .04 if the true response rate is 5%, so if three or more patients respond (an observed response rate of at least 19%), we will conclude that this regimen is potentially efficacious and worthy of further study.

12.4 Secondary and exploratory analyses

Progression free and overall survival will be estimated using the method of Kaplan and Meier, with time zero the time of first T cell infusion. Given the small size of the trial, many of the secondary and exploratory endpoints are descriptive or hypothesis generating in nature, and are thus underpowered for formal statistical analysis. Standard methods will be used to estimate each of the secondary endpoints.

12.5 Sample Size and Accrual

This study is a phase I, non-blinded interventional trial. Our target sample size is 15-18 patients (8-12 per year/1.5-2 years).

13.0 DATA AND SAFETY MONITORING PLAN

13.1 Overall Scope of Monitoring Activities

Institutional support of trial monitoring will be in accordance with the Fred Hutch/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan (DSMP). Under the provisions of this plan, Fred Hutch Clinical Research Support coordinates data and compliance monitoring conducted by consultants, contract research organizations, or Fred Hutch employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), Fred Hutch Scientific Review Committee (SRC) and the Fred Hutch/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating patients. The IRB reviews the study

progress and safety information to assess continued acceptability of the risk-benefit ratio for human patients. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines. The conduct of this trial will be further monitored by an independent Data and Safety Monitoring Board (DSMB) in accordance with an approved DSMB Charter.

13.2 Monitoring the Progress of Trial and Safety of Participants

The first level of trial oversight for this protocol will be provided by the Principal Investigator, the Research Nurse, and Research Coordinator(s), who will provide continuous oversight of the trial. These individuals will meet at least monthly to review recently acquired data, stopping rules, and adverse events. Serious adverse events will be reviewed upon occurrence to ensure prompt and accurate reporting to the IND Sponsor, appropriate committees, and regulatory agencies as described above. The data recorded in the research charts and protocol database will be compared with the actual data available from the medical record and/or clinical histories. Data detailed in the research case report forms (CRFs) will include the nature and severity of all grade 3-5 adverse events. The Principal Investigator and all other investigators on the protocol have received formal training in the ethical conduct of human research.

The IND Sponsor will ensure routine trial monitoring as described above, and will review Serious Adverse Events and other reports of safety issues promptly upon receipt from the PI.

A DSMB will be in place and will meet after all patients in a given dose level have received treatment (or sooner if requested) to review the data as it relates to AEs and determine whether trial may proceed to phase II dose level. The purpose of the DSMB meetings is to review the conduct of the trial to date and assess safety and toxicity of the study intervention. The DSMB will review all grade 3 or greater NIH CTC v5.0 toxicities and SAEs and determine whether the study should be prematurely discontinued due to excessive toxicity consistent with **Section 13**. Ad hoc meetings may be scheduled as needed.

14.0 DATA MANAGEMENT/CONFIDENTIALITY

The medical record containing information regarding treatment of the patient will be maintained as a confidential document, within the guidelines of the Fred Hutchinson Cancer Research Center, the University of Washington Medical Center, and the Seattle Cancer Care Alliance.

The investigator will ensure that data collected conform to all established guidelines for coding collection, key entry and verification. Each patient is assigned a unique patient number to assure patient confidentiality. Information forwarded to the FDA, NIH, NCI or other agencies about patients on this protocol refers to patients by a coded identifier and not by name. Patients will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the

patient receives medical care, maintains all original inpatient and outpatient chart documents. Additional clinical data may be made available from the Fred Hutch core database which is managed and verified independent of the research group.

The research team will maintain Case Report Forms (CRFs) and associated research documentation for each patient treated under the protocol. This documentation includes both clinical data and study-specific documents for each patient. The Principal Investigator or a designee will verify completed CRFs against source documentation on an ongoing basis as they are completed for individual patients. Data required for analysis of patients treated on this protocol will be maintained in a password-protected study-specific database. Data from the CRFs are keyed directly into the database by authorized research staff and verified on an ongoing basis.

15.0 TERMINATION OF STUDY

The study will terminate after the last treated patient has completed 15 years of follow-up as described in this protocol.

The PI or IND Sponsor may terminate the study at any time. The IRB and FDA also have the authority to terminate the study should it be deemed necessary.

16.0 REFERENCES

1. Schmid P, Adams S, Rugo HS, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. *N Engl J Med.* 2018;379(22):2108-2121.
2. Balar AV, Galsky MD, Rosenberg JE, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet.* 2017;389(10064):67-76.
3. Balar AV, Castellano D, O'Donnell PH, et al. First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. *Lancet Oncol.* 2017;18(11):1483-1492.
4. Powles T, Duran I, van der Heijden MS, et al. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or metastatic urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised controlled trial. *Lancet.* 2018;391(10122):748-757.
5. Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. *N Engl J Med.* 2018;378(24):2288-2301.
6. Rittmeyer A, Barlesi F, Waterkamp D, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet.* 2017;389(10066):255-265.
7. Reck M, Rodriguez-Abreu D, Robinson AG, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N Engl J Med.* 2016;375(19):1823-1833.
8. ASCO Abstract 3044: Augmentation of adoptive T-cell therapy for Merkel cell carcinoma with avelumab. 2017.
9. Mrklic I, Spagnoli GC, Juretic A, Pogorelic Z, Tomic S. Co-expression of cancer testis antigens and topoisomerase 2-alpha in triple negative breast carcinomas. *Acta histochemica.* 2014;116(5):740-746.
10. Gure AO, Chua R, Williamson B, et al. Cancer-testis genes are coordinately expressed and are markers of poor outcome in non-small cell lung cancer. *Clin Cancer Res.* 2005;11(22):8055-8062.
11. Kim YD, Park HR, Song MH, et al. Pattern of cancer/testis antigen expression in lung cancer patients. *Int J Mol Med.* 2012;29(4):656-662.
12. Sharma P, Shen Y, Wen S, et al. Cancer-testis antigens: expression and correlation with survival in human urothelial carcinoma. *Clin Cancer Res.* 2006;12(18):5442-5447.
13. Hofmann O, Caballero OL, Stevenson BJ, et al. Genome-wide analysis of cancer/testis gene expression. *Proc Natl Acad Sci U S A.* 2008;105(51):20422-20427.
14. Obenaus M, Leitao C, Leisegang M, et al. Identification of human T-cell receptors with optimal affinity to cancer antigens using antigen-negative humanized mice. *Nat Biotechnol.* 2015;33(4):402-407.
15. Fijak M, Meinhardt A. The testis in immune privilege. *Immunol Rev.* 2006;213:66-81.
16. Uhlen M, Fagerberg L, Hallstrom BM, et al. Proteomics. Tissue-based map of the human proteome. *Science.* 2015;347(6220):1260419.
17. Uhlen M, Zhang C, Lee S, et al. A pathology atlas of the human cancer transcriptome. *Science.* 2017;357(6352).
18. Morgan RA, Chinnasamy N, Abate-Daga D, et al. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. *J Immunother.* 2013;36(2):133-151.

19. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. *Nature.* 2012;490(7418):61-70.
20. Cancer Genome Atlas Research N. Comprehensive genomic characterization of squamous cell lung cancers. *Nature.* 2012;489(7417):519-525.
21. Cancer Genome Atlas Research N. Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature.* 2014;507(7492):315-322.
22. Pascolo S, Schirle M, Guckel B, et al. A MAGE-A1 HLA-A A*0201 epitope identified by mass spectrometry. *Cancer Res.* 2001;61(10):4072-4077.
23. Specht JM LS, Turtle C, et al. A phase I study of adoptive immunotherapy for ROR1+ advanced triple negative breast cancer (TNBC) with defined subsets of autologous T cells expressing a ROR1-specific chimeric antigen receptor (ROR1-CAR). 2018 San Antonio Breast Cancer Symposium; December 4-8, 2018; San Antonio, Texas.
24. Chapuis AG, Roberts IM, Thompson JA, et al. T-Cell Therapy Using Interleukin-21-Primed Cytotoxic T-Cell Lymphocytes Combined With Cytotoxic T-Cell Lymphocyte Antigen-4 Blockade Results in Long-Term Cell Persistence and Durable Tumor Regression. *J Clin Oncol.* 2016.
25. Chapuis A, Daniel N. Egan, Merav Bar, Thomas M Schmitt, Megan McAfee, Maurizio Perdicchio, Gunnar B. Ragnarsson, Cecilia C.S. Yeung, Hieu Nguyen, Lara Kropp, Luca Castelli, Felecia Wagener, Daniel Hunter, Kristen Cohen, Aaron Seese, Margaret (Julie) McElrath, Kieu-Thu Bui, Deborah E Banker, Natalie Duerkopp and Philip D Greenberg. EBV-Specific Donor Cells Transduced to Express a High-Affinity WT1 TCR Can Prevent Recurrence in Post-HCT Patients with High-Risk AML. *Blood.* 2016;128:1001.
26. Bar M, Chapuis AG, Schmitt TM, et al. Transferred Donor-Derived Virus Specific CD8+T Cells That Have Been Transduced to Express a WT1-Specific T Cell Receptor Can Persist and Provide Anti-Leukemic Activity in AML Patients Post-Transplant. *Blood.* 2014;124(21).
27. Nauerth M, Weissbrich B, Knall R, et al. TCR-ligand koff rate correlates with the protective capacity of antigen-specific CD8+ T cells for adoptive transfer. *Sci Transl Med.* 2013;5(192):192ra187.
28. Stone JD, Kranz DM. Role of T cell receptor affinity in the efficacy and specificity of adoptive T cell therapies. *Frontiers in immunology.* 2013;4:244.
29. Stone JD, Harris DT, Kranz DM. TCR affinity for p/MHC formed by tumor antigens that are self-proteins: impact on efficacy and toxicity. *Curr Opin Immunol.* 2015;33:16-22.
30. Hebeisen M, Allard M, Gannon PO, Schmidt J, Speiser DE, Rufer N. Identifying Individual T Cell Receptors of Optimal Avidity for Tumor Antigens. *Frontiers in immunology.* 2015;6:582.
31. Linette GP, Stadtmauer EA, Maus MV, et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. *Blood.* 2013;122(6):863-871.
32. Stromnes IM, Schmitt TM, Chapuis AG, Hingorani SR, Greenberg PD. Re-adapting T cells for cancer therapy: from mouse models to clinical trials. *Immunological reviews.* 2014;257(1):145-164.
33. Greenberg PD, Cheever MA, Fefer A. Eradication of disseminated murine leukemia by chemoimmunotherapy with cyclophosphamide and adoptively transferred immune syngeneic Lyt-1+2- lymphocytes. *The Journal of experimental medicine.* 1981;154(3):952-963.
34. Sommermeyer D, Hudecek M, Kosasih PL, et al. Chimeric antigen receptor-modified T cells derived from defined CD8+ and CD4+ subsets confer superior antitumor reactivity in vivo. *Leukemia.* 2016;30(2):492-500.

35. Turtle CJ, Hanafi LA, Berger C, et al. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. *The Journal of clinical investigation.* 2016;126(6):2123-2138.
36. Swain SL, McKinstry KK, Strutt TM. Expanding roles for CD4(+) T cells in immunity to viruses. *Nature reviews Immunology.* 2012;12(2):136-148.
37. Soto CM, Stone JD, Chervin AS, et al. MHC-class I-restricted CD4 T cells: a nanomolar affinity TCR has improved anti-tumor efficacy in vivo compared to the micromolar wild-type TCR. *Cancer immunology, immunotherapy : CII.* 2013;62(2):359-369.
38. Hunder NN, Wallen H, Cao J, et al. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. *N Engl J Med.* 2008;358(25):2698-2703.
39. Tran E, Turcotte S, Gros A, et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. *Science.* 2014;344(6184):641-645.
40. Thibodeau J, Bourgeois-Daigneault MC, Lapointe R. Targeting the MHC Class II antigen presentation pathway in cancer immunotherapy. *Oncoimmunology.* 2012;1(6):908-916.
41. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell.* 2017;168(4):707-723.
42. Gattinoni L, Lugli E, Ji Y, et al. A human memory T cell subset with stem cell-like properties. *Nat Med.* 2011;17(10):1290-1297.
43. Berger C, Jensen MC, Lansdorp PM, Gough M, Elliott C, Riddell SR. Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates. *J Clin Invest.* 2008;118(1):294-305.
44. Wherry EJ, Ahmed R. Memory CD8 T-cell differentiation during viral infection. *J Virol.* 2004;78(11):5535-5545.
45. Rossi M, Botta C, Arbitrio M, Grembiale RD, Tagliaferri P, Tassone P. Mouse models of multiple myeloma: technologic platforms and perspectives. *Oncotarget.* 2018;9(28):20119-20133.
46. Andreatta M, Nielsen M. Gapped sequence alignment using artificial neural networks: application to the MHC class I system. *Bioinformatics.* 2016;32(4):511-517.
47. Ghandi M, Huang FW, Jane-Valbuena J, et al. Next-generation characterization of the Cancer Cell Line Encyclopedia. *Nature.* 2019;569(7757):503-508.
48. Qasim W, Gaspar HB, Thrasher AJ. Progress and prospects: gene therapy for inherited immunodeficiencies. *Gene Ther.* 2009;16(11):1285-1291.
49. Jones S, Peng PD, Yang S, et al. Lentiviral vector design for optimal T cell receptor gene expression in the transduction of peripheral blood lymphocytes and tumor-infiltrating lymphocytes. *Hum Gene Ther.* 2009;20(6):630-640.
50. Scholler J, Brady TL, Binder-Scholl G, et al. Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. *Science translational medicine.* 2012;4(132):132ra153.
51. Robbins PF, Morgan RA, Feldman SA, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J Clin Oncol.* 2011;29(7):917-924.
52. Maruggi G, Porcellini S, Facchini G, et al. Transcriptional enhancers induce insertional gene deregulation independently from the vector type and design. *Mol Ther.* 2009;17(5):851-856.
53. Recchia A, Bonini C, Magnani Z, et al. Retroviral vector integration deregulates gene expression but has no consequence on the biology and function of transplanted T cells. *Proc Natl Acad Sci U S A.* 2006;103(5):1457-1462.

54. Baum C. Insertional mutagenesis in gene therapy and stem cell biology. *Curr Opin Hematol.* 2007;14(4):337-342.
55. Montini E, Cesana D, Schmidt M, et al. The genotoxic potential of retroviral vectors is strongly modulated by vector design and integration site selection in a mouse model of HSC gene therapy. *The Journal of clinical investigation.* 2009;119(4):964-975.
56. June CH, Sadelain M. Chimeric Antigen Receptor Therapy. *N Engl J Med.* 2018;379(1):64-73.
57. Wherry EJ, Blattman JN, Murali-Krishna K, van der Most R, Ahmed R. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. *J Virol.* 2003;77(8):4911-4927.
58. Lugli E, Dominguez MH, Gattinoni L, et al. Superior T memory stem cell persistence supports long-lived T cell memory. *The Journal of clinical investigation.* 2013;123(2):594-599.
59. Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science.* 2002;298(5594):850-854.
60. Dudley ME, Wunderlich JR, Yang JC, et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol.* 2005;23(10):2346-2357.
61. Dudley ME, Yang JC, Sherry R, et al. Adoptive Cell Therapy for Patients With Metastatic Melanoma: Evaluation of Intensive Myeloablative Chemoradiation Preparative Regimens. *J Clin Oncol.* 2008.
62. Wrzesinski C, Paulos CM, Gattinoni L, et al. Hematopoietic stem cells promote the expansion and function of adoptively transferred antitumor CD8 T cells. *J Clin Invest.* 2007;117(2):492-501.
63. Kochenderfer JN, Dudley ME, Kassim S, et al. Chemotherapy-Refractory Diffuse Large B-Cell Lymphoma and Indolent B-Cell Malignancies Can Be Effectively Treated With Autologous T Cells Expressing an Anti-CD19 Chimeric Antigen Receptor. *Journal of Clinical Oncology.* 2015;33(6):540-549.
64. Kochenderfer JN, Kassim SH, Somerville R, et al. Treatment of Chemotherapy-Refractory B-Cell Malignancies with Anti-CD19 Chimeric Antigen Receptor T Cells. Presented at the Annual Meeting of the American Society of Gene and Cell Therapy; 2014; New Orleans, LA.
65. Turtle CJ, Hanafi LA, Berger C, et al. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8+ and CD4+ CD19-specific chimeric antigen receptor-modified T cells. *Science translational medicine.* 2016;8(355):355ra116.
66. Fry TJ. CD22 CAR Update and Novel Mechanisms of Leukemic Resistance. Paper presented at: Annual Meeting of the American Society of Clinical Oncology2016; Chicago, IL.
67. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood.* 2014;124(2):188-195.
68. Coiffier B, Altman A, Pui CH, Younes A, Cairo MS. Guidelines for the management of pediatric and adult tumor lysis syndrome: an evidence-based review. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2008;26(16):2767-2778.
69. Howard SC, Jones DP, Pui CH. The tumor lysis syndrome. *The New England journal of medicine.* 2011;364(19):1844-1854.
70. Schulert GS, Grom AA. Pathogenesis of Macrophage Activation Syndrome and Potential for Cytokine-Directed Therapies. *Annual Review of Medicine.* 2015;66:145-159.
71. Weber JS, Kahler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. *J Clin Oncol.* 2012;30(21):2691-2697.

72. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin Cancer Res.* 2009;15(23):7412-7420.
73. Brahmer JR, Lacchetti C, Thompson JA. Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline Summary. *J Oncol Pract.* 2018;14(4):247-249.

APPENDIX A: Schedule of Evaluations

Assessment	Screening	Pretreatment Evaluation	Lymphodepleting Chemotherapy (Day -4 to -2)	T cell Infusion (Day 0)	1	3	7	14	21	28	*see Note
Window		-30 days of leuka		Up to 7 days post-LD chemo	+/- 1 d	+/- 1 d	+/- 1 d	+/- 3 d	+/- 3 d	+/- 3 d	Day 60 +/- 14 days, Day 90-365 +/- 30 days
Informed consent/HIPAA	X	X									
HLA typing	X[a]										
MAGE A1 evaluation	X										
I/E criteria		X									
Medical history[a]		X			X		X	X	X	X	
Physical exam		X	X	X	X		X	X	X	X	
Height/weight[b]		X		X							
ECOG performance status		X	X	X							
Pregnancy test		X		X[g]							
Vitals including O_2 sat				X[c]							
Brain imaging[h]		X									
12-lead ECG		X									
MUGA/ECHO[d]		X									

Assessment	Screening	Pretreatment Evaluation	Lymphodepleting Chemotherapy (Day -4 to -2)	T cell Infusion (Day 0)	1	3	7	14	21	28	*see Note
Window		-30 days of leuka		Up to 7 days post-LD chemo	+/- 1 d	+/- 1 d	+/- 1 d	+/- 3 d	+/- 3 d	+/- 3 d	Day 60 +/- 14 days, Day 90-365 +/- 30 days
CT/PET scan[i]		X									Performed at the following timepoints if clinically indicated or suspicion of progression: • Approximately 1 month after each T cell infusion • Approximately 3 months after each T cell infusion • Approximately 6 months after the last T cell infusion • Approximately 9 months after the last T cell infusion • Approximately 12 months after the last T cell infusion Before the patient receives additional antitumor therapy
Tumor biopsy (in subjects with accessible disease)		X						X	Performed at week 12 after each infusion then as clinically indicated.,		
Leukapheresis		X									
Lymphodepleting chemotherapy			X								
T cell admin				X							
AEs		X	X	X	X	X	X	X	X	X	X
Neurological exam		X									

Assessment	Screening	Pretreatment Evaluation	Lymphodepleting Chemotherapy (Day -4 to -2)	T cell Infusion (Day 0)	1	3	7	14	21	28	*see Note
Window		-30 days of leuka		Up to 7 days post-LD chemo	+/- 1 d	+/- 1 d	+/- 1 d	+/- 3 d	+/- 3 d	+/- 3 d	Day 60 +/- 14 days, Day 90-365 +/- 30 days
CBC, differential, platelet count		X	X	X	X	X	X	X	X	X	Concurrent with restaging as clinically indicated Concurrent with T-cell persistence evaluation
Renal, hepatic function with LDH, Mg		X	X	X	X	X	X	X	X	X	Concurrent with restaging as clinically indicated
Uric acid		X		X	X	X	X				
Serum ferritin		X									As clinically indicated
CRP		X									As clinically indicated
IL-6		X									As clinically indicated
DIC panel without platelets		X									As clinically indicated
Quantitative IgG[f]		X									Approximately monthly
Virology panel		X									
ABO blood typing; antibody screen		X									
G6PD screening		X									
T cell persistence		X	X		X	X	X	X	X	X	Approx. 6 weeks and 2, 3, 6, 9, and 12 months after the T cell infusion and at the time of tumor relapse
T cell cellular immune response		X								X	Approx 2, 3, 6, 9 and 12 months after the T cell infusion

Assessment	Screening	Pretreatment Evaluation	Lymphodepleting Chemotherapy (Day -4 to -2)	T cell Infusion (Day 0)	1	3	7	14	21	28	*see Note
Window		-30 days of leuka		Up to 7 days post-LD chemo	+/- 1 d	+/- 1 d	+/- 1 d	+/- 3 d	+/- 3 d	+/- 3 d	Day 60 +/- 14 days, Day 90-365 +/- 30 days
RCL testing by VSVG qPCR		X									Approximately 3, 6, and 12 months after the last T cell infusion
Serum archive		X	X		X	X	X	X	X	X	Approximately at 3, 6, 9 and 12 months after the last T cell infusion
Citrate plasma archive		X	X		X	X	X	X	X	X	Approximately at 3, 6, 9 and 12 months after the last T cell infusion
Tumor Biopsy		X						X			Prior to enrollment, 2 weeks after and 12 weeks after the first T cell infusion.

*NOTE: Protocol time points represent guidelines for performance of required evaluations. Due to numerous factors influencing scheduling (subject and provider availability, testing services limitations, etc.), variation in evaluation performance dates is anticipated and acceptable to the protocol (i.e., within ± 4 days of time points < day 30; ± 30 days for timepoints > day 30. Evaluations do not need to be repeated if results from previous test are available and within protocol window

[a] Medical history to include hematologic, cytogenetic, flow cytometric, and histologic findings at diagnosis and time of enrollment as well as prior therapies and response to therapy.

[b] Height required only at Pretreatment Evaluation.

[c] Vital signs and O_2 saturation being recorded before, approximately **every 15 minutes during**, at completion of the infusion, and hourly after the infusion for at least 2 hours after infusion.

[d] ECHO required if patients ≥60 years of age within 60 days of enrollment.

[e] Diagnostic CT/PET scan to include neck chest, abdomen and pelvis, as clinically indicated by disease.

[f] In patient who receive lymphodepleting chemotherapy, quantitative IgG to be measured approximately monthly until levels are normal without IVIG replacement. If levels are still abnormal at the end of follow-up, IgG should continue to be assessed by the treating physician.

[g] Pregnancy test within 72 hours of cell infusion

[h] If history of brain metastasis or neurologic symptoms
[i] Repeat imaging if not within 45 days of infusion day

APPENDIX B: Research Sample Checklist

Please note that many of the listed research studies are optional pending clinical course, and may be replaced by newer or alternate assays that answer the same general question. Note that the selected studies will be batched and results are not anticipated to be available in "real-time". Leftover materials from all time points may be archived.

RECIPIENT RESEARCH EVALUATIONS BEFORE T CELL INFUSION

SAMPLE	TIME	TEST	TUBE	VOL. (Approx)	LAB
Blood	At Enrollment	T cell persistence and functional studies, epitope spreading, scRNAseq, clonality	Lavender top	60 mL	Research Cell Bank (RCB)
Blood	At Enrollment	Cytokine levels	Serum separator	20 ml	RCB
Blood	At Enrollment	RCL Testing	Lavender top	10 ml	RCB
Tumor	At Enrollment	Single cell RNA sequencing	RPMI	3 cores	Chapuis D3-235
Tumor	At Enrollment	T cell localization and tumor microenvironment characteristics	Formalin	1 core; in addition to clinical core	Chapuis D3-235
Blood	Within 2 days prior to LD (or cell infusion if not receiving LD)	Cytokine levels (research)	Serum separator	10 ml	RCB
Blood	Within 2 days prior to LD (or cell infusion if not receiving LD)	T cell baseline persistence, function	Lavender top	30 ml	RCB

RECIPIENT RESEARCH EVALUATIONS AFTER T CELL INFUSION

SAMPLE	TIME	TEST	TUBE	VOL. (Approx)	LAB
Blood	Day 1	Cytokine levels (research)	Serum separator	20 ml	RCB
Blood	Day 1	T cell persistence, phenotype	Lavender top	60 ml	RCB
Blood	Day 3	Cytokine levels (research)	Serum separator	20 ml	RCB
Blood	Day 3	T cell persistence	Lavender top	60 ml	RCB
Blood	Day 7	Cytokine levels (research)	Serum separator	20 ml	RCB
Blood	Day 7	T cell persistence	Lavender top)	60 ml	RCB
Blood	Day 14	Cytokine levels (research)	Serum separator	20 ml	RCB
Blood	Day 14	T cell persistence and functional studies, epitope spreading, scRNAseq, clonality	Lavender top	60 ml	RCB
Blood	Day 21	Cytokine levels (research)	Serum separator	20 ml	RCB
Blood	Day 21	T cell persistence	Lavender top	60 ml	RCB
Blood	Day 28	Cytokine levels (research)	Serum separator	20 ml	RCB
Blood	Day 28	T cell persistence, immunophenotype	Lavender top	60 ml	RCB
Blood	Day 56	Cytokine levels (research)	Serum separator	20 ml	RCB
Blood	Day 56	T cell persistence, immunophenotype	Lavender top	60 ml	RCB
Blood	Day 84	T cell persistence, epitope spreading, scRNAseq, immunophenotype, clonality, RCL	Lavender top	60 ml	RCB
Blood	Day 84	Cytokines, research serologies	Serum separator	20 ml	RCB
If second infusion, repeat pattern above/please refer to study schedule					

SAMPLE	TIME	TEST	TUBE	VOL. (Approx)	LAB
Blood	Day 180	T cell persistence, epitope spreading, immunophenotype, clonality, RCL	Lavender top	60 ml	RCB
Blood	Day 180	Cytokines, research serologies	Serum separator	20 ml	RCB
Blood	Day 270	T cell persistence, epitope spreading, immunophenotype, clonality, RCL	Lavender top	60 ml	RCB
Blood	Day 270	Cytokines, research serologies	Serum separator	20 ml	RCB
Blood	Day 365	T cell persistence, epitope spreading, immunophenotype, clonality, RCL	Lavender top	60 ml	RCB
Blood	Day 365	Cytokines, research serologies	Serum separator	20 ml	RCB
Blood	Annual years 2-15	T cell persistence, RCL	Lavender top	20 ml	RCB
Blood	Clinical events	T cell persistence, epitope spreading, immunophenotype, clonality, RCL, scRNAseq (all optional/TBD)	Lavender top	Up to 60 ml	Discuss with study staff
Blood	Clinical events	Cytokine levels	Serum separator	20 mL	Discuss with study staff
Tumor	Day + 14	Single cell RNA sequencing	RPMI	3 cores or equiv	Chapuis D3-235
Tumor	Day + 14	T cell localization and tumor microenvironment characteristics	Formalin	1 core or equiv	Chapuis D3-235
Tumor	Day +14	Archive	RPMI	Any add'l tissue	Chapuis D3-235
Tumor	Day + 84	Single cell RNA sequencing	RPMI	3 cores or equiv	Chapuis D3-235

SAMPLE	TIME	TEST	TUBE	VOL. (Approx)	LAB
Tumor	Day + 84	T cell localization and tumor microenvironment characteristics	Formalin	1 core or equiv; in addition to clinical core(s)	Chapuis D3-235
Tumor	Day + 84	Archive	RPMI	Any add'l tissue	Chapuis D3-235
Tumor	Clinical event	Single cell RNA sequencing	RPMI	3 cores	Chapuis D3-235
Tumor	Clinical event	T cell localization and tumor microenvironment characteristics	Formalin	1 core; in addition to clinical core	Chapuis D3-235

APPENDIX C: KARNOFSKY PERFORMANCE STATUS SCALE

KARNOFSKY PERFORMANCE STATUS SCALE

General	Index	Specific Criteria
Able to carry on normal activity; no special care needed	100	Normal, no complaints, no evidence of disease
	90	Able to carry on normal activity, minor signs or symptoms of disease
	80	Normal activity with effort, some signs or symptoms of disease
Unable to work, able to live at home and care for most personal needs, varying amount of assistance needed	70	Care for self, unable to carry on normal activity or to do work
	60	Requires occasional assistance from others but able to care for most needs
	50	Requires considerable assistance from others and frequent medical care
Unable to care for self, requires institutional or hospital care or equivalent; disease may be rapidly progressing	40	Disabled; requires special care and assistance
	30	Severely disabled, hospitalization indicated, death not imminent
	20	Very sick, hospitalization necessary, active supportive treatment necessary
	10	Moribund
	0	Dead

APPENDIX D: ECOG Performance Status Scale

ECOG Performance Status Scale

GRADE	SCALE
0	Fully active, able to carry out all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead

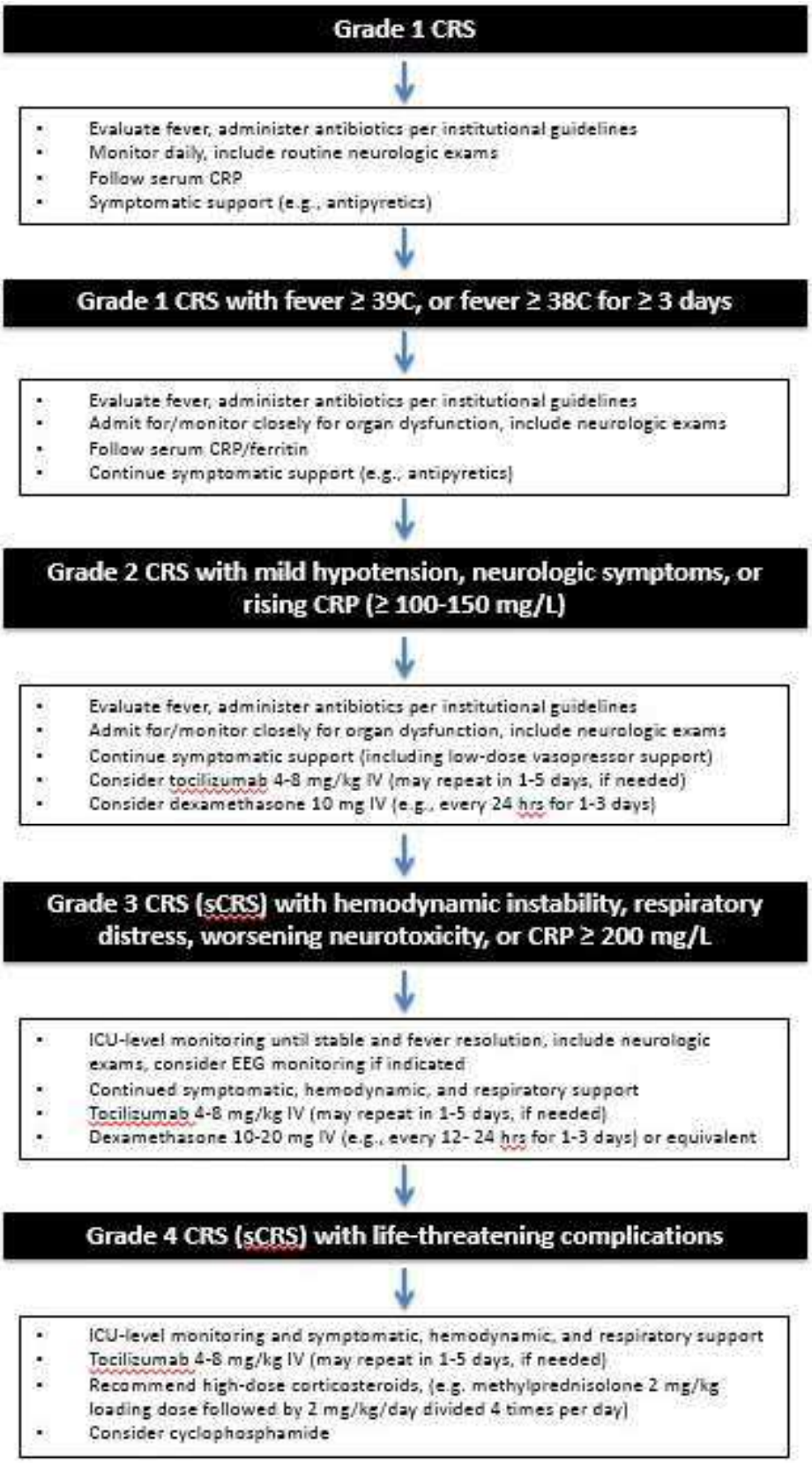
APPENDIX E: CRS Grading Criteria (CTCAE v. 5.0)

Definition: A disorder characterized by fever, tachypnea, headache, tachycardia, hypotension, rash, and/or hypoxia caused by the release of cytokines.

Grade	Description of Symptoms
1: Mild	Fever with or without constitutional symptoms
2: Moderate	Require and respond to moderate intervention: • Oxygen requirement < 40%, or • Hypotension responsive to fluids,
3: Severe	Require and respond to aggressive intervention: • Oxygen requirement ≥ 40% O2, or • Hypotension requiring a single vasopressor
4: Life-threatening	Life-threatening; urgent intervention indicated
5: Fatal	Death

[a] Organ toxicity excludes neurotoxicity, which will be evaluated separately

APPENDIX F: Recommended Management of CRS



ASTCT CRS Consensus Grading

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

Organ toxicities associated with CRS may be graded according to CTCAE v5.0 but they do not influence CRS grading.

*Fever is defined as temperature ≥38°C not attributable to any other cause. In patients who have CRS then receive antipyretic or anticytokine 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.

†CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a patient with temperature of 39.5°C, hypotension requiring 1 vasopressor, and hypoxia requiring low-flow nasal cannula is classified as grade 3 CRS.

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

ASTCT ICANS Consensus Grading for Adults

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE Score*	7-9	3-6	0-2	0 (patient is unarousable and unable to perform ICE)
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
Depressed Level of Consciousness †	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 without intervention.	Life-threatening prolonged seizure (>5 min); or repetitive clinical or electrical seizures without return to baseline in between.
Motor Findings‡	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis
Elevated ICP/Cerebral Edema§	N/A	N/A	Focal/local edema on neuroimaging	Diffuse Cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad

ICANS grade is determined by the most severe event (ICE score, level of consciousness, seizure, motor findings, raised 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 grade 3 ICANS.

N/A indicates not applicable

* A patient with an ICE score of 0 may be classified as grade 3 ICANS if awake with global aphasia, but a patient with an ICE score of 0 may be classified as grade 4 ICANS If unarousable.
†Depressed level of consciousness should be attributable to no other cause (e.g., No sedating medications)
‡ Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading.
§ 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 v 5.0

APPENDIX G: Long Term Follow-Up

Study participants should be asked to participate in long term follow-up, as directed by the FDA Guidance for Industry – Gene Therapy Clinical Trials: Observing Subjects for Delayed Adverse Events. (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm072957.htm#5).

Long term follow-up should commence one year after the final T cell infusion. The planned recommendations for follow-up are as follows:

Years 1 - 15:

1. Recommendation that patients undergo at least annual history and physical examination with their primary physician:
    - Adverse event screening guidance for the primary physician in the form of a gene therapy LTFU-directed screening survey may be available.
    - A request for the study team to be notified of all new malignancies and unexpected illnesses.
    - The primary physician may be provided with a blood draw courier kit to enable samples to be returned to the Chapuis Lab for archival purposes, and for analysis for transgene and vector persistence, and RCL, as dictated by studies of transferred T cell persistence.
    - If the patient develops a late recurrence, request for up to 50 cc of research blood as well as leftover or archival biopsy/surgical tissue, in order to evaluate for mechanisms of late recurrence (may be collected remotely with a courier kit)

2. Annual phone call survey or questionnaire to the participant to screen for adverse events.

3. Offer the opportunity to return to Fred Hutch for an annual LTFU clinic visit.

4. Compliance with 21 CFR 312.32 in adverse event reporting.

5. Research bloodwork: 30 cc of blood annually for persistence and RCL monitoring

APPENDIX H: DOSE ASSESSMENT COMMITTEE

The Dose Assessment Committee (DAC) will be comprised of investigators directly involved in the study and therefore most knowledgeable regarding balancing safety and efficacy. Members will include:

Michael Schweizer, MD (Chair)
Aude Chapuis, MD, PhD (co-Chair)
William Rayford Gwin III, MD
Sylvia Lee, MD
Ted Gooley, PhD (Biostatistician)

Cellular therapies may not function in dose dependent manner and unique escalation/de-escalation are required to optimally select safe and effective doses. In the event a particular dose is consistently effective or has a substantial number of minor toxicities that do not individually trigger de-escalation, a particular dose in the algorithm may be explored further with additional patients. In such an event, the DAC may override the overall algorithm.

APPENDIX I: MAGE-A1-SPECIFIC IMMUNOHISTOCHEMISTRY (IHC) ASSAY

1. Description of the assay

The MAGE-A1 selection assay is a standard single-color chromogenic immunohistochemistry assay developed for use on formalin-fixed paraffin-embedded tissue. Monoclonal antibody MA454 is specific to MAGE-A1 and is detected with an anti-mouse polymer conjugated to horse radish peroxidase. DAB chromogen creates a brown pigment visible using standard brightfield microscopy. Results are provided via interpretation by a board-certified pathologist.

2. Application of assay results to the clinical trial

MAGE-A1-specific IHC will be used as one of the subject eligibility criteria for treatment with engineered autologous T cell therapy, FH-MagIC TCR-T. FH-MagIC TCR-T is specific to a Class I HLA A*0201-restricted MAGE-A1 epitope. The rationale is that the engineered autologous T cell product will target tumor cells with MAGE-A1 antigen to be present before TCR will be able to deliver immunomodulating therapeutic effects.

A positive MAGE-A1 IHC result is required for enrollment. A positive result is any tumor with >10%, 1+ or higher staining intensity, membranous, nuclear or cytoplasmic expression of MAGE-A1.

3. Description of the population and information regarding what is known about the prevalence of the biomarker being evaluated in the patient population

MAGE-A1 plays a role in transcriptional control of cancers and have been shown to inhibit the activity of tumor suppressor microRNA Let7a. Overexpression of this protein has been noted in multiple solid tumors and have become an attractive therapeutic target. The MAGE-A1 IHC assay detects expression of MAGE-A1 protein on cells in the tumor tissue, a pathologist will interpret if the expression of the protein is seen in tumor cells, stromal cells, or inflammatory cells and if the protein is likely membranous, cytoplasmic, and or nuclear.

4. Specimen type(s) to be collected for investigational IVD testing, including the anatomical site

MAGE-A1 IHC selection assay will be performed on archival FFPE tumor specimens. Biopsies will not be conducted exclusively for patient selection testing.

5. Risk Analysis

Aside from immune-privileged testes, MAGE-A1 is undetectable in most normal tissues. MAGE-A1 IHC shows 93% specificity, 69% sensitivity, and 100% precision (reproducibility) when calculated from the validation sample set. This represents a low likelihood of false positive test results, and a 31% possibility of false negative results. Since this assay will be used for patient

selection in a safety phase clinical trial with unknown efficacy, there is minimal risk associated with a false negative test result.