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ATTAMAGE-A1.: Phase I/II study of Autologous CD8+ and CD4+ Transgenic T cells expressing high affinity MAGE-A1-specific T-Cell Receptor (TCR) combined with Atezolizumab in patients with metastatic MAGE-A1 expressing cancer

Current date: 12/28/2021
Previous date: 08/25/2021

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IND number:	027041
Investigational Agent:	FH-MagIC TCR-T: Autologous CD8+ and CD4+ T cells transduced with transgenic TCR-MagIC
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Protocol Synopsis

Protocol Number: RG1007463	**Product Names:** FH-MagIC TCR-T
Title of Study: *ATTAMAGE-A1: Phase I/II study of Autologous CD8+ and CD4+ Transgenic T cells expressing high affinity MAGE-A1-specific T-Cell Receptor (TCR) combined with Atezolizumab in patients with metastatic MAGE-A1 expressing cancer*	
Sponsor: Fred Hutch Cancer Research Center	**Phase of Development:** I
IND Number: 027041	

Study Endpoints:

Primary: Safety, Efficacy (Response Rate by RECIST criteria)

Secondary: Progression-free survival, overall survival at 1 year

Exploratory: T cell persistence, T cell phenotype, T cell function, T cell localization into tumor, epitope spreading of T cell responses

Study Purpose and Rationale:

Metastatic triple negative breast cancer (TNBC), urothelial, and non-small cell lung cancer (NSCLC) are solid tumor malignancies with abysmal survival rates following progression on initial therapies. There are currently few consistently effective treatments for individuals with these cancers following treatment with PD-L1 axis blockade. We have developed an engineered autologous T cell therapy targeting cancer testes antigen MAGE-A1. In a phase I trial, we seek to determine the safety and potential efficacy of this T cell therapy in addition to an FDA approved therapeutic standard anti-PD-L1 therapeutic (e.g., atezolizumab) for patients with advanced triple negative breast cancer, urothelial, or non-small cell lung cancers whose tumors express high level of MAGE-A1 and have become resistant to PD-L1 axis treatments.

Study Population:

Inclusion Criteria:

1. Patient must have a confirmed diagnosis of metastatic or advanced inoperable disease of one of the following disease types:
    a. triple negative breast cancer
    b. urothelial carcinoma
    c. non-small cell lung cancer
2. Tumor tissue staining positive by immunohistochemistry for MAGE-A1 antigen (see **Appendix I**)
3. Patient must have measurable disease by RECIST 1.1 criteria.
4. Patients with NSCLC who have actionable somatic mutations or alterations in EGFR, ROS1 and ALK with available FDA-approved drug therapy options will be eligible for study after prior treatment with targeted therapies for those mutations have been offered or received. Patients with urothelial carcinoma who are candidates for enfortumab vedotin-ejfv will be eligible for study after prior treatment with enfortumab vedotin-ejfv has been offered or received.
5. Patients will be eligible for study after having been offered or received a PD-1 axis inhibitor. If received, they must have either developed progression or still have detectable disease and not have developed CTCAE grade 3 or higher toxicity while on treatment.
6. Patient must be HLA type HLA-A*02:01.
7. Patient must be 18 years of age or older.
8. Patient must be capable of understanding and providing a written informed consent.
9. Patient must be willing to comply with reproductive requirements.

10. ECOG performance status of 0, 1, or 2.
11. Tumor tissue amenable to safe biopsy and subject willing to undergo serial tumor biopsies:
    a. Should there be no tumor tissue that is accessible for biopsy, patients will still be considered for participation, at discretion of the sponsor and in consultation with the investigator. Similarly, should an investigator determine that a biopsy cannot be performed safely for clinical reasons biopsies may be cancelled or retimed after confirming plan with the sponsor.
12. Patients must be at least three weeks from last systemic treatment for metastatic disease.
13. Patient must have acceptable organ function.

Exclusion Criteria:

1. Patients who are HLA type B*49:01
2. Women who are pregnant or breastfeeding.
3. Patients with active autoimmune disease requiring immunosuppressive therapy.
4. Participants who have had a solid organ transplant (with the exception of kidney transplants, which will be considered on a case-by-case basis), or who have a history of allogeneic stem cell transplant.
5. Patients who receive corticosteroid therapy at a dose equivalent of >0.5 mg/kg prednisone per day.
6. Concurrent use of other investigational anti-cancer agents.
7. Patients with CLL or other hematologic malignancy.
8. Patients with an active uncontrolled infection.
9. Patients with an uncontrolled concurrent illness.
10. Patients with untreated brain metastases.
11. For patients in Phase 1/2, Grade 3 or higher immune-mediated toxicity to any prior PDL1 axis blocking agent
12. Participants receiving ongoing treatment for prior serious immune related adverse events.
13. Patients with significant underlying neurologic disease.
14. Other medical, social, or psychiatric factor that interferes with medical appropriateness and/or ability to comply with study, as determined by PI.

Test Product, Dose, and Mode of Administration:

FH-MagIC TCR-T: Autologous CD8+ and CD4+ T cells transduced with transgenic TCR-MagIC

Safety Assessments: Ongoing and continuous; with evaluation for transition to next dose level after three patients have been treated with lymphodepleting chemotherapy followed by FH-MagIC TCR-T.

DLT Definition:

DLTs will be assessed for 14 days following the 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
Efficacy Assessments: At completion of study
Statistical Methods: Phase I interventional trial, not masked, dose-escalation. Safety assessment by CTCAE version 5.0 with a goal treatment related unexpected toxicity rate of <35%; likelihood of stoppage if true rate 20% is 0.06. Response determination by RECIST 1.1 with goal response rate statistically >5%; power to detect this if true response rate is 20% is 0.84. All secondary and exploratory endpoints exploratory.
Sample size: 15-18 participants

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1.0 BACKGROUND

This document is a clinical research protocol and the described study will be conducted in compliance with the IRB-approved protocol.

Most patients with metastatic solid tumors will succumb to those cancers. In this protocol, the focus is on three of the most deadly and common metastatic solid tumors: triple negative breast cancer, urothelial and non-small cell lung cancer.

Triple negative breast cancer (TNBC), a subtype of breast cancer not expressing the estrogen and progesterone receptors or over-expressing the HER2 receptor, is considered one of the most virulent forms of breast cancer. Once metastatic, de novo or relapsed, survivability is rare. In women, TNBC alone is responsible for 10% of all cancer deaths in the United States. Historically, first line therapy for metastatic disease has commonly been single agent chemotherapy and, upon progression, additional chemotherapy agents. Recently, a small subset (<10%) of patients with metastatic TNBC who harbor germline mutated homologous recombination repair genes (BRCA1 or BRCA2) have been shown to have improvement in progression-free survival with PARP inhibitor therapy but without an overall survival advantage. Following FDA approval in early 2019, a first line checkpoint immunotherapy (CPI) option targeting the PD-1/PD-L1 axis has emerged as the preferred treatment approach for newly diagnosed metastatic TNBC as evaluated in the IMpassion130 trial.[1] Available to the approximately 60% subset of TNBC patients whose cancers express the PD-L1 receptor on >1% of the immune component of the tumor, this treatment utilizes atezolizumab (anti-PD-L1 monoclonal antibody) in combination with nab-paclitaxel chemotherapy. Though the overall survival data are immature from this trial, there is a statistically significant improvement in patients on combination CPI + chemotherapy in patients expressing PD-L1 on the immune component of the tumor. However, upon progression at a median of 7.5 months from PD-L1 targeted therapy, patients are primarily again left with single agent chemotherapies as the primary treatment option and most patients are likely to succumb quickly with a median overall survival of 25 months. Therefore, with no other targeted agents presently available for the substantial majority of patients, metastatic TNBC remains a solid tumor that represents a high unmet need for innovative and new treatments.

Metastatic or advanced urothelial cancer, much like metastatic TNBC, is approached commonly with immunotherapy. Atezolizumab and pembrolizumab (a PD-1 inhibitory monoclonal antibody) have both been FDA-approved for treatment of urothelial cancer based on phase II studies for patients who are ineligible for platinum therapy in the first-line setting based on the IMvigor 210 trial.[2,3] In this study, patients historically with very poor survival on multiagent chemotherapy (13-16 months) were reported to have an overall survival of 16 months on atezolizumab and notable regardless of PD-1/PD-L1 expression. In patients who are eligible for platinum-based chemotherapy in the first-line setting, upon progression, CPI (nivolumab, pembrolizumab, durvalumab, atezolizumab, and avelumab) are available as treatment with FDA approvals. Only pembrolizumab and atezolizumab are approved based on Phase III clinical trial data. Single agent atezolizumab was compared to single agent chemotherapy of investigator's choice in the IMvigor 211 trial[4] and demonstrated a median overall survival of 11 months and not statistically significantly better than standard chemotherapy in the patients whose cancers intermediately or strongly expressed PD-L1 on the immune component of the tumor. However,

duration of response and tolerability were significantly better in the atezolizumab-treated patients. The other single-agent CPI similarly demonstrated median survivals in the range of 11-13 months.

In patients with NSCLC, treatment with CPI is available in a number of clinical contexts with several agents having achieved FDA approval. In chemotherapy-naïve patients without actionable driver mutations (ALK, ROS-1, EGFR, RAS/MEK, HER2, etc.) with PD-L1 expression below 50%, addition of pembrolizumab to standard chemotherapy or atezolizumab to standard chemotherapy+/-bevacizumab are common clinical options. Focusing on atezolizumab, when added to chemotherapy in first line non-driver mutation patients with non-squamous biology, the median PFS is 8.3 months and OS is 19 months as demonstrated in the Impower 150 randomized phase III trial.[5] In patients with non-squamous NSCLC without driver mutations who have not received first-line immunotherapy, a group of patients numerically rare now with access to CPI early in care, nivolumab, pembrolizumab and atezolizumab are all FDA approved as single agent treatments. With regards to atezolizumab, patients unselected for PD-L1 achieved a 14-month OS in a randomized phase III trial compared to single agent docetaxel chemotherapy (OAK trial).[6] Nivolumab and pembrolizumab in this clinical context are reported to have OS between 9-13 months depending on specific clinical context. In patients with strongly expressing PD-L1 >50% (about 1/3 of all patients with metastatic NSCLC), single agent pembrolizumab provides a median OS of 30 months in the KEYNOTE-024 phase III clinical trial compared to 14 months with platinum doublet chemotherapy.[7] Summarizing the experience of immunotherapy in NSCLC, median overall survivals remain low in most contexts with most patients succumbing quickly to therapy. Of note, tolerability in every study was comparable when comparing to chemotherapy alone regimens. Furthermore, when comparing single agent CPI to chemotherapy options, CPI was better tolerated in all studies.

There are no prior treatment protocols for any solid malignancy targeting MAGE-A1 using the current engineered T cell approach developed in this protocol; however, there is experience that is informative in patients who have been diagnosed with advanced Merkel Cell Carcinoma (MCC).[8] Prior FHCRC protocol 9245, combination treatment of patients with advanced MCC with avelumab (another FDA-approved PD-L1 inhibitor), class I HLA upregulation, and autologous bulk expanded endogenous T cells targeting MCPyV (the MCC viral antigen)[13] was shown to be safe with no patients experiencing unexpected toxicities. Moreover, several of these patients had durable complete responses (CRs) and non-durable partial response (PR). In protocol 9845 ATTAC-MCC trial, which is ongoing, is a phase I/II trial of autologous in patients with advanced MCC that has progressed on or after treatment with immune checkpoint (PD-1 or PD-L1 inhibitor) therapy. The ATTAC-MCC regimen is a combination of the FH- MCVA2TCR autologous transgenic CD8+ and CD4+ T cells carrying the transgenic TCR A2-MCC1 targeting MCPyV, avelumab, and class I MHC upregulation to the tumor tissue with single fraction radiation therapy (SFRT). Although this MCC trial is ongoing and data is being acquired, infusion of the first T cell dose corresponding to ~5 x 10^8 in a patient resulted without toxicity.

2.0 STUDY PURPOSE AND RATIONALE

Knowing the high mortality following progression after early line CPI-based therapies for metastatic TNBC, urothelial and NSCLC, novel therapies that might prolong and improve on immunotherapy

responses are desperately needed. Notably, prior experience with transgenic antigen-specific TCR therapies even in combination with CPI have demonstrated safety in other diseases with manageable immune-related adverse events (e.g., transient cytokine release syndrome <48 hours). In prior trials with these therapies against acute leukemia and MCC, efficacy was also observed in patients for whom other therapies had failed. Buoyed by these demonstrations of safety and efficacy in early phase studies in other disease contexts, it is imperative to extend the benefits of antigen-specific transgenic T-cell based therapies to common and deadly solid malignancies such as TNBC or NSCLC.

2.1 Rationale for Investigation of FH-MagIC TCR-T

2.1.1 Scientific basis for development of TCR against MAGE-A1

MAGE-A1 is an ideal target for cancer T cell immunotherapy. The MAGE-A protein is classified within the "testis restricted" cancer testis antigens and is broadly expressed in a wide range of malignancies (**Figure 1A**), including 69.1% of TNBCs (85.7% of Grade III cases),[9] 27-46% of NSCLCs[10,11] and 30% of urothelial neoplasms.[12] Importantly, MAGE-A1 is undetectable in most normal tissues exclusive of the immune privileged testes. The MAGE-A1 epitope targeted by FH-MagIC TCR-T is specific to MAGE-A1 therefore the identified TCR does not demonstrate cross-reactivity to other highly homologous MAGE-A family members with less restricted somatic expression.[13,18] We have developed a MAGE-A1 specific IHC laboratory assay to screen for tumor expression on FFPE patient biopsy samples (**Figure 1B-C**).

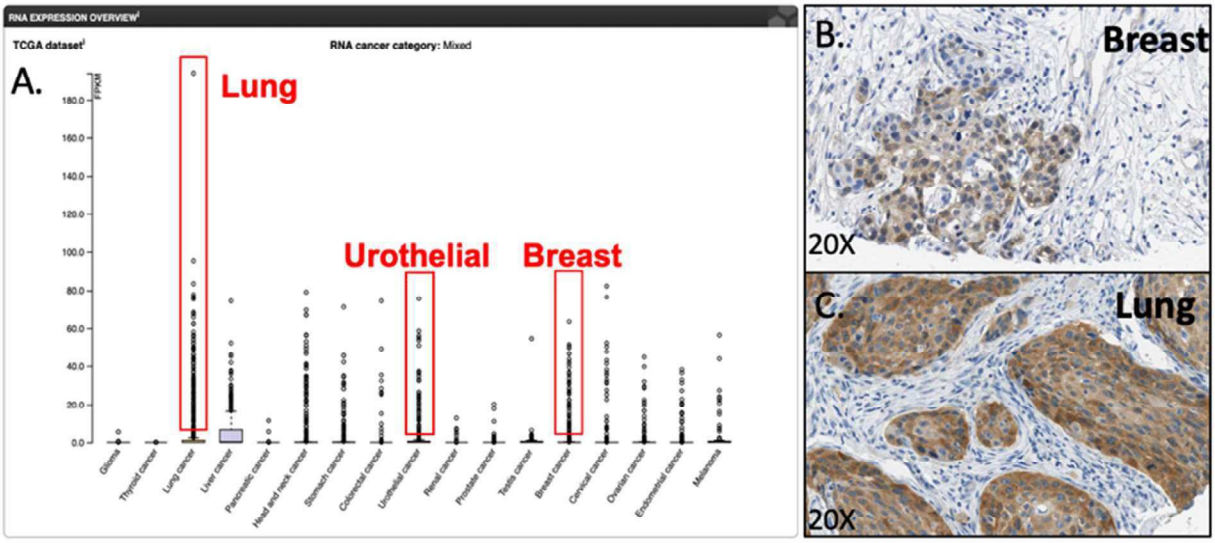


Figure 1: Somatic MAGE-A1 protein expression is limited to malignant cells, reducing chance of on-target off-tissue toxicity. A] MAGE-A1 expression (RNA FPKM) from the cancer genome atlas[19-21] (TCGA) for various malignancies. MAGE-A1 positive tumor cells (Brown) as determined by IHC using monoclonal antibody MA454 in FFPE **B]** TNBC and **C]** NSCLC patient biopsies.

2.1.2 Target epitope: HLA-A02 restricted "KVLEYVIKV" MAGE-A1 Epitope

TCR-T targeting of MAGE family proteins is required due to their intracellular expression but the high homology of MAGE proteins can allow for the generation of peptides that are shared or highly homologous by multiple MAGE-A family members thus leading to the potential development of cross-reactive TCRs toward multiple MAGE-A family members. In order to target only MAGE-A1 and no other MAGE family members, FH-MagIC TCR-T is specific to the Class I HLA A*0201-restricted MAGE-A1$_{278-287}$ epitope (KVLEYVIKV) identified by mass spectrometry on the surface of KS24 breast cancer cells.[22] MAGE-A1 KVL epitope is a particularly appealing immunotherapy target because the immunogenic peptide is unique to MAGE-A1 among all other MAGE family genes that have demonstrated less restrictive somatic expression patterns.[18]

2.1.3 Clinical experience to date with cell therapies for TNBC and NSCLC

No large systematic studies of adoptive genetically modified T cell therapies have been completed in either TNBC, urothelial, or NSCLC. At a recent major breast cancer symposium, Specht et al. presented data from the first 7 patients enrolled on a phase I dose escalation study utilizing a chimeric antigen T cell construct (scFv/4-1BB/CD3ζ) targeting embryonic antigen ROR-T, an orphan tyrosine kinase expressed in TNBC and NSCLC.[23] One patient experienced cytokine release syndrome easily managed with tocilizumab and dexamethasone with no major off-target toxicity. In these heavily pre-treated patients, one patient had a partial response of 14 weeks following a second infusion but most had transient disease stability or progression as best response. The early work suggests exhaustion in the CAR-T cells. The NSCLC cohort has not been presented or published but continues to enroll.

2.1.4 Benefits of a transgenic approach

Because of limited success with endogenous T cell therapy, a switch to a transgenic approach is necessary to improve efficacy. A transgenic approach offers four major advantages: time, potency, dose, number of patients, and cost. These are further detailed: **Time to T cell product**: On a previous ex vivo expanded T cell trial for MCC patients, the time to T cell product production ranged from 49-80 days. Given the rapid growth of aggressive cancers, this delay is significant and clinically unacceptable. A transgenic approach allows for T cells to be produced in less than one-quarter the time, approximately 14 days. **Ability to deliver a more potent T cell product:** With an ex vivo expanded T cell product, the avidity of T cells (ability of T cells to bind MAGE-A1 expressing tumor cells) varies greatly between patients. A highly avid T cell offers the best opportunity to "stick to" and kill a cell that has potentially weak expression of the class I HLA genes, such as advanced solid tumors like TNBC or NSCLC which commonly down-regulate MHC as a means of immune evasion. By encoding a highly avid T-cell receptor specifically targeting MAGE-A1 peptide, we can improve the ability of transferred T cells to respond to tumor. **Ability to deliver a larger number of transgenic T cells:** Deliverable dose is directly related to T cell persistence which may be essential for long term tumor control for T cell therapy.[24] Therefore, it is important to reach as close to our target dose as possible. By starting with a larger number of cells and transducing/gene-modifying them, target doses are more achievable.

2.1.5 Safety of TCR transgenic T cells for other cancers

There is prior experience preparing and delivering transgenic T cells with natural TCR isolated with similar approaches and in the same clinical vector in other disease processes. In clinical trial # NCT01640301,[25] 24 acute myeloid leukemia patients post allogeneic stem cell transplant were treated with transgenic CD8+ T cells targeting the WT1 antigen. Apart from expected grade I-II cytokine release

syndromes (<24 hour duration) and transient lymphopenia (<10 days), no immediate or delayed off-target or on-target/off-tissue toxicity was observed, and clinical benefit observed.[26]

2.1.6 Rationale for isolating a TCR from the peripheral repertoire of HLA matched healthy donors.

High avidity T cells are generally most effective in mediating antitumor responses.[27,28] As TCR affinity largely determines T cell avidity,[29,30] infusion of T cells expressing a high affinity, transgenic TCR could be effective in patients with low or absent endogenous $CD8^+$ T cell responses. We developed a methodology to identify low frequency, physiologic high-affinity TCRs from healthy donors (**See Section 8.2**). This is in contrast to modifying TCRs with low affinity through mutagenesis *in vitro* to artificially enhance affinity. The latter has shown toxicity since affinity-enhanced TCRs have not been vetted by thymic selection, and these carry the risk of having acquired cross-reactivity with additional unknown self-antigens.[31] Additionally TCRs generated in antigen negative transgenic mice[14,18] that also do not undergo human-specific thymus selection may have an increased chance of off-target cross reactivities.[18,31] In light of these challenges, our approach was to isolate thymus vetted TCRs from the highest affinity tumor/self-antigen-specific T cells within the peripheral repertoire of matched healthy donors.

2.1.7 Why include transgenic CD4+ T cells in addition to transgenic CD8+ T cells?

TCR-based immunotherapies have primarily exploited $CD8^+$ T cells, which recognize tumor antigens in the context of Class I presentation/restriction.[32] However, co-transferred $CD4^+$ T cells can provide major direct and indirect anti-tumor effects, including promoting expansion and survival of tumoricidal $CD8^+$ T cells, as was shown in an FBL murine leukemia model.[33] More recently this has been shown with therapeutic CD19-CAR T cells,[34,35] and supports the concept of engaging both subsets to target the same tumor. Tumor-specific, HLA Class II-restricted $CD4^+$ T cells promote Class I-restricted $CD8^+$ T cell proliferation, survival, and effector functions in part by producing IL-2, and facilitate activation of dendritic cells (DC) for *de novo* immune responses.[36,37] $CD4^+$ T cells expressing Class II-restricted TCRs exhibit direct cytolytic activity in metastatic melanoma and have had anti-tumor activity against human cholangiocarcinoma;[38,39] however, Class II expression is rare in solid tumors[40]. An alternative is to confer function to $CD4^+$ T cells through expression of a high-affinity Class I TCR ($TCR_{Class\text{-}I}$), that would co-localize the engineered $CD8^+$ and $CD4^+$ T cells on the same tumor target without requiring Class II expression. These T cells can both perform traditional CD4 functions (production of T cell supportive cytokines, DC activation) and have direct tumoricidal effect. Similar approaches have been safely employed for melanoma by other groups, in T cell therapy trials targeting the MART-1 antigen.[41]

2.1.8 TCR-MagIC is a highly potent natural TCR that effectively lyses cell lines expressing MAGE-A1

FH-MagIC TCR-T cell products for murine experiments were generated according to the products destined for patients (see **CMC Section 7**). Briefly, PBMC first underwent a positive magnetic bead selection for CD4+. The flow through containing a majority of CD8+ T cells was then positively selected for CD62L+ cells. This assures CD8+ T cell subsets that have demonstrated improved in vivo persistence including naïve, central memory and stem cell memory CD8+ are selected for transduction.[42-44] Pooled CD4+ and CD4-CD62L+ cells at a ratio of 1:3 obtained from frozen PBMC of three individual healthy donors were then stimulated with IL-2 and TransAct (aCD3/CD28) two days prior to transduction with the TCR-MagIC PRRLSIN lentivirus. FH-MagIC TCR-T cell mock products were harvested on day 14 post stimulation. FH-MagIC-TCR T cell products from all three donors specifically killed low-density MAGE-

A1 expressing breast lines (**Figure 2A**) and high-density MAGE-A1 expressing melanoma (**Figure 2B**) cell lines over a period of 5 days (IncuCyte assay).

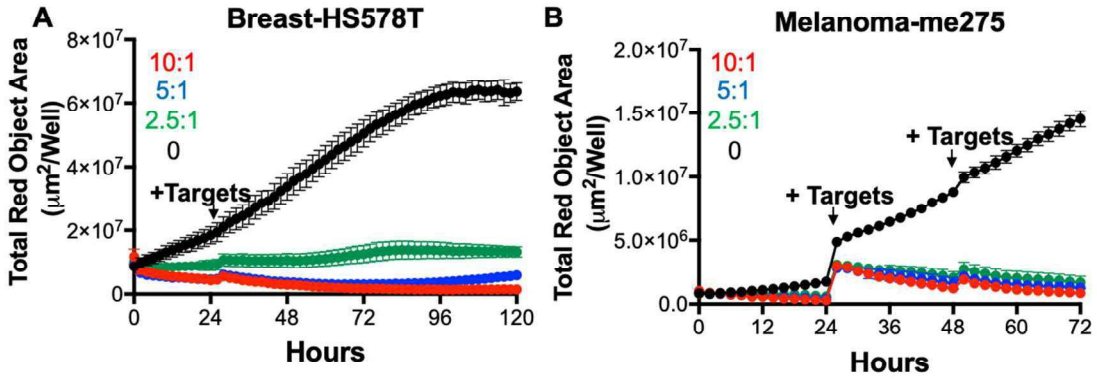


Figure 2: FH-MagIC TCR-T specifically kill HLA-A2 MAGEA1 positive tumor cell lines. [A,B] *In vitro* cytotoxic activity of FH-MagIC TCR-T cells at decreasing E:T ratios generated from three healthy donors measured by dynamic killing of red labeled [**A**] HS578T breast cell line with a single re-challenge at 24 hours or [**B**] me275 melanoma cell line re-challenged at 24 and 48 hours (n= 3 donors).

Furthermore, we utilized an immunodeficient NSG xenograft mouse model of MAGE-A1+ myeloma[45] to test the *in vivo* efficacy of FH-MagIC TCR-T. Briefly, mice were engrafted with $2x10^6$ U266 myeloma cells intravenous 7 days prior to FH-MagIC TCR-T cell transfer. The U266 myeloma cell line was selected due to its native HLA-A2*01 expression, MAGE-A1 protein expression, and the ability for the cells to grow specifically and consistently in the bone marrow as a xenograft in NSG mice. Furthermore, serial tumor burden assessments in individual mice can be performed by serum quantification of secreted IgE by the U266 cells. To evaluate the *in vivo* efficacy of each T cell population, $15x10^6$ TCR-MagIC transduced CD8+, CD4+ or CD8+ and CD4+ T cells (proposed FH-MagIC TCR T formulation) were transferred separately and all showed tumor control (**Figure 3A**). All groups containing TCR-MagIC transduced T cells demonstrated *in vivo* persistence and tumor control resulting in eradication of U266 cells in tumor engrafted mice (**Figure 3B**). These results highlight the potency of both TCR-MagIC transduced CD8+ and CD4+ T cells. As solid tumors with complex tumor microenvironments will be targeted in the clinical trial planned here, we expect effective TCR-T function will require the synergy of both CD8+ and CD4+ T cell subsets.

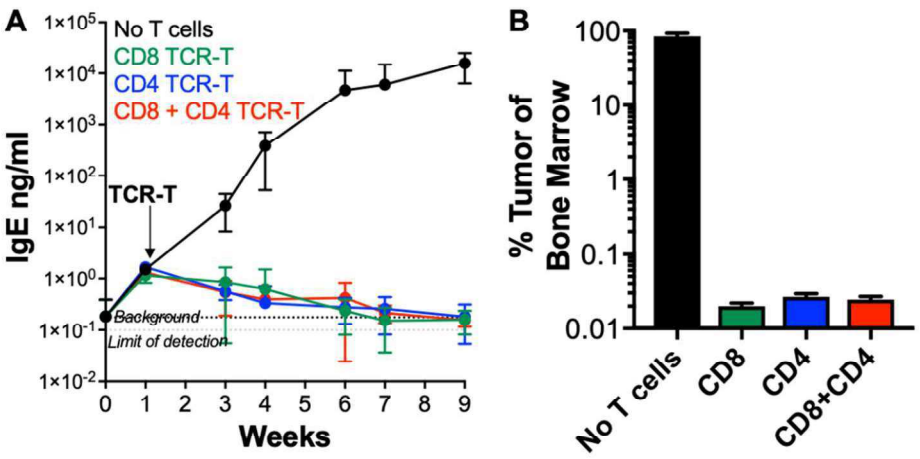


Figure 3: FH-MagIC TCR-T controls HLA-A2+ MAGEA1+ U266 xenograft in vivo.[A] IgE levels over time in the serum (ng/ml) of U266 (iv injected) tumor bearing NSG mice with and without FH-MagIC TCR-T cells transfer. **[B]** Tumor burden in the bone marrow 9 weeks post U266 engraftment in FH-MagIC TCR-T cell treated mice as compared to non-treated control mice (n=4 mice/group).

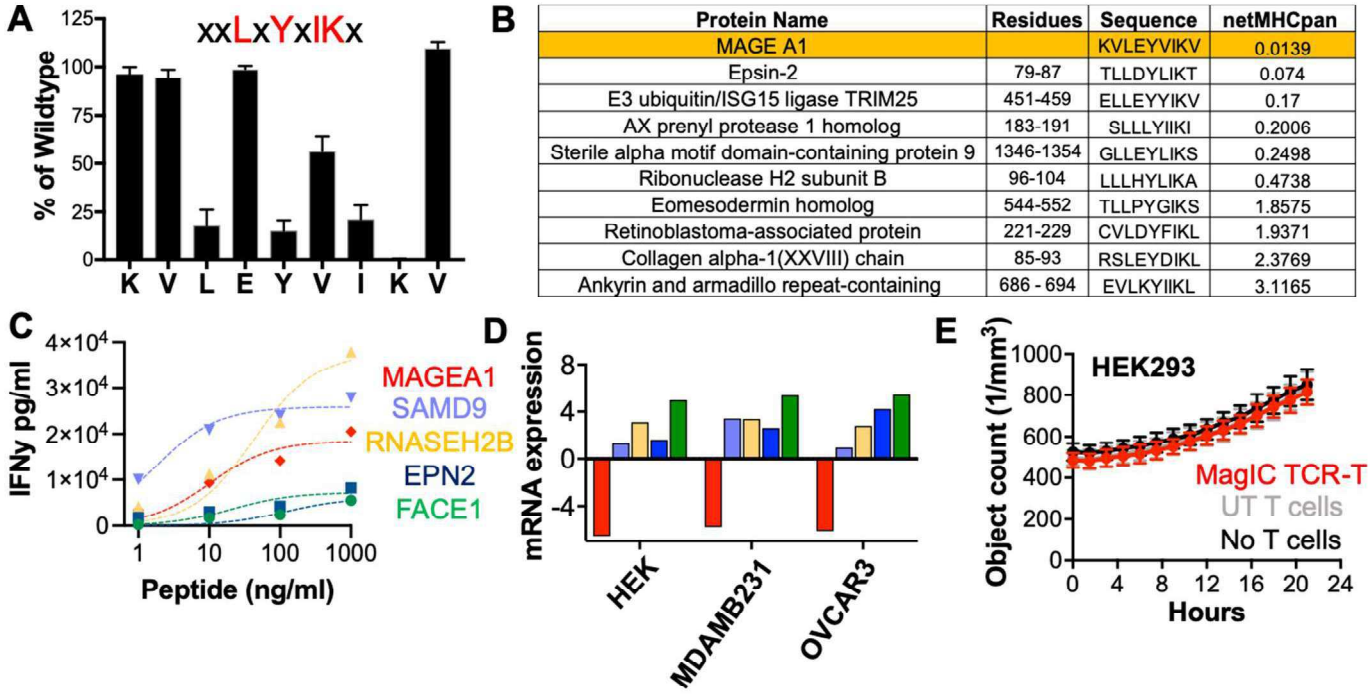
2.1.9 Evaluation of FH-MagIC TCR-T potential for off-target activity

To assess whether TCR MagIC is cross-reactive and can recognize HLA-A2+ restricted peptides from other self/human proteins, an alanine scan was performed using FH-TCR MagIC mock cell products.[37] Peptides spanning the MAGE-A1$_{278-287}$ [KVLEYVIKV] epitope were generated in which every amino-acid was substituted by an alanine.[31] Target peptide recognition performed for FH-MagIC-TCR T cells containing a 1:1 ratio of CD4+ and CD8+ identified four residues critical for binding to the TCR (**Figure 4A**). A BLAST search of the human genome for peptides with allowed substitutions (xxLxYxIKx) at each non-critical residue identified nine potentially recognizable self-peptides that were predicted to be held by HLA-A*02:01 using netMHC 4.0[46] (**Figure 4B**). HLA-A2+ TAP-deficient B-lymphoblastoid cell lines (T2 cells) loaded with a high concentration of the identified peptides were incubated with FH-MagIC TCR-T and recognition was assessed by interferon-gamma intracellular cytokine staining (ICS). Initial screening resulted in four positive responses from synthesized peptides, in addition to the targeted KVLEYVIKV peptide (positive control). These results were confirmed with cytokine media assay, using titrated amounts of loaded self-peptide which resulted in a positive response for the targeted MAGEA1 peptide and significant responses to two of the four tested self-peptides (**Figure 4C)**. Thus, additional investigation into the identified self-proteins (SAMD9 and RNASEH2B) was initiated.

Using available RNAseq and protein-expression datasets[16,47] we profiled the expression patterns of SAMD9 and RNASEH2B and found them to be widely expressed in healthy human tissues (including CD8+ and CD4+ T cells) and tumor cell lines (**Figure 4D**). Since TCR-M0 had undergone negative selection in the thymus of the healthy adult it was isolated from, and we had never observed any T cell-T cell recognition of transduced HLA-A2+ donors during initial functional assays, we hypothesized that the *in silico* determined peptides were not endogenously processed and presented. FH-MagIC TCR-T reactivity to endogenously processed peptide was evaluated by co-culture of HLA-A2+ cells lines negative for MAGE-A1 expression (HEK293T, MDAMB231and OVCAR3) with FH-MagIC TCR-T over 24

hours. Using the IncuCyte live cell imaging platform, no significant monolayer destruction was observed compared to untraduced T cells of identical donors and untreated wells (**Figure 4E** - 239T cell line shown). FH-MagIC TCR-T cells were also evaluated for activation after co-culture with HEK293, MDAMB231 and OVCAR3 by intracellular cytokine production and no detectable T cell activation was observed (not shown). To ascertain the epitopes derived from these proteins were not presented, HLA-A2+ lines were transduced with lentiviral vectors encoding SAMD9 and RNAH2B linked to a GPF reporter. Again, no recognition, as measured by intracellular cytokine production, of GFP sorted K562 and LCLs were identified during co-culture of FH-MagIC TCR-T cells by (not shown).

FH-MagIC TCR-T was also tested against a panel of HLA-A2 positive normal cells (**see section 2.1.10**) and no unexpected cross-reactivity was observed. Our data support an absence of antigen processing of the *in silico* identified cross-reactive peptides since FH-MagIC TCR-T only recognized the screened self-peptides after exogenous loading, but not endogenous antigen processing.



Protein Name	Residues	Sequence	netMHCpan
MAGE A1		KVLEYVIKV	0.0139
Epsin-2	79-87	TLLDYLIKT	0.074
E3 ubiquitin/ISG15 ligase TRIM25	451-459	ELLEYYIKV	0.17
AX prenyl protease 1 homolog	183-191	SLLLYIIKI	0.2006
Sterile alpha motif domain-containing protein 9	1346-1354	GLLEYLIKS	0.2498
Ribonuclease H2 subunit B	96-104	LLLHYLIKA	0.4738
Eomesodermin homolog	544-552	TLLPYGIKS	1.8575
Retinoblastoma-associated protein	221-229	CVLDYFIKL	1.9371
Collagen alpha-1(XXVIII) chain	85-93	RSLEYDIKL	2.3769
Ankyrin and armadillo repeat-containing	686 - 694	EVLKYIIKL	3.1165

Figure 4: In Silico analysis of MagIC TCR-T cross-reactivity. A: Alanine scan identifies critical residues (in red) for peptide-MHC recognition. **B**: Peptides identified by *in silico* analyses and rank on HLA-A2 binding scoring system. **C**: Reactivity of MagIC TCR-T to titrated amounts of exogenously loaded peptide. Targeted MAGEA1 KVL peptide is shown as positive control. **D**. Expression of SAMD9 and RNASEH2B in HLA-A2+ MAGEA1-negative cell lines. **E**. Growth of HEK293 T cells during co-culture with MagIC TCR-T (red), Un-transduced T cells (gray) or no T cells (black).

2.1.10 Evaluation of FH-MagIC TCR-T potential for cross- and allo-reactivity

We performed unbiased testing of a panel of commercially available HLA-A2 positive induced pluripotent stem cell (IPSC) derived human cell lines, cryopreserved normal human bronchial fibroblasts, normal human bronchial and renal epithelial cell lines. These cell lines reflected critical tissues including heart/cardiac, lung, endothelium, liver/hepatocytes, brain (astrocytes and GABA neurons), and kidney (renal epithelium). As these lines are not all HLA-A2:01 expressing, they were lentivirally transduced with HLA-A2 prior to TCR-T co-culture. FH-MagIC-TCR T did not express detectable IFNγ or exhibit cytotoxicity as measured by live cell imaging when co-cultured in the presence of all tested cell lines with or without upregulation of HLA molecules and alterations in peptide processing by pretreatment of IFN_{gamma} (**Figure 5**).

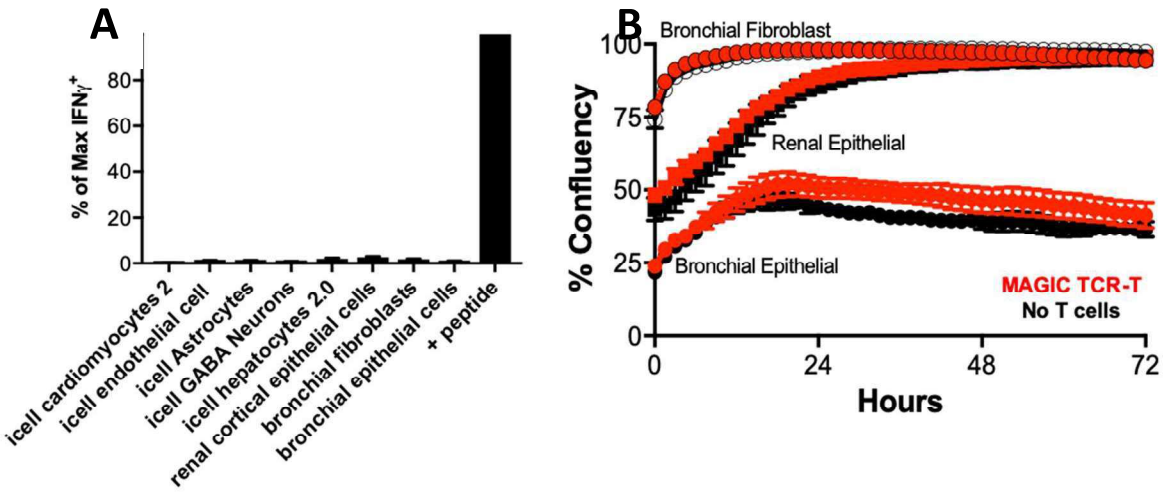


Figure 5: MagIC TCR-T does not recognize self-peptides presented by HLA-A2*01 on healthy human cell lines. **A**: Cytokine production by MagIC TCR-T after co-culture of HLA-A2*01 iPS derived human cell lines or harvested normal human cell lines (determined by ICS or ELISA). Independent experiments performed from 3-5 healthy human donors. Cytokine production normalized to amount/percentage produced after co-culture with MAGEA1 peptide loaded cells. **B**: No evidence of monolayer destruction or limit to healthy normal cell growth during co-culture with MagIC-TCR T (Red, n=3 donors) as measured by IncuCyte live cell imaging.

A bank of EBV-transformed human lymphoblastoid cell lines (LCL), which express abundant class I HLA, were used to assess potential class I HLA alloreactivity of FH-MagIC TCR-T cells. As the B cell lineage-derived LCLs do not express MAGE-A1, they would not be expected to trigger FH-MagIC TCR-T recognition unless loaded with cognate peptide. Reactivity of the FH-MagIC TCR construct to twenty-six lines representing >60 of the most common HLA types was assessed both by a nur77- reporter jurkat cell line expressing CD8 and the FH-MagIC-TCR construct and normal donor-derived FH-MagIC-TCR T cells via an interferon-gamma cytokine release assay. Alloreactivity towards a single LCL-line with one unique HLA as compared to the other tested lines (**Table 1**). The identified reactivity to HLA-B49*01

was confirmed by positive recognition of HLA-A2 negative, HLA-B49 positive Namalwa cell line. (**Figure 6**). As a consequence of these findings, patients expressing HLA-B49*01 will be excluded from trials that use the FH-MagIC TCR construct.

Table 1: HLA typing of cell lines tested.

Cell Line	HLA-A		HLA-B		HLA-C	
LCL-1	0203	1102	4601	380201	0702	1202
LCL-2	24020101		4801	5401	0803	0102
LCL-3	3001	6802	4201		1701	
LCL-4	2402	2901	2702	0705	1505	020202
LCL-5	0206	2402	5502	4002	0102	1502
LCL-6	0205	6802	1402	5801	0802	0701
LCL-7	2402	310102	350101	520101	0401	1202
LCL-8	02	680101	5802	4501	0602	1601
LCL-9	3601	7401	5301	5703	0401	0701
LCL-10	3402	7401	0801	1503	0701	020204
LCL-11	3001	3301	5301	8101	04	08
LCL-12	3101	0201	4001	1801	03041	0701
LCL-13	0205	0201	4901	1501	0701	0303
LCL-14	0301	0201	070201	5701	0702	0602
LCL-15	2402	0201	390601	1501	0702	0303
LCL-16	0301	0301	5601	2705	0102	020202
LCL-17	0301	0101	3501	0801	0401	0701
LCL-18	2501	0201	0801	4402	0701	0501
LCL-19	0206	2601	3501	3801	0401	1203
LCL-20	0101	1101	5101	5001	1502	0602
LCL-21	6601		3801		1203	
LCL-22	110101	0253N	3701	5801	0302	0602
LCL-23	3201	6802	0801	44020101	0102	07
LCL-24	0201	2902	1516	440301	1402	1601
LCL-25	2301	2301	4101	5101	1701	1502
LCL-26	0201	2402	1302	070201	0602	0702
Namalwa	301	6802	701	4901	701	

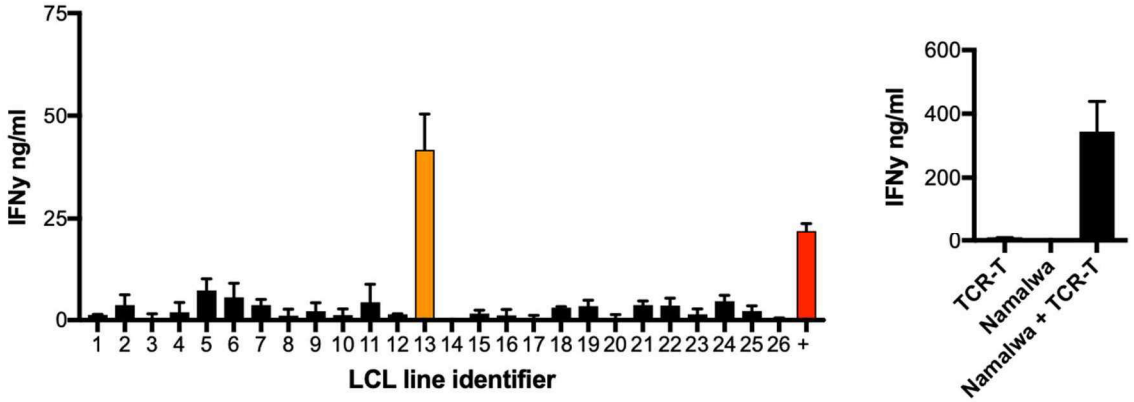


Figure 6: FH-MagIC TCR-T Recognition of HLA-B49*01. A: Cytokine production by FH-MagIC TCR-T after co-culture with a panel of B-LCL cell lines (determined by ICS or ELISA). Independent experiments (n=2). **B**: Cytokine production by FH-MagIC TCR-T after co-culture of an HLA-B49^{+} tumor cell line Namalwa (n=3 donor T cell products).

2.1.11 Why use a third-generation lentiviral vector to transduce TCR-MagIC into autologous T cells?

The theoretic risk of insertional mutagenesis with retroviral vectors became realized during two X-linked severe combined immunodeficiency gene therapy trials in which five patients developed acute

lymphoblastic leukemia, shown to reflect transactivation of either the LMO2 or CCND2 gene by the retroviral insert in transduced CD34+ hematopoietic stem cells (HSCs). This highlighted the propensity of gamma-retroviral vectors (γ-RV) to preferentially insert near genes that are actively transcribed, which may be particularly problematic in transduced HSC that express genes that confer self-renewal capacity and a proliferative/survival advantage and are then transplanted into a setting in which they are driven to extensively expand.[48]

By contrast, the risks of insertional oncogenesis or other vector-related cellular toxicities are extremely low when targeting peripheral blood T cells that lack the same self-renewal capacity of HSC, with no significant toxicities reported to date in any clinical trial. As described below, this risk can be further decreased with the use of a third-generation self-inactivating (SIN) lentivirus (LV), in which the promoter regions of the long terminal repeats (LTRs) have been truncated and the insert, such as a TCR gene, is expressed under control of an internal murine stem cell virus (MSCV)-based promoter.[49]

Insertional oncogenesis was not observed in the long-term results from three clinical trials evaluating γ-RV-engineered T cells for patients with HIV.[50] The transduced cells persisted long term, in some cases >10 years, and clinical monitoring of the patients at yearly intervals for cumulatively >540 patient-years of observation has not detected evidence of retroviral genotoxicity. In more recent trials with less extensive retrospective data, no toxicities attributable to the administration of T cells transduced to express HLA*0201-restricted MART1 or gp100-specific TCRs using the MSGV1-based RV were observed in 33 patients with metastatic melanoma. The MSGV1-based RV uses the same promoter from the LTR of MSCV that will be used in the lentiviral vector in our trial.[51] The MSGV1-based γ-RV was also used to transduce autologous T cells to express an NY-ESO-1-specific TCR that was infused in 17 patients with metastatic synovial sarcoma or melanoma[51] and to transduce cells to express a carcinoembryonic antigen (CEA)-specific TCR in T cells that were infused into three patients. Again, no insertional mutagenesis events were identified.

Transcriptionally active enhancer/promoter elements may influence, expression of cellular genes at a distance from the insertion site, independently of the vector type used to introduce the gene (RV vs LV; LTR-based or SIN).[52] However, γ-RVs have a predilection toward integrating in the immediate proximity of transcription start sites and deoxyribonuclease I hypersensitivity sites, increasing the probability that the viral LTR transcriptional enhancer will interfere with gene regulation and potentially activate cancer-causing genes.[53] In contrast, LVs commonly insert into introns, and thus are more likely to integrate further away from the transcription start sites of active transcription units, making them less likely to induce transcriptional activation.[54] In vivo genotoxicity assays based on the transplantation of transduced tumor-prone Cdkn2a-/- murine hematopoietic progenitor cells have also directly compared the effect of promoter location within γ-RVs and SIN lentiviral constructs on the oncogenic potential. Placing the strong LTR-based spleen focus-forming virus promoter as an internal promoter in a construct containing a SIN LTR rather than as a component of the LTR further reduced the propensity for insertional mutagenesis and lymphoid tumors.[55]

Importantly, >1000 patients have been treated with TCR transgenic or CAR-T cells on protocols nationwide that include T cell modifications with similar vectors. A recent review in the New England

Journal of Medicine determined that among these >1000 individuals no occurrence of an oncogenic transformation has yet been reported.[56]

Despite the risk of insertional mutagenesis being extremely low, it remains justified to initially examine safety/toxicity and potential efficacy of therapy with transduced T cells in patients with solid cancer on this protocol. The potential toxicities are listed in the protocol consent form and will be reviewed with patients as a part of the consent process. Eligible patients who elect to enroll on the protocol will be monitored and managed for potential toxicities as per current guidelines, which are outlined in **Section 12.** Stopping rules will also be applied, as described in **Section 4.1**.

2.1.12 Why transduce CD62L+ ("young" but well differentiated) CD8+ T Cells?

Establishing a persistent functional population of antigen-specific T cells after adoptive transfer will likely be necessary to eliminate all tumor cells and prevent recurrence. Previous trials of T cell therapies in multiple malignancies have often been limited by the inability of transferred T cells to expand and persist post- transfer. The in vivo fate of transferred T cells is dependent in part on the intrinsic properties of the T cells from which the infused cells are derived,[43,57] and we can choose the T cell type used for adoptive cell therapy.

Conventional CD8+ T cells can be divided into naïve and antigen-experienced, memory T cell subtypes (TN, TM). TM cells can be further divided into T_{CM} and effector memory T cell (T_{EM}) subsets, which have distinct transcriptional programs associated with defined characteristics, including homing, phenotype, and function. When T_{EM} cells are stimulated in vitro, they differentiate largely into short-lived effector cells that kill targets, but generally have limited proliferative capacity and fail to persist for long periods of time after transfer.[58]

Studies of transferred purified CD8+ T cell subsets in a murine virus infection model revealed that transferred TCM provide enhanced protective immunity from in vivo challenge compared to T_{EM}. Although TCM cells expand and also differentiate into effector cells in response to in vitro stimulation, adoptive transfer studies in non-human primates revealed that TCM–derived effectors had been imprinted and retained some of the beneficial properties of their parent TCM cell, in particular the capacity for self-renewal, which translates into improved in vivo persistence and response to antigen challenge.[43]

For this proposed trial, we will employ CD62L expressing CD8+ T cells. This will include lymphocytes subsets that are T_{CM}, T_N and T_{SCM} ("young" but well differentiated, with increased persistence potential). In exploratory analyses, we will determine which subset of cells was associated with best persistence and tumor localization, and use this information to help to better refine T cell therapies.

2.2 FH-MagIC TCR-T Dose Rationale

Our prior endogenous cell therapy trials in MCC, melanoma and leukemia targeted an infused dose of 10 billion (10^{10}) tumor-specific endogenous CD8+ T cells per square meter, or approximately 20 billion ($2*10^{10}$) CD8+ T cells per infusion. No dose limiting toxicities were observed, and maximum actual infused dose was 26 billion for protocol 2586 (HLA upregulation + T cells) and 17.7 billion for protocol 9245 (HLA upregulation + T cells + avelumab).

For the present study, our planned goal doses are initially 1 billion (1 x 10^9) and eventually 5 billion (5 x 10^9) MAGE-A1-specific TCR transgenic CD3+ T cells for the full dose infusion This final dose was selected for several reasons: it is achievable in a 10-14 day production, it is lower than or similar to dosing for TCR transgenic T cells for other disease processes (e.g., MCC and AML), and it offers a safety window by starting at a substantial dose reduction from our previous dosing experience. All patients included in this study will be adults and are thus expected to have similar body surface areas, and therefore we have selected a flat rather than weight-based dosing; this approach is used with dosing with that of other commercially approved transgenic T cell products for adults (e.g., Kymriah, Gilead Inc.).

Our product will include both CD8+ and CD4+ transgenic T cells. Dose will be measured based on quantity of transgenic CD3+ cells. Purity will be required to be at least 10% of cells transgenic for TCR-MagIC and is expected to range from 20-80%, more typically from 30-50%. Although we will target a 1:1 ratio of CD8+ and CD4+ transgenic T cells in the final product, cells are co-cultured and there is inherent variability in infused products.

2.3 Rationale for Lymphodepleting Chemotherapy

Standard lymphodepleting chemotherapy will be administered to subjects prior to the T-cell infusion to reduce tumor burden, minimize the risk of tumor lysis syndrome, and induce lymphopenia to improve T-cell persistence. Clinical trials of T-cell therapy for melanoma at the National Cancer Institute (NCI) have demonstrated that administering lymphodepleting chemotherapy such as fludarabine and cyclophosphamide, or fludarabine, cyclophosphamide, and total body irradiation, prior to the transfer of 10^{10} to 10^{11} polyclonal melanoma-specific T cells improved the survival of a subset of the transferred T cells and antitumor efficacy [59-61]. The size of the T-cell pool is subject to homeostatic regulation, and the induction of lymphopenia results in less competition for cytokines such as IL-15 and IL-7 that promote lymphocyte proliferation and survival, and thus leads to the proliferation of residual T cells including those that are adoptively transferred. Lymphodepleting chemotherapy may also eliminate $CD4^+$ $CD25^+$ regulatory T cells and activate antigen-presenting cells that may promote the function of transferred T cells. Studies in murine models subsequently confirmed the human data indicating that lymphodepletion improves the persistence and antitumor efficacy of transferred effector T cells [62].

Substantial data have been presented for subjects treated with three different targeted CAR-T-cell products; however, there is no consensus on the optimal lymphodepleting chemotherapy regimen to use. Encouraging antitumor activity has been observed in 11 subjects with NHL who received lymphodepleting chemotherapy with a combination of high doses of cyclophosphamide (60 to 120 mg/kg) and fludarabine (25 mg/m^2/day for 5 days) [63]. However, significant cardiac toxicity, neurotoxicity, and death were reported at the highest CAR-T-cell dose level (5×10^6 CAR-T cells/kg). When the CAR-T-cell dose was reduced to 1×10^6 CAR-T cells/kg and a lower-dose regimen of cyclophosphamide (300 mg/m^2/day for 3 days) and fludarabine (30 mg/m^2/day for 3 days) was used, the severe cardiac and neurotoxicity was eliminated, and although transient neurotoxicity (aphasia and ataxia) was still evident, encouraging antitumor activity was still observed [64].

Current data with JCAR014 show improved CAR-T-cell expansion, persistence, and antitumor activity in subjects with ALL and NHL who received lymphodepleting chemotherapy with high-dose cyclophosphamide (60 mg/kg) and fludarabine (25 mg/m^2/day for 3 to 5 days) compared with those treated with cyclophosphamide alone or in combination with etoposide. The improved CAR-T-cell expansion and persistence in subjects who received Cy/Flu lymphodepletion was due a combination of mitigation of an anti-CAR-Transgene immune response in the Cy/Flu-treated subjects and enhancement in homeostatic IL-7 and IL-15 concentrations after Cy/Flu lymphodepletion compared to lymphodepletion without Flu [35,65]. Current data in subjects with NHL and ALL support the tolerability of JCAR014 at doses of 2×10^5 and 2×10^6 CAR-T cells/kg after Cy/Flu conditioning.

Unless clinical circumstances suggest otherwise, the current trial will use a lower cyclophosphamide dose (300 mg/m^2 for 3 days rather than 60 mg/kg) and a comparable dose of fludarabine (30 mg/m^2 for 3 days). This regimen has resulted in good cell expansion and persistence, and a favorable toxicity profile in ongoing studies of CAR-T cells in hematopoietic malignancies [66] (NCT #02631044).

3.0 STUDY OBJECTIVES

3.1 Primary Objectives

1) Safety: To evaluate the safety of adoptive T cell therapy using FH-MagIC TCR-T (defined as an observed treatment-related unexpected grade 3 or higher toxicity rate consistent with a true rate <= 35%).
2) Efficacy: To demonstrate clinical activity of FH-MagIC TCR-T (defined as an observed response rate that statistically exceeds 5%). Without treatment, metastatic TNBC, urothelial cancer or NSCLC are near uniformly fatal. Response rates to TNBC specifically in the setting of post-progression are unknown as no clinical trials have been reported in this setting. Please see biostatistical section for further details on efficacy assessment and power calculations.

3.2 Secondary Objectives

1) Persistence: Persistence of infused transgenic T cells in peripheral blood.
2) Migration: Migration of infused transgenic T cells in tumor tissue.
3) Progression free and overall survival: Determine progression-free and overall survival of treated individuals.
4) Response rate by immune RECIST: Determine objective response rates by immune-related RECIST criteria.

3.3 Exploratory Objectives

Our study includes a number of exploratory objectives. These include phenotype of infused T cells that persist and localize to tumor, functional capacity of infused transgenic T cells, determination of the presence or absence of epitope spreading (broadening of immune responses) in the peripheral blood and/or tumor environment, and evaluation of changes in the tumor tissue and microenvironment that may correlate with success and/or failure of the ATTAC regimen.

We will also examine feasibility of generating the FH-MagIC TCR-T product. Feasibility may be assessed by determining: i) the proportion of patients able to receive the TCR product, ii) the proportion of patients able to receive a TCR product meeting all pre-specified release parameters, iii) the proportion of patients able to receive a product not meeting pre-specified release parameters, and iv) the proportion of patients unable to receive the FH-MagIC TCR-T product due to manufacturing failure.

These exploratory objectives are not anticipated to result in direct conclusions but instead develop hypotheses for future trials.

3.4 Projected Target Accrual

All racial groups and ethnicities will be included and participation of members of under-represented groups welcomed. These targeted/planned enrollment numbers are based on relative percentages of race/ethnicity in NSCLC, urothelial carcinoma and TNBC in the catchment area of the Seattle Cancer Care Alliance. The trial is first come, first served basis and no specific allocation for a specific cancer type is being made. Assuming equal distribution among the three cancers studied, there will be a slight skew in enrollment of women because TNBC is statistically all women.

Table 2: Targeted / Planned Enrollment

Ethnic Category	**Patient Numbers**		
	Females	**Males**	**Total**
Hispanic or Latino	1	1	2
Not Hispanic or Latino	9	7	16
Ethnic Category Total of All Patients	10	8	18
Racial Category			
American Indian / Alaska Native	0	0	0
Asian	1	0	1
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	2	1	3
White	6	7	13
More than One Race	1	0	2
Racial Categories: Total of All Patients	10	8	18

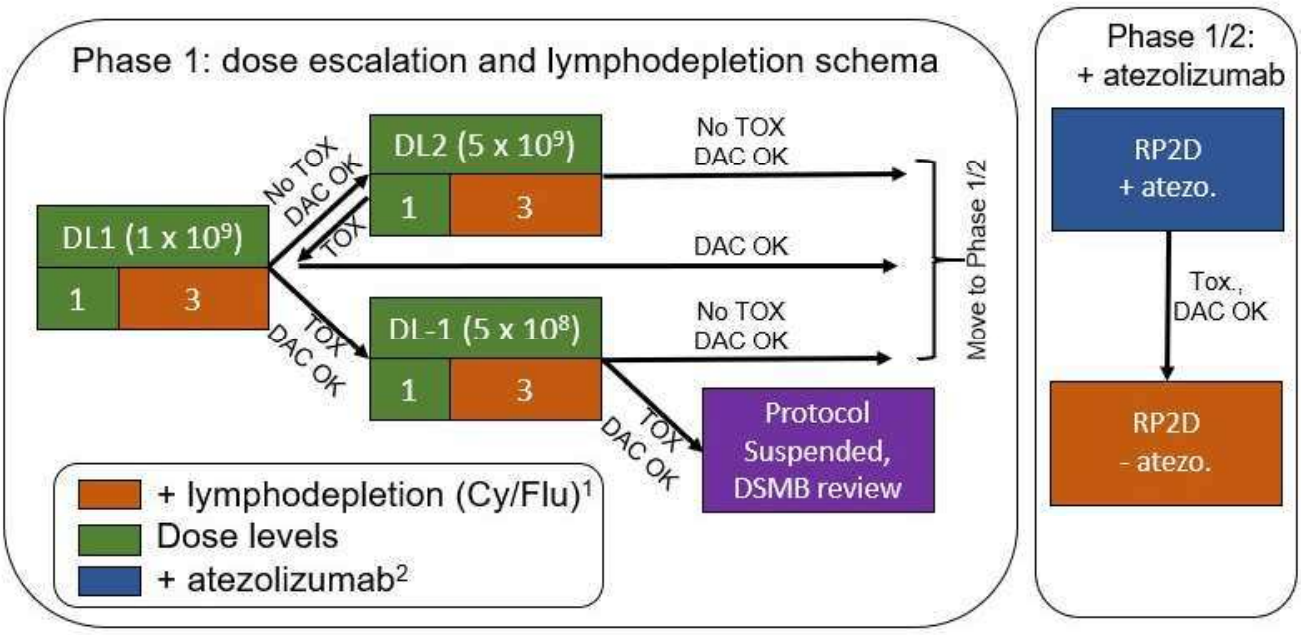
4.0 STUDY DESIGN AND INVESTIGATIONAL PLAN

4.1 Overall Study Design: Phase I/II single arm dose escalation non-masked interventional study

The study will be a phase I, open-label interventional study aimed at treating 15-18 HLA*A0201 patients with metastatic TNBC, urothelial carcinoma or NSCLC (please refer to specific inclusion criteria in **Section 5.1**). TCR-T cells are investigational. An anti-PD(L)1 inhibitor will be provided as standard of care in those patients enrolled on to the Phase 1/2 portion of the trial (**Figure 7**). In the event atezolizumab (preferred) is not available another FDA-approved PD1-axis inhibitor for the specific clinical indication may be substituted (e.g., durvalumab or pembrolizumab in NSCLC, or avelumab,

durvalumab, or pembrolizumab in urothelial cancer). Clinically, all the PD1-axis inhibitors are indistinguishable and multiple standards of care are available in most disease settings. Duration of the PD1-axis inhibitors is provided as a suggestion only and may be adapted at the discretion of the treating oncologist.

Figure 7: Plan of Treatment



[1] Lymphodepletion Dosing: Cyclophosphamide 300mg/m² + Fludarabine 30mg/m² both IV days -4, -3, -2 followed by T cell infusion Day 0
[2]Atezolizumab 1200mg IV q3w.

Figure 7 demonstrates the guidelines for progression through the protocol after the approval from the DAC when no DLT are observed. There are three components to the plan of treatment: cell dose, lymphodepletion, and CPI treatment with atezolizumab. All decisions for dosing through the protocol will be reviewed by the Dose Assessment Committee (DAC). For the initial phase of the trial, at each cell dose level, one patient will be treated without lymphodepletion and if no DLT is observed, the next three patients will receive lymphodepletion therapy. The first four patients will be treated with a 1 x 10^9 CD3+ cells (DL1) during their first and second infusion approximately 6-12 weeks later, with the second infusion being considered in subjects with disease progression who have not experienced DLTs. If all four patients complete the first infusion without DLT, the next 4 patients will be treated on the next dose level of 5 x 10^9 CD3+ cells (DL2) and a second infusion approximately 6-12 weeks later will be considered in subjects with disease progression who have not experienced DLTs. If there are no DLT and no evidence for progression by RECIST criteria. If there is toxicity observed at DL1 that is attributable to cellular therapy following review by the DAC, the next four patients will be treated at 5 x 10^8 CD3+ cells (DL-1) with the first patient receiving cellular therapy only and the next three patients receiving lymphodepletion followed by cellular therapy. The RPD2 is targeted to be 5 x 10^9 CD3+ cells with lymphodepletion; however, a lower dose may be selected by the DAC for the RP2D based on the totality of toxicity data.

The Phase 1/2 portion of the trial will build on the RP2D with the addition of PD1-axis therapy (e.g., atezolizumab 1200mg IV q3weeks). Of note, patients enrolled to this portion of the trial must have been offered or received a PD-1 axis inhibitor. If received, they must have either developed progression or still have detectable disease and not have developed serious adverse events. If no DLT are observed, up to 6 patients may be treated with the combination. The DAC will review the data after every 2 patients in this portion of the study and if PD1-axis attributable toxicity is observed, expansion of the number of patients at RP2D may continue without PD1-axis therapy.

Lymphodepletion will comprise of cyclophosphamide (Cy) 300mg/m^2 and fludarabine (Flu) 30mg/m^2 intravenously (IV) on days -4, -3, and -2 before each investigational T cell infusion. The DAC committee may recommend lymphodepletion de-escalation in the event of DLT attributable Cy/Flu.

At any dose level per the schema above, if one patient experiences a DLT, 3 additional patients may be recruited at that dose level (6 patients total). As long as no additional DLT are observed (i.e., 2 or more patients with DLT in the total of 6 patients), escalation may proceed to the next dose level. If 2 or more of the 6 patients experience a DLT, de-escalation (if possible) to the lower dose will take place with addition of 3-6 additional patients enrolled at the de-escalated dose level as trial resources may permit. In the event of an on-study death that is at least possibly related to the investigational agent(s) or study procedures, enrollment will be paused pending review by the DAC.

Any patient experiencing treatment related grade 3 or 4 toxicity (except for anticipated toxicity specifically excepted/outlined in section 12) will immediately stop receiving T cell infusions and/or atezolizumab. They will receive treatment as appropriate for their condition (see **Section 7** and **Section 12**). They will continue to be followed for response/outcome.

As a criterion for enrollment into the trial, participants must agree to biopsies of tumor tissue if medically feasible. A minimum of 3 biopsies will be performed as clinical status allows: prior to enrollment, 2 weeks after and 12 weeks after the first T cell infusion. However, should there be no tumor tissue that is accessible for biopsy, patients will still be considered for participation, at discretion of the investigator. Similarly, should an investigator or treating physician determine that a biopsy cannot be performed safely for clinical reasons (e.g., no safe approach), biopsies may be cancelled or retimed at the investigators' or treating physician's discretion.

4.2 Protocol Enrollment

Enrollment is expected to take approximately 18-24 months. Study treatment with scheduled follow-up for all patients is expected to be approximately 1 year. All patients who receive treatment with FH-MagIC TCR T should be followed for at least 15 years after the final infusion for safety evaluations in the long-term follow-up (LTFU) portion of the study. The anticipated duration of the study, excluding initial LTFU assessments, is approximately 3.5 years.

4.3 Staggering of Patients within each Dose Level

Each patient in each dose level must complete their DLT evaluation period (14 days after their T-cell infusion) before the next patient can receive his/her first infusion. Staggering restrictions will be lifted

14 days after the last patient has received the first infusion and the protocol has proceeded to phase 1/2.

5.0 STUDY POPULATION

The target study population consists of adult patients with metastatic TNBC, urothelial, or NSCLC that has progressed on or after prior treatment with a PD-L1 axis immune checkpoint inhibitor. Patients must meet all of the inclusion criteria and none of the exclusion criteria to be enrolled in this study.

Results of tests and/or procedures conducted as per standard of care purposes may be used for research purposes if conducted within the protocol-defined window prior to screening/leukapheresis and/or T-Cell Therapy.

5.1 Inclusion Criteria

1) **Tissue confirmation of TNBC, urothelial carcinoma or NSCLC:** Participants must have metastatic disease. Confirmation of diagnosis must be or have been performed by internal pathology review of archival, initial or subsequent biopsy or other pathologic material at Fred H/SCCA/UWMC. Patients with TNBC must meet the American Society of Clinical Oncology – College of American Pathologists (ASCO-CAP) definition of negative estrogen, progesterone and HER2 receptor expression.
2) **Expression of MAGE-A1:** Baseline tissue will be stained to confirm MAGE-A1 expression (see **Appendix I**).
3) **Measurable disease by RECIST 1.1 criteria:** Participants must have measurable disease, defined as at least one target lesion that can be measured in at least one dimension (longest diameter to be recorded) as ≥10 mm, unless lymph node in which case short axis must be >= 15 mm. Baseline imaging with RECIST read (for example diagnostic CT chest/abdomen/pelvis and imaging of the affected extremity as appropriate). Brain imaging is only indicated if history of brain metastasis or neurologic symptoms concerning for brain metastasis are present (MRI or CT scan). Repeat imaging will be required if not within 45 days prior to first infusion of FH-MAGEA1-A2TCR. MRI can be substituted for CT in patients unable to have CT contrast. Measurable disease is not required for purposes of leukapheresis /cell manufacturing storage for patients who meet HLA and expression criteria but have not received SOC therapies as outlined below (i.e. inclusion criteria 4 and 5).
4) **Previous treatment with SOC FDA-approved therapies.** Patients with NSCLC who have actionable somatic mutations or alterations in EGFR, ROS1 and ALK with FDA-approved drug therapy options will be eligible for study only after treatment with targeted therapies for those mutations have been offered or received. Patients with urothelial

carcinoma who are candidates for enfortumab vedotin-ejfv will be eligible for study after prior treatment with enfortumab vedotin-ejfv has been offered or received.
Note: Participants will be eligible to enroll before standard therapy is received in order to expedite leukapheresis/cell manufacturing as long as the standard of care agent is administered prior to lymphodepletion and/or cell infusion.

5) **Previous treatment with PD-1 axis inhibitor:** Patients in both dose escalation (Phase 1) and dose expansion (Phase 2) must have progressed on or after prior treatment with an FDA-approved PD-1 axis inhibitor (e.g., PD-1 or PD-L1 inhibiting monoclonal antibody such as pembrolizumab, nivolumab, avelumab, atezolizumab, durvalumab) if indicated as treatment, or have refused or not tolerated such therapies. If received, they must have either developed progression or still have detectable disease and not have developed CTCAE grade 3 or higher toxicity while on treatment. Patients may have received 1 or more prior systemic regimens for metastatic TNBC or NSCLC. There is no upper limit on prior regimens. Patients may have received prior anti-PD-1/anti-PD-L1 in the neoadjuvant or adjuvant setting.
6) **HLA type HLA-A*02:01:** Participants must be HLA-A*02:01 in order for infused transgenic T cells in order to insure recognition of antigen-MHC complexes. HLA typing should be determined though a molecular approach in a clinical laboratory licensed for HLA typing.
7) **Life expectancy must be anticipated to be >3 months at trial entry**
8) **18 years or older**
9) **Capable of understanding and providing a written informed consent**
10) **If fertile, willingness to comply with reproductive requirements:** Outlined in section 5.3.
11) **ECOG performance status of 0, 1, or 2:** Please see Appendix D for ECOG performance status.
12) **Tumor tissue amenable to safe biopsy and subject willing to undergo serial tumor biopsies:** Should there be no tumor tissue that is accessible for biopsy, patients will still be considered for participation, at discretion of the sponsor and in consultation with the investigator. Similarly, should an investigator determine that a biopsy cannot be performed safely for clinical reasons biopsies may be cancelled or retimed after confirming plan with the sponsor.
13) **Participants must be at least three weeks from last systemic treatment at the time of cell collection:** At least 3 weeks must have passed since any: immunotherapy (for example, T-cell infusions, immunomodulatory agents, interleukins, vaccines), small molecule or chemotherapy cancer treatment, other investigational agents. There is no washout period for radiation, so long as radiated lesion is not the lesion being evaluated for RECIST measurements on the protocol. Bisphosphonates are permitted but the concurrent treatment with RANK-ligand inhibitors (i.e., denosumab) is not permitted within 8 weeks before treatment.

14) **Acceptable organ function-** Acceptable organ function is defined as:
- Renal: serum creatine <2.5 mg/dL or eGFR > 30 mL/min
- Hepatic: tBili < 3.0 mg/dL, AST, and ALT <5x upper limit of normal (ULN). Patients with suspected Gilbert syndrome may be included if Tbili >3 but no other evidence of hepatic dysfunction
- Pulmonary: ≤ grade 1 dyspnea and SaO2 ≥ 92% on ambient air. If PFTs are performed based on the clinical judgement of the treating physician, patients with FEVI ≥ 50% of predicted and DLCO (corrected) of ≥ 40% of predicted will be eligible.
- Cardiac: Patients 60 years of age or older are required to have left ventricular ejection fraction (LVEF) evaluation performed within 60 days prior to enrollment. LVEF may be established with echocardiogram or MUGA scan, and left ejection fraction must be ≥ 35%. Cardiac evaluation for other patients is at the discretion of the treating physician.
- Hematologic: ANC > 500 cells/ mm3.

5.2 Exclusion Criteria

1) **Expression of HLA B*4901**: participants will be excluded due to the risk of alloreactivity.
2) **Pregnancy or lactation:** Participants of childbearing potential must have a negative serum pregnancy test within 14 days prior to enrollment. Childbearing potential is defined as women who have not been surgically sterilized and who are not post-menopausal (free of menses for at least 1 year).
3) **Active autoimmune disease:** Patients with active autoimmune disease requiring immunosuppressive therapy are excluded. Case-by-case exemptions are possible with approval by PI.
4) **Prior solid organ transplant or allogenic hematopoietic stem cell transplant:** Kidney transplant patients will be considered on a case-by-case basis requiring discussion with PI. If kidney transplant, patient must have dialysis access, dialysis plan, supportive nephrologist, willingness to stop transplant immunosuppression, and express understanding that rejection is possible outcome. Dialysis or costs related to transplant kidney will not be supported by the study. Participants having had any other solid organ transplants will be excluded, as will those with any history of allogeneic stem cell transplant.
5) **Corticosteroid therapy at a dose equivalent of > 0.5 mg/kg of prednisone-equivalent per day**
6) **Concurrent use of other investigational anti-cancer agents**
7) **CLL or other active hematologic malignancy**
8) **Active uncontrolled infection:** HIV positive participants on HAART with a CD4 count > 500 cells/mm3 are considered controlled, as are individuals with a history of hepatitis C

who have successfully completed antiviral therapy with an undetectable viral load, and those with hepatitis B who have, per standard practice, hepatitis well-controlled on medication (e.g., AST and ALT <5x ULN).

9) **Uncontrolled concurrent illness:** Participants may not have uncontrolled or concurrent illness including, but not limited to, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
10) **Untreated brain metastases:** Participants with small asymptomatic brain metastases (<1 cm) or those with brain metastases previously treated with surgery or radiotherapy will be considered for inclusion at discretion of principal investigator, so long as other eligibility criteria are met.
11) **For patients in Phase 1/2, Grade 3 or higher immune-mediated toxicity to any prior PD-L1 axis blocking agent**
12) **Active treatment for prior immune related adverse event to any immunotherapy:** Participants receiving ongoing treatment for prior serious immune related adverse events are excluded, with exception of hormone supplementation or corticosteroid therapy at equivalent of up to 0.5 mg/kg prednisone per day, unless otherwise approved by PI.
13) **Significant underlying neurologic disease:** Study participants must not have significant active underlying neurologic disease, unless approved by PI. Neuropathy related to diabetes or prior chemotherapy is acceptable.
14) **Other medical, social, or psychiatric factor that interferes with medical appropriateness and/or ability to comply with study, as determined by the PI.**

5.3 Reproductive Potential and Contraception Requirements

Any female patient who does not meet at least one of the following criteria will be considered to have reproductive potential:

- Post-menopausal for at least 12 consecutive months (i.e., no menses), or
- Undergone a sterilization procedure (hysterectomy, salpingectomy, or bilateral oophorectomy; tubal ligation is not considered a sterilization procedure)

Pregnancy test for females of reproductive potential must be negative within 72 hours before MagIC TCR-T infusion.

Female subjects with reproductive potential who are not sexually abstinent and male subjects who are sexually active with females of reproductive potential must agree to use a suitable method of contraception for the duration of the study, for example:

- Condom with spermicidal agent
- Diaphragm or cervical cap with spermicidal agent

- Intrauterine device
- Hormonal contraceptives in combination with either a condom, diaphragm, or cervical cap

6.0 TREATMENT PLAN

6.1 Leukapheresis

Leukapheresis collection will be performed on each patient to obtain peripheral blood mononuclear cells (PBMCs) for the production of the FH-MagIC TCR-T investigational product. At least 3 weeks must have passed since any: immunotherapy (for example, T-cell infusions, immunomodulatory agents, interleukins, vaccines), small molecule or chemotherapy cancer treatment, other investigational agents. If a patient is clinically stable on another agent by their treating provider, they will be permitted to pause treatment for 3 weeks and then proceed with leukapheresis to help expedite generation of the FH-MagIC TCR-T cell product. Following leukapheresis these patients should restart therapy and continue to be followed as outlined in the **Section 6.2: Bridging Therapy**.

The leukapheresis will be performed by the Seattle Cancer Care Alliance (SCCA) Apheresis Unit using departmental standard operating procedures (SOPs) for obtaining PBMCs. If a technical issue arises during the procedure or in the processing of the product, or if insufficient FH-MagIC TCR-T cells are manufactured for the prescribed FH-MagIC TCR-T dose, the patient may undergo additional collection procedures.

Subjects ineligible for a vein-to-vein apheresis may elect to have a percutaneous central venous access catheter inserted to support this collection.

The leukapheresis product will be delivered to the Cell Processing Facility (CPF) at the Fred Hutch for handling according to methods outlined in the Chemistry, Manufacturing and Controls (CMC) section of the Investigational New Drug (IND) application to the FDA. PBMCs from the leukapheresis and cell selection products may be archived for research.

The FH-MagIC TCR-T-cell product for each patient will be stored at the Fred Hutchinson CPF and Seattle Cancer Care Alliance Cellular Therapy Laboratory (CTL) until required for infusion. Quality control and release testing will be performed on the FH-MagIC TCR-T-cell product prior to its release for patient infusion.

6.2 Bridging Therapy

Patients may receive treatment after leukapheresis to control disease during production of FH-MagIC TCR-T. Decisions regarding therapy in this interval should be discussed with the study PI. If a patient is responding favorably to bridging therapy, they may continue on this as long as deemed medically appropriate. Patients should have a washout of the time equivalent to one treatment cycle prior to moving forward with lymphodepletion. Baseline imaging studies should be completed following bridging therapy or, in the absence of bridging therapy, if imaging was completed ≥8 weeks prior to undergoing lymphodepletion or cell infusion (for those not receiving LD chemotherapy).

6.3 Lymphodepletion

For Phase 1 of the trial, at each cell dose level, one patient will be treated without lymphodepletion and if no DLT is observed, the next three patients will receive lymphodepletion therapy. Lymphodepletion will comprise of cyclophosphamide (Cy) 300mg/m^2 and fludarabine (Flu) 30mg/m^2 intravenously (IV) on days -4, -3, and -2 before each investigational T cell infusion. The DAC committee may recommend lymphodepletion de-escalation in the event of DLT attributable Cy/Flu.

6.4 FH-MagIC TCR-T Treatment

Patients will receive either 1 or 2 infusions of FH-MagIC TCR-T starting at $1x10^9$ (one billion) TCR-MagIC transgenic CD3+s per study design (section 4.1). At each T cell dose level evaluated, the first patient will receive cells alone while the 2^{nd}-4^{th} patient will also receive lymphodepleting with Cy/Flu as per lymphodepletion plan. Cy/Flu lymphodepletion will be administered according to institutional guidelines and with standard supportive care therapies (e.g., anti-emetics).

T-cell infusions and lymphodepletion therapies will be given at the Seattle Cancer Care Alliance (SCCA) or at the University of Washington Medical Center (UWMC). All patients will be observed for 2 hours post infusion and will be released if no adverse events requiring hospitalization have occurred.

T cells will be delivered to the patient's bedside as a frozen product, which will be thawed immediately prior to infusion (see Product Administration Manual). T cells will then be infused intravenously over approximately 15-30 minutes via nonfiltered tubing at the specified dose. The infusion bag will be gently mixed periodically during the infusion.

6.4.1 FH-MagIC TCR-T Combination Therapy

6.4.1.1 Atezolizumab (or another PD1-Axis Inhibitor)

Atezolizumab is a fully humanized monoclonal antibody which targets human PD-L1 and inhibits its interaction with its receptors. Atezolizumab should be administered as standard of care starting 24-72 hours after the T cell infusion. Currently, the FDA recommended dosing as a single agent is 1200mg IV fixed dose every 3 weeks. If an alternative PD1-axis inhibitor is instead available for a patient per formulary according to NCCN guidelines, it may be substituted instead.

Procedures will be performed per physician's discretion and typical practice at the SCCA: A physical exam is usually performed by an APP or MD within 48 hours before infusion and blood work is obtained. Typically, this blood work includes a CBC with differential, basic metabolic panel, liver function panel, and LDH with each infusion, and a serum cortisol, TSH, free T4, amylase, and lipase with every other infusion. Cortisol, TSH, free T4, amylase and lipase do not often result prior to that day's infusion, however these are followed longitudinally to determine whether there is a need for hormone replacement.

The first dose of atezolizumab should be infused initially within 24-72 hours after T cell infusion. Atezolizumab doses may be held, rescheduled, or dosages reduced at the discretion of the provider in keeping with standard clinical practice. If immune-related adverse event secondary to atezolizumab is suspected or confirmed, the provider will discuss management with the study team and a joint management decision established. In general, the first choice for partner to FH-MagIC TCT-T therapy

for TNBC and NSCLC is atezolizumab as it is standard of care for these indications. For urothelial carcinoma, the first choice for partner will be pembrolizumab. However, if the commercial supplier of the checkpoint inhibitor agrees to only provide an alternative PD1-axis inhibitor to atezolizumab, substitution is permitted.

6.4.2 Preparation of Cell Product

All manufacturing activities are conducted using approved written procedures performed by trained, qualified personnel in accordance with cGMP and current Good Tissue Practices (cGTP) guidelines. Quality assurance oversight and review is provided to assure regulatory compliance and safety of therapeutic products.

The formulated cell dose refers to TCR+ CD3+ T cells determined by binding to HLAA2-MAGE-A1 tetramer. The intent for each infusion is to provide a cell product that contains approximately 50% of TCR-modified CD4+ T cells and CD8+ T cells (i.e., TCR-modified T cells in 1:1 CD4+/CD8+ ratio). Although a cell ratio of 1:1 will be targeted, as cells will be co-cultured actual ratios will vary. Any ratio of CD4 to CD8 will be acceptable.

If a product cannot be formulated to meet this target cell dose specification because of low transduction efficiency, suboptimal growth of one of the subsets, or failure of either the CD4+ or CD8+ T-cell product to meet release criteria, the cell product should be infused at or as close as possible to the total T-cell dose and CD4+:CD8+ transgenic T-cell ratio. A product will be considered evaluable if it contains ≥10% of the scheduled CD3+ transgenic T cell dose.

6.4.3 LD Chemotherapy and T Cell Infusions

Patients should undergo clinical evaluation on both the day of scheduled LD chemotherapy (in patients receiving LD chemotherapy only) and the day of T cell infusion in order to determine appropriateness to proceed with LD chemotherapy and T cell administration.

Patients deemed not appropriate for LD chemotherapy may have this deferred and if necessary receive supportive care or bridging therapy (see Section 6.2) as needed. Patients deemed not appropriate for T cell administration as originally planned may receive a T cell infusion outside the specified window within 60 days after the originally planned initial infusion if they subsequently resolve the clinical and/or laboratory concerns which deemed them inappropriate for the infusion as originally planned..

FH-MagIC TCR-T will be provided as a single cell product at the assigned cell dose.

The final product will be prepared and labeled according to departmental standard operational procedures in the Cellular Processing Facility. The cell product will be transported to the infusion facility by a delegated staff member. During the time of transportation, the cell product will be kept frozen in a CryoPod™. The product will be thawed at bedside (see Product Administration Manual) and a nurse will then administer the cells to the patient using standard institutional precautions for blood borne pathogens and follow institutional standard for administration of cryopreserved Immune Effector Cells.

The infusion should be administered IV over approximately 15-30 minutes. All patients will be monitored as outlined in the protocol Product Administration Manual.

6.4.4 Acute infusion reactions

Acute infusion reactions may occur with administration of FH-MagIC TCR-T. Guidelines for the monitoring and treatment of acute infusion reactions are provided in **Section 7.1**.

6.4.5 Guidelines for the determination of 1 or 2 cycles of T cell therapy

Each "cycle" of T cell therapy consists of one T cell infusion. Each patient may receive up to two cycles of T cell therapy.

Approximately 12 weeks after first T cell infusion, restaging, and evaluation of peripheral blood persistence of infused transgenic T cells, will be performed. Patients will be considered for a second infusion of transgenic T cells (cycle 2). If a second T cell infusion is planned, this will occur at the highest dose level that has been determined to be safe by the DAC. In addition, if LD chemotherapy and/or anti-PD1/PDL1 therapy has been found to be safe, these may be utilized during cycle 2 even if not administered with cycle 1 for an individual patient. Decision-making surrounding this will be at the discretion of the investigator and will be generally made following along the below guidelines:

1. Individuals with **progressive disease** and non-persisting transgenic TCR T cells may be considered for a second infusion. Patients can receive debulking radiation/chemotherapy per standard of care and remain on protocol to be considered for a second T cell infusion. In this event, 3 weeks must have passed for cells to be given if chemotherapy is administered. PD-1 axis blockade can continue leading up to the second T cell infusion. The second cycle may be given as early as 6 weeks and later than 12 weeks in patients who have persistent disease and <5% transgenic TCR-T cells amongst CD3 positive cells in the blood.

2. Individuals with **progressive disease** and persistent transgenic TCR T cells (>5% of CD3) will no longer receive study treatments (T cells, atezolizumab) for reasons of progression. They will not receive additional T cells, nor atezolizumab on study. They will leave the treatment phase of the study and transition to long term follow-up.

3. Individuals with **complete response** will remain on the treatment phase of the study and will continue to receive atezolizumab to complete one full year of treatment. They will not, however, receive additional T cells. For these patients, T cell product will be cryogenically preserved, and they may be considered for a second cycle of T cell therapy in the future, at the discretion of the investigator, should their metastatic disease recur.

4. Individuals with **partial response** or **stable disease** (per RECIST 1.1) will remain on study and will continue to receive atezolizumab to complete at minimum a year of treatment. They will have evaluation of the persistence of their transgenic T cell. Should they have poor to moderate persistence (<5% TCR MagIC CD3+s), they will be considered for a second T cell infusion.

6.5 Recommended Supportive Care, Additional Treatment, and Monitoring

Prophylactic treatment/measures are recommended for subjects at risk for tumor lysis syndrome (TLS) according to institutional or clinical standards. Supportive care for the management of CRS is detailed in **Section 7.3**.

The use of red blood cells and platelet transfusions, and/or colony-stimulating factors is permitted according to institutional or clinical standards.

The use of prophylactic or empiric anti-infective agents (e.g., trimethoprim/sulfamethoxazole for pneumocystis pneumonia [PCP] prophylaxis, broad spectrum antibiotics, antifungals, or antiviral agents for febrile neutropenia) is permitted according to institutional standards. Prophylactic anti-seizure medication should be considered in some subjects (see **Section 7.4**).

Hospitalization may be required after study treatment to manage treatment-associated toxicities.

7.0 POTENTIAL RISKS AND TOXICITY MONITORING

Acute infusion toxicity may occur during or shortly after T-cell infusion. In addition, CRS, TLS, and neurologic toxicity may occur due to the infusion of genetically modified products. Management of these complications is addressed in the sections below. A table of proposed grading criteria for CRS is provided in **Appendix E.**

7.1 Acute Toxicity Associated with T-Cell Infusion

Examples of potential symptoms and signs associated with T-cell infusion reactions and their initial management are listed below:

- Fever, chills, and temperature elevations > 38.2°C may be managed with acetaminophen 650 mg PO every 4 to 6 hrs. All subjects who develop fever > 38.2°C or chills should have a blood culture drawn and the PI or designee should be notified.
- Headache may be managed with acetaminophen.
- Nausea and/or vomiting may be managed with ondansetron 8 mg po or other antiemetics (excluding corticosteroids).
- Hypotension should be managed initially by fluid administration.
- Hypoxemia should be managed initially with supplemental oxygen.

If an infusion is terminated due to acute toxicity, the residual T cells should be returned to Fred Hutch Therapeutic Products Program (TPP) Quality Control Department for analysis. Investigation of possible causes of observed signs should proceed and, if necessary, additional medical treatment will be instituted.

Subjects requiring discontinuation of the infusion may be eligible for re-treatment if the cause is deemed not related to the infusion.

If an infusion is terminated due to acute toxicity, the residual T cells should be returned to Fred Hutch Therapeutic Products Program Quality Control Department for analysis. Investigation of possible causes of observed signs should proceed and, if necessary, additional medical treatment will be instituted.

Patients requiring discontinuation of the infusion may be eligible for re-treatment if the cause is deemed not related to the infusion.

7.2 Acute Toxicity Associated with Atezolizumab or Other PD1-Axis Inhibitor Infusion

As with all monoclonal antibody therapies, atezolizumab carries a risk of allergic reaction. If hypersensitivity reaction occurs, the participant must be treated according to the best available medical practice.

Atezolizumab: special precautions

Infusion-related reactions

Symptoms

- Fever
- Chills
- Rigors
- Diaphoresis
- Headache

Management

Table 3. Treatment modification for symptoms of infusion-related reactions caused by PD-1 Axis Inhibitors

NCI-CTCAE Grade	Treatment Modification
Grade 1 – mild Mild transient reaction; infusion interruption not indicated; intervention not indicated.	Decrease the infusion rate by 50% and monitor closely for any worsening. The total infusion time should not exceed 120 minutes.
Grade 2 – moderate Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (for example, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hours.	Stop infusion. Resume infusion at 50% of previous rate once infusion-related reaction has resolved or decreased in severity to ≤ Grade 1, and monitor closely for any worsening.
Grade 3 or Grade 4 – severe or life -threatening Grade 3: Prolonged (for example, not rapidly responsive to symptomatic medication and/or brief interruption of	Stop the infusion immediately and disconnect infusion tubing from the participant.

NCI-CTCAE Grade	Treatment Modification
infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae related to PD-1 axis inhibitor. Grade 4: Life-threatening consequences; urgent intervention indicated.	Participants must be withdrawn immediately from the PD-1 axis inhibitor treatment and must not receive any further treatment with that particular PD-1 axis inhibitor treatment.

Definitions: IV=intravenous, NCI-CTCAE=National Cancer Institute-Common Terminology Criteria for Adverse Event, NSAIDs=nonsteroidal anti-inflammatory drugs

Once the infusion rate has been decreased by 50% or interrupted due to an infusion-related reaction, it must remain decreased for all subsequent infusions. If the participant has a second infusion-related reaction of Grade ≥ 2 on the slower infusion rate, the infusion should be stopped, and the participant should be removed from atezolizumab treatment. If a participant experiences a Grade 3 or 4 infusion-related reaction at any time, the participant must discontinue that particular PD-1 axis inhibitor.

Management of severe hypersensitivity reactions and flu-like symptoms due to atezolizumab infusions

If hypersensitivity reaction occurs, the participant must be treated according to the best available medical practice. Participants should be instructed to report any delayed reactions to the Investigator immediately.

Symptoms of severe hypersensitivity reaction

Impaired airway
Decreased oxygen saturation (< 92%)
Confusion
Lethargy
Hypotension
Pale / clammy skin
Cyanosis

Management

Epinephrine injection and dexamethasone infusion
Patient should be placed on monitor immediately
Alert ICU for possible transfer, if required

7.3 Cytokine Release Syndrome

Administration of transgenic T cells may be associated with CRS. CRS may be characterized by high fever, fatigue, nausea, headache, dyspnea, tachycardia, rigors, hypotension, hypoxia, myalgia/arthralgia, anorexia, coagulation abnormalities, organ dysfunction, and neurologic abnormalities.

If a patient becomes febrile or develops symptoms of CRS, cytokine levels, serum ferritin, C-reactive protein (CRP), coagulation studies, and/or markers of tumor lysis syndrome (e.g., chemistry, uric acid, lactate dehydrogenase [LDH]) may be measured, and persistence and/or phenotype of the transgene-expressing cells may be evaluated, as clinically indicated.

Any patient who develops clinical evidence of symptoms related to CRS will have a work-up to exclude infection or other causes, as clinically appropriate. Initial treatment should consist of supportive measures as dictated by the clinical and laboratory findings, and may include fluid replacement, medications to support blood pressure, antipyretics, oxygen supplementation, anti-seizure medications, and broad-spectrum antibiotics if infection cannot be excluded as a potential etiology for the signs and symptoms.

The CTCAE version 5.0 CRS grading scale will be utilized to quantify T cell associated CRS [67], as detailed in **Appendix E.**

Guidelines for management of CRS are provided in **Appendix F.** Other cytokine-directed therapies may be considered after discussion with the PI.

7.4 Neurologic Toxicity

Neurotoxicity, manifested as delirium, seizures, focal neurologic deficits, and/or coma, has been reported after CAR T-cell therapy. Although neurotoxicity has not been reported after transgenic TCR therapy, if neurotoxicity develops, management will be given analogous to CAR therapy. Neurotoxicity to CAR-T is usually reversible but can be irreversible or fatal.

For patients who develop mild neurologic manifestations (CTCAE Grade 1), symptomatic care and levetiracetam are recommended. Discussion with the PI or designee is recommended. For patients with worsening neurologic changes, the addition of corticosteroids should be considered (e.g., dexamethasone 10 mg IV every 6 to 24 hours). Tocilizumab (4-8 mg/kg IV) or other cytokine-directed therapies may be used based on clinical judgment; at this time, it is unclear if these approaches are of benefit to patients with neurotoxicity. Cerebrospinal fluid (CSF) assessments and CNS imaging should be considered if clinically indicated.

7.5 Tumor Lysis Syndrome

Although rarely reported for TNBC, urothelial cancer, and NSCLC, FH-MagIC TCR-T may cause tumor lysis syndrome (TLS) in patients with high disease burden. Patients with large bulky tumors will be considered for allopurinol prophylaxis to start prior to infusion and continued for as long as the medical team determines appropriate after the infusion, unless contraindications. Patients may receive additional hydration for the first 2 weeks after infusion. If TLS develops, as defined by the Cairo Bishop criteria, [68] the attending physician will direct patient management with guidance from the study staff. [69] Conservative therapy, including allopurinol and IV fluid hydration may be instituted immediately for suspected TLS. Hyperkalemia may be treated with potassium-binding resins, diuresis, or insulin/dextrose therapy. Hyperphosphatemia may be treated with phosphate-binding resins. In severe cases, rasburicase (in non-G6PD-deficient individuals) or renal dialysis may be necessary.

7.6 Macrophage Activation Syndrome

Macrophage activation syndrome (MAS) is a serious disorder potentially associated with uncontrolled activation and proliferation of T cells and subsequent activation of macrophages. MAS is typically characterized by high-grade, non-remitting fever, cytopenias, and hepatosplenomegaly, and may be difficult to distinguish from CRS. Laboratory abnormalities found in MAS include elevated inflammatory cytokine levels, serum ferritin, soluble IL-2 receptor (sCD25), triglycerides, and decreased circulating NK cells. Other findings include variable levels of transaminases, signs of acute liver failure, coagulopathy, and disseminated intravascular coagulopathy. While there are no definitive diagnostic criteria for MAS, it is typically diagnosed using published criteria for hemophagocytic lymphohistiocytosis [70].

Patients treated with FH-MagIC TCR-T should be monitored for MAS, and cytokine-directed therapy or corticosteroids should be considered as clinically indicated.

7.7 Adrenal Toxicity

Immune-mediated damage to the adrenal gland is a potential toxicity, and could manifest as primary mineralocorticoid or glucocorticoid deficiency.

If mineralocorticoid and/or glucocorticoid insufficiency should occur, diagnosis may be difficult in the acute setting of CRS, and attention should be given to ensuring there is sufficient replacement therapy. For patients with hypotension in the absence of fever that is unresponsive to fluid boluses, fludrocortisone or other mineralocorticoid administration should be considered. If CRS requiring intervention with tocilizumab and dexamethasone should occur, the activity of dexamethasone 10 mg/bid will likely be sufficient; however, if hypotension recurs following cessation of dexamethasone or persists despite dexamethasone, stress-dose hydrocortisone is recommended. Samples will be assayed for renin, aldosterone and cortisol levels. To detect subclinical hypoadrenalism, an adrenocorticotropic hormone (ACTH) stimulation test will be performed at baseline and approximately 4 weeks after infusion

7.8 Immune-Mediated Adverse Events

Atezolizumab is associated with a known risk of immune-related adverse events (irAEs). Patients will also be closely monitored for potential irAEs,[71,72] which may manifest earliest after weeks of treatment. Such events may consist of persistent rash, diarrhea and colitis, autoimmune hepatitis, arthritis, glomerulonephritis, cardiomyopathy, or uveitis and other inflammatory eye conditions. The spectrum of hypothetical irAEs also includes formation of auto-antibodies like anti-nuclear antibodies (ANAs) or antineutrophil cytoplasmic antibodies (ANCAs). Severe IRAEs will be managed with steroids. Where possible, IRAEs definitely, probably, or possibly secondary to atezolizumab will be managed by the Attending Physician in consultation with the study team in accordance with ASCO society guidelines.[73]

7.9 Persistent Uncontrolled T-cell Proliferation

Uncontrolled proliferation of TCR-modified T cells has not been observed in clinical trials to date.[56] However, in the unlikely event that clinically significant uncontrolled and persistent proliferation of T cells occurs in a study patient, initial therapy may involve treatment with corticosteroids (e.g.,

methylprednisolone 1 g IV). If there is a progressive increase in TCR T cells to greater than 30% of T cells at more than 3 months after the last infusion is observed, an analysis for clonal expansion by deep sequencing of the T-cell receptor (TCR) beta gene (Adaptive Biotechnology) may be conducted.

7.10 Replication-Competent Lentivirus

All patients will be followed in this study for presence of replication-competent lentivirus (RCL) and in blood for up to 15 years following the last dose of FH-MagIC TCR-T. Research studies to detect presence and phenotype T cells may be conducted on samples of tissue biopsies or blood performed for clinical conditions occurring during follow-up considered possibly related to FH-MagIC TCR-T.

7.11 Management of Other Toxicities

If a new onset CTCAE v5 Grade ≥ 3 toxicity is observed following infusion, the patient will receive investigation and medical treatment appropriate for the physiological abnormalities. Grade ≥ 3 toxicity that is attributed to the infusion may be treated with corticosteroids (e.g., dexamethasone 10 mg IV every 4 to 12 hours), tocilizumab, or other cytokine-directed therapy after discussion with the PI or designee.

8.0 INFORMED CONSENT OF SUBJECT AND DONOR

8.1 Patient screening

A Screening Consent, separate from the treatment consent, and HIPAA Consent will be used for the patient to provide consent for records review, pathology review, MAGE-A1 testing and HLA typing. Where possible, consent for evaluation will be performed in person. However, for many patients this will not be possible for travel/logistical reasons. It is anticipated that some patients reside outside of the area and may have their screening blood draws completed locally. Screening blood draws for HLA typing may be performed by the patient's local provider and shipped to the SCCA or SCISCO for resulting.

8.2 Treatment

Patients will be seen at the SCCA/UWMC for consideration of treatment options for their disease and be offered to sign the Treatment Consent. The protocol will be discussed thoroughly with the patient and other family members if appropriate, and all known and potential risks to the patient will be described. The procedure and alternative forms of therapy will be presented as objectively as possible, and the risks and hazards of the procedure explained to the patient. Treatment Consent from the patient will be obtained using forms approved by the Fred Hutch IRB. A summary of the clinic visit detailing what was covered will be dictated for the medical record.

9.0 SUBJECT REGISTRATION

Potential subjects will be identified and registered into the Institution's system (CTMS and Gateway) by the Data Management office or the protocol Research Coordinator. Each subject is assigned a Unique Patient Number (UPN).

Subjects will be evaluated for eligibility and enrolled on to the main study after data related to the inclusion and exclusion criteria are reviewed by the PI or delegate. Following enrollment patients will undergo leukapheresis.

A study number will be allocated to each patient and a log of enrolled subjects will be maintained.

Patients enrolled in the protocol are eligible for reimbursement of up to $3500 for travel expenses (i.e., airfare, mileage, parking and overnight accommodations) related to their participation in this study from the time of consent to 28 days post immunotherapy infusion.

10.0 CLINICAL AND LABORATORY EVALUATIONS

A tabular schedule of events is provided in **Appendix A.** The proposed days of all treatments and assessments are approximate and may vary due to scheduling, clinical or other factors.

10.1 Screening Evaluations

1) HLA Typing
2) MAGE-A1 expression

10.2 Pre-treatment Evaluations

All assessments must be performed within 30 days before enrollment unless otherwise noted. Results of tests and procedures conducted as per standard of care purposes prior to screening may be used for research purposes if conducted within the protocol-defined window.

The Treatment Consent must be signed before any non-standard of care evaluations are performed.

1) Medical history and physical examination, including prior therapies and response to therapy, if known, height/weight and ECOG performance status.
2) Laboratory tests, including:
    a. CBC, differential, platelet count
    b. Renal, hepatic function with LDH, Mg
    c. SCCA (BWNW) Patient/recipient virology testing (should be performed within 30 days of leukapheresis)
    d. Serum ferritin
    e. Serum CRP
    f. Uric acid
    g. Serum IL-6
    h. DIC panel without platelets.
    i. Serum pregnancy test for females of childbearing potential within 14 days of planned enrollment
    j. Quantitative IgG (for patients receiving LD chemotherapy)
    k. ABO blood typing
    l. G6PD