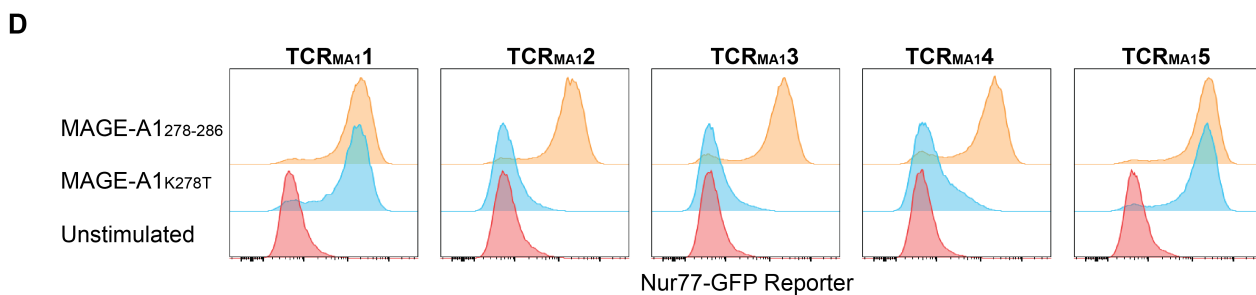
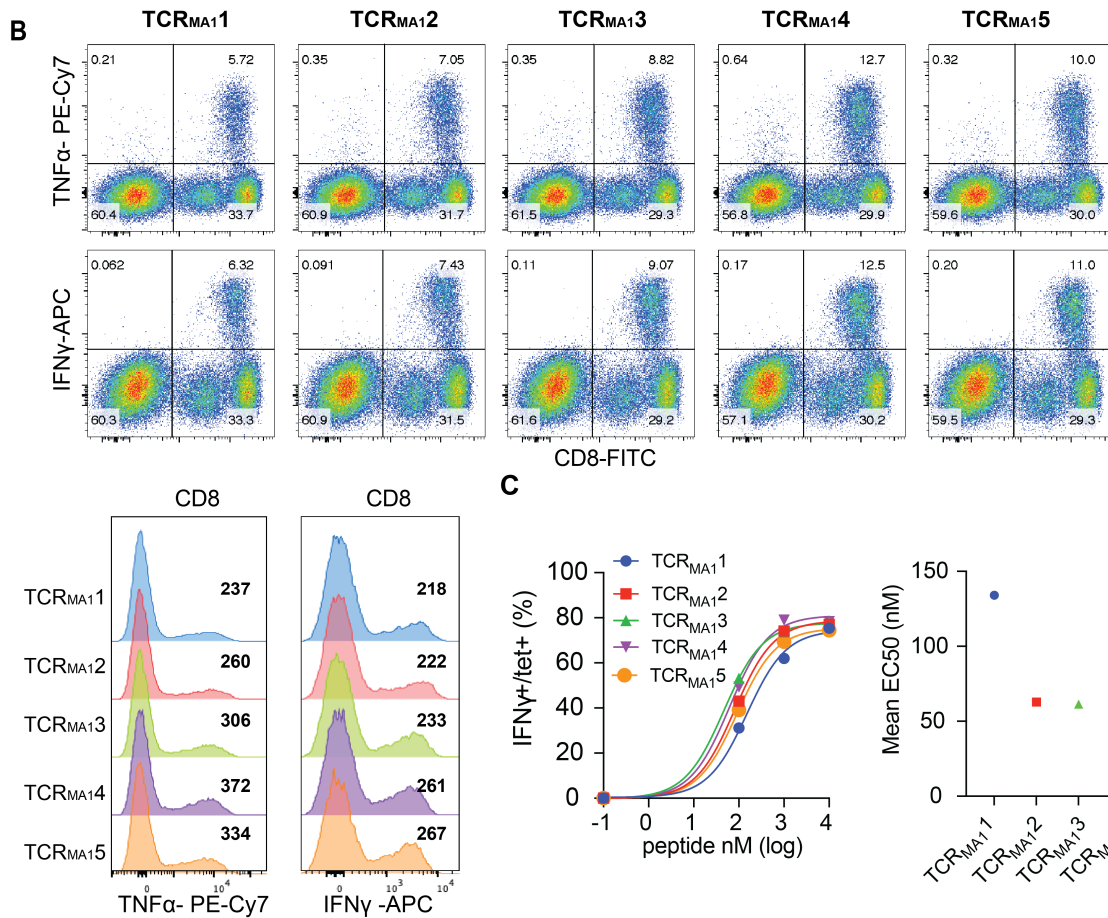
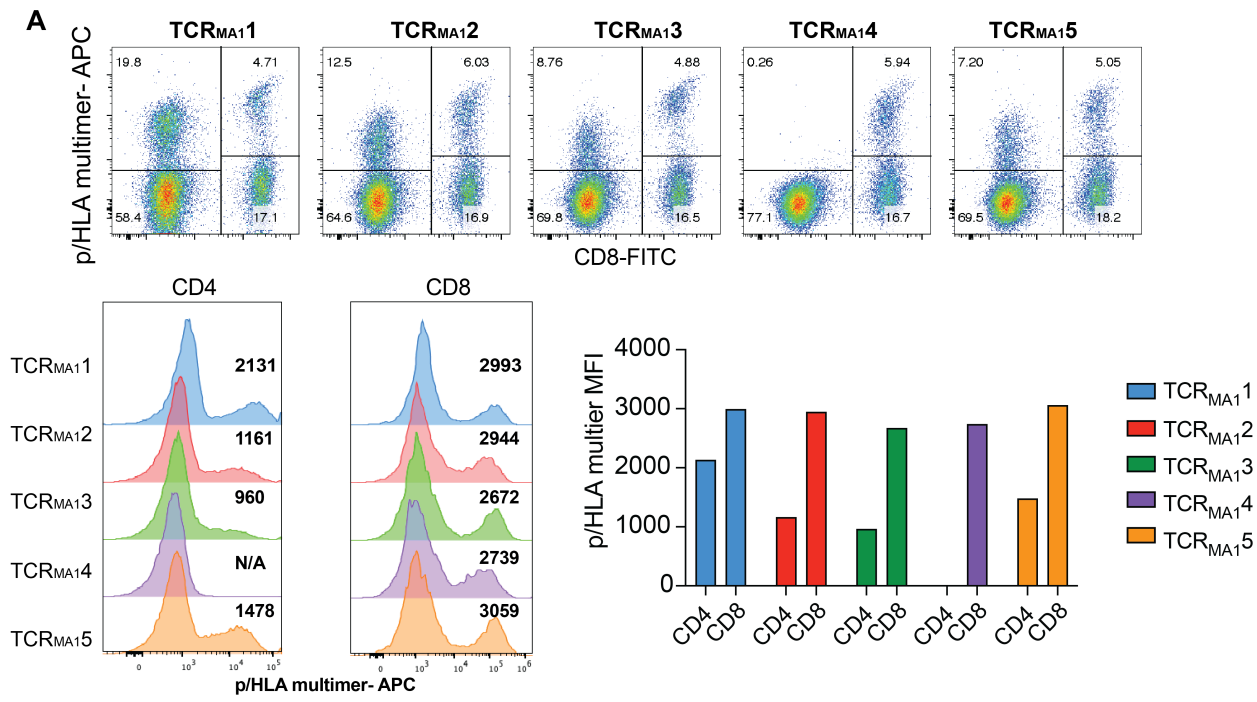


Supplementary Information for
A CD8 $\alpha\beta$ co-receptor modified to contain an intracellular CD28 signaling tail
enhances TCR-engineered T cell function independent of solid-tumor-associated co-
stimulatory ligands

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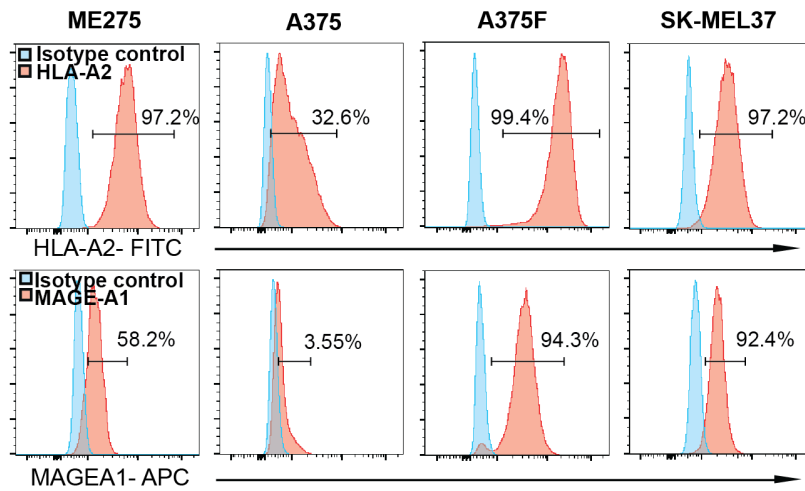
The PDF file includes:

Supplementary Fig. 1 to 15
Supplementary Table 1-8
Supplementary Note 1



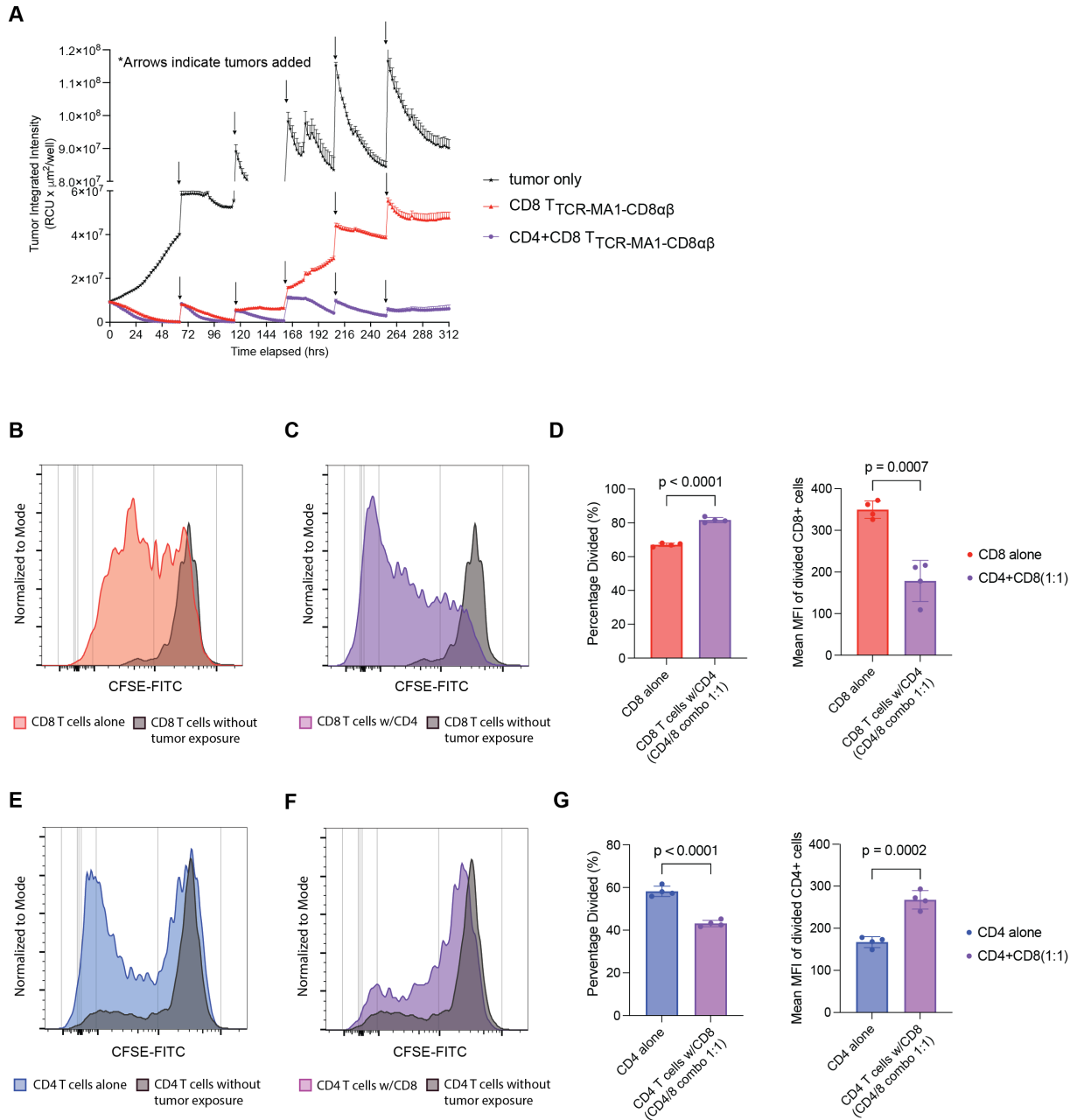
Supplementary Fig. 1: Identification of an HLA A*0201-restricted MAGE-A1₂₇₈₋₈₆ specific TCR for clinical translation.

(A) Dot plot of CD8 and p/MHC multimer binding (top row), histograms (bottom left) and bar graphs (bottom right) of p/MHC multimer mean fluorescence intensities (MFI) of TCR_{MA1}1-5 transduced donor PBMC CD8⁻ (representing CD4⁺ T cells) and CD8⁺ T cell populations. **(B)** TNF α (top row) and IFN γ (bottom row) intracellular levels in CD8⁻ (representing CD4⁺ T cells) and CD8⁺ T cells, histograms (bottom panel) representing TNF α and IFN γ MFI of CD8⁺ T cells transduced to express TCR_{MA1}1-5 after an 18-hour incubation with 1 μ M cognate peptide. **(C)** Dose response of MAGE A1₂₇₈₋₈₆ peptide stimulated CD8⁺ T cells transduced with TCR_{MA1}1-5 TCRs, indicated by IFN γ levels (left) and mean half-maximal effective concentrations required to induce 50% IFN γ expression (EC50) (right). **(D)** Recognition of wildtype MAGE-A1₂₇₈₋₈₆ peptide or MAGE-A1₂₇₈₋₈₆ K278T mutant peptide [loaded on TAP-deficient T2 B lymphoblastoid cell lines (T2-BLCL)] by the TCR-deficient Nur-77 reporter Jurkat cells transduced with TCR_{MA1}1-5. Histograms represent GFP expression downstream of Nur77-GFP triggered when the TCR is engaged.



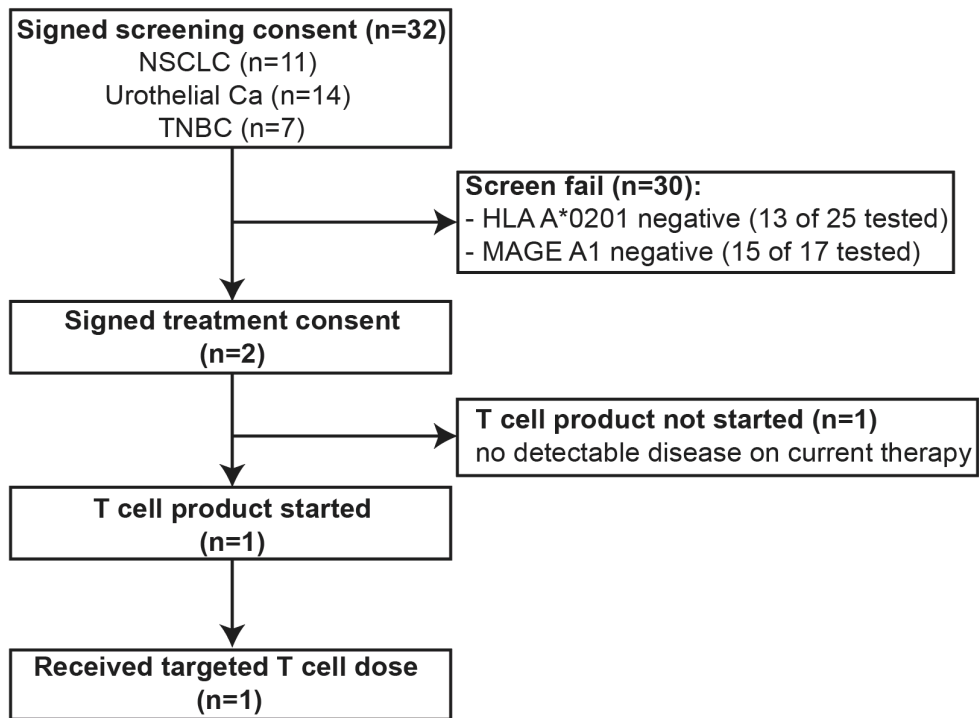
Supplementary Fig. 2: Tumor cell line HLA-A2 and MAGE-A1 expression.

Expression of extracellular HLA-A2 (top row) and intracellular MAGE-A1 (bottom row) by the melanoma tumor cell lines ME275, A375, A375F(MAGEA1 and HLA-A2 overpressed) and SK-MEL-37. Histogram plots show representative staining (red) and isotype control (blue).



Supplementary Fig. 3: $CD8^+$ $T_{TCR-MA1-CD8\alpha\beta}$ cell enhanced proliferation with presence of $CD4^+$ $T_{TCR-MA1-CD8\alpha\beta}$.

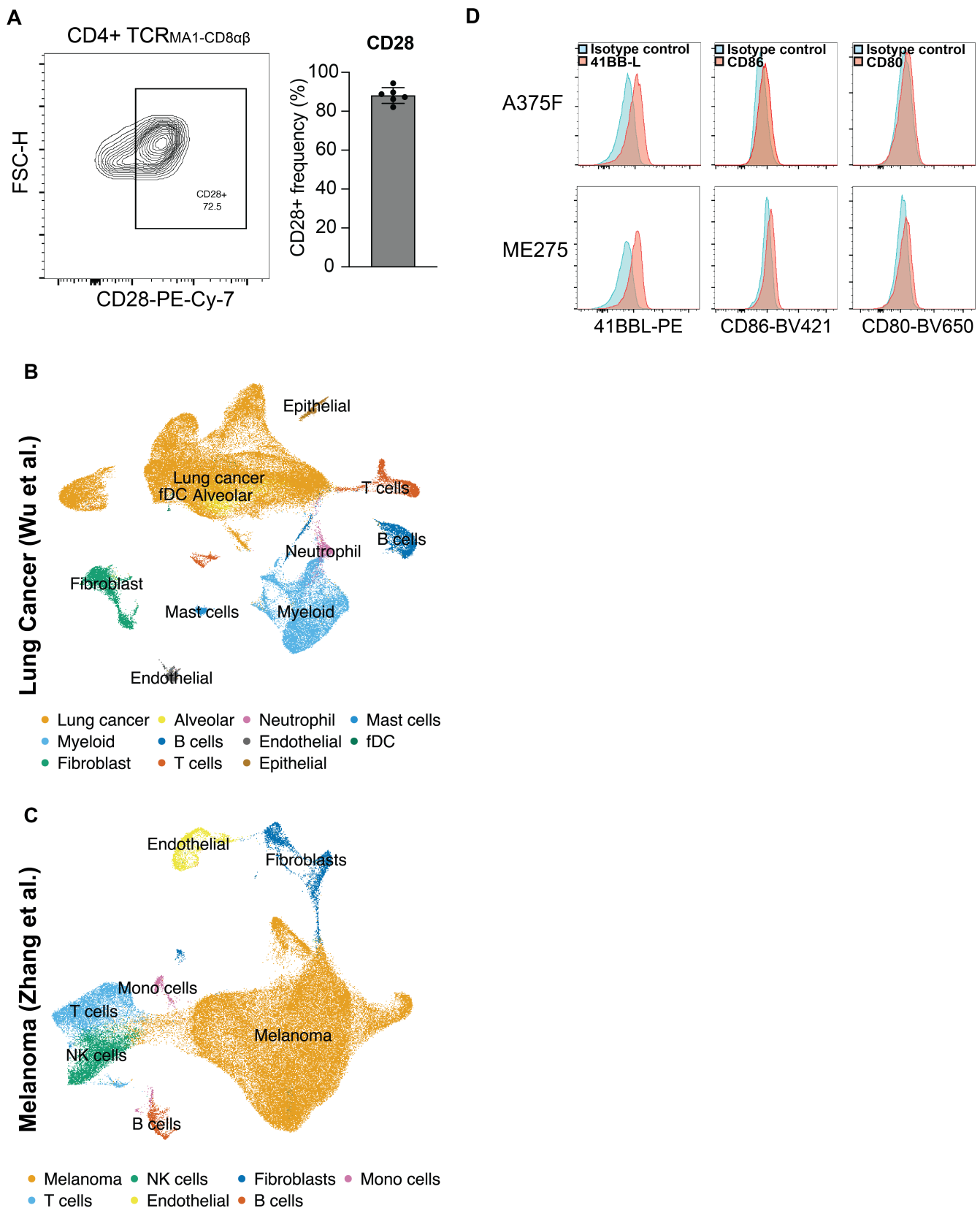
(A) Growth kinetics for the ME275 cell line in a live tumor visualization assay for 312 hours (13 days) (Incucyte S3) in the absence (black line) or presence of equal total numbers of seeded $CD8^+$ $T_{TCR-MA1-CD8\alpha\beta}$ (red line) or $CD4^+$ $CD8^+$ $T_{TCR-MA1-CD8\alpha\beta}$ (purple line). The E:T ratio was 2:1, and arrows indicate the addition of tumor cells to the culture. The mean $\pm$ SD of triplicate wells is shown. (n=3 wells/group). (B-G) Co-culture of ME275 with $CD4^+$ $T_{TCR-MA1-CD8\alpha\beta}$ (blue), $CD8^+$ $T_{TCR-MA1-CD8\alpha\beta}$ (red), or $CD4^+$ $CD8^+$ $T_{TCR-MA1-CD8\alpha\beta}$ (purple) at E:T ratio of 5:1 for 7 days. (B-C) Histogram representing cell division between $CD8^+$ $T_{TCR-MA1-CD8\alpha\beta}$ alone without tumor stimulation (gray), $CD8^+$ $T_{TCR-MA1-CD8\alpha\beta}$ alone (red) or $CD8^+$ $T_{TCR-MA1-CD8\alpha\beta}$ with $CD4^+$ $T_{TCR-MA1-CD8\alpha\beta}$ (purple) with tumor stimulation identified by CFSE proliferation dye. (D) Percentage divided (left panel) and mean MFI of divided cells (right) of $CD8^+$ $T_{TCR-MA1-CD8\alpha\beta}$ alone (red) compared with $CD8^+$ $T_{TCR-MA1-CD8\alpha\beta}$ (purple) from $CD4^+$ / $CD8^+$ $T_{TCR-MA1-CD8\alpha\beta}$ combination group. The mean $\pm$ SD of duplicated wells from two experiments is shown (n=4). p value determined by unpaired two-tailed t-test. (n=4 wells) (E-F) Histogram representing cell division between $CD4^+$ $T_{TCR-MA1-CD8\alpha\beta}$ alone without tumor stimulation (gray), $CD4^+$ $T_{TCR-MA1-CD8\alpha\beta}$ alone (red) or $CD4^+$ $T_{TCR-MA1-CD8\alpha\beta}$ with $CD8^+$ $T_{TCR-MA1-CD8\alpha\beta}$ (purple) with tumor stimulation identified by CFSE proliferation dye. (n=4 wells/group). (G) Percentage divided (left panel) and mean MFI of divided cells (right) of $CD4^+$ $T_{TCR-MA1-CD8\alpha\beta}$ alone (red) compared with $CD4^+$ $T_{TCR-MA1-CD8\alpha\beta}$ (purple) from $CD4^+$ / $CD8^+$ $T_{TCR-MA1-CD8\alpha\beta}$ combination group. The mean $\pm$ SD of duplicated wells from two experiments is shown. p value determined by unpaired two-tailed t-test. (n=4 wells/group).



Supplementary Fig 4: Patient consort diagram

Supplementary Fig. 5: MAGE-A1 IHC from patient biopsy and *in vivo* TILs analysis.

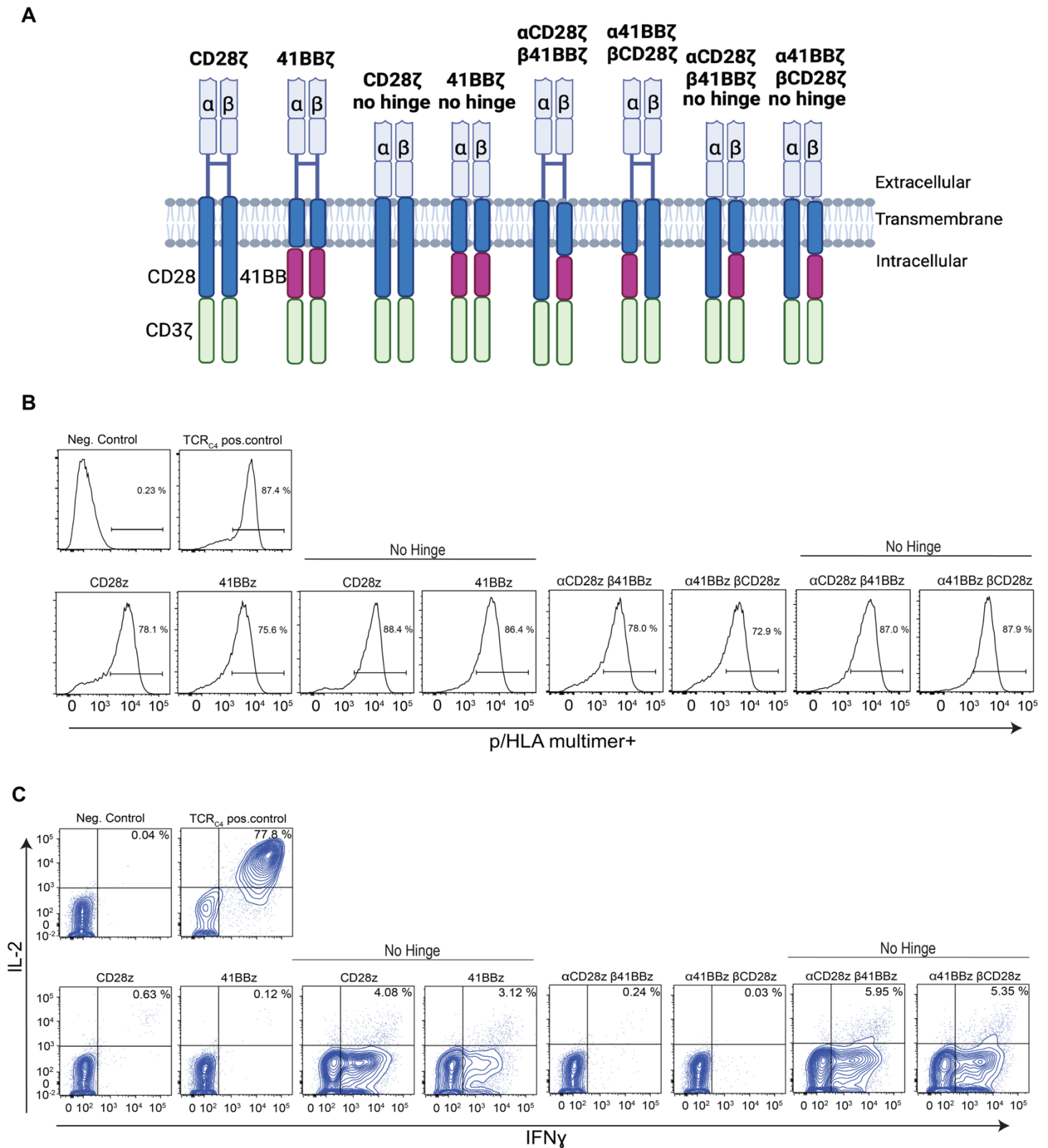
(A) MAGE-A1 expression by IHC of tumor core biopsies for a semiquantitative H score of 20 obtained from the patients before the first infusion. Scale bars represents 50 μ M. **(B)** Co-expression of PD-1 (x-axis) and Tim-3 (y-axis) in CD4⁺ (left 3 panels) and CD8⁺ (right 3 panels) T cells identified in spleen (top panels) or tumor (second row panels) for the indicated conditions. Co-expression of CD39⁺ (x-axis) and Tim-3⁺ (y-axis) (third row panels) and expression of 2B4⁺ (fourth row panels) in CD4⁺ T_{TCR-MA1-CD8 α β} (left 3 panels) and CD8⁺ T_{TCR-MA1-CD8 α β} (right 3 panels). Single cell suspensions were obtained at sacrifice, 8 days after T cell infusion from **(Fig. 2D)**. **(C)** Immunohistochemistry analysis of FOXP3 intranuclear expression in CD4⁺ T_{TCR-MA1-CD8 α β} between the CD4⁺ alone and the CD4⁺ + CD8⁺ combination (1:1 ratio) groups from **(Fig. 2D)**. The mean and $\pm$ SEM of 6 tumor is shown (n=6). p value determined by unpaired two-tailed t-test. (n=3 mice, 2 tumors/mouse).



Supplementary Fig. 6: Melanoma and lung cancer patient *in silico* analysis.

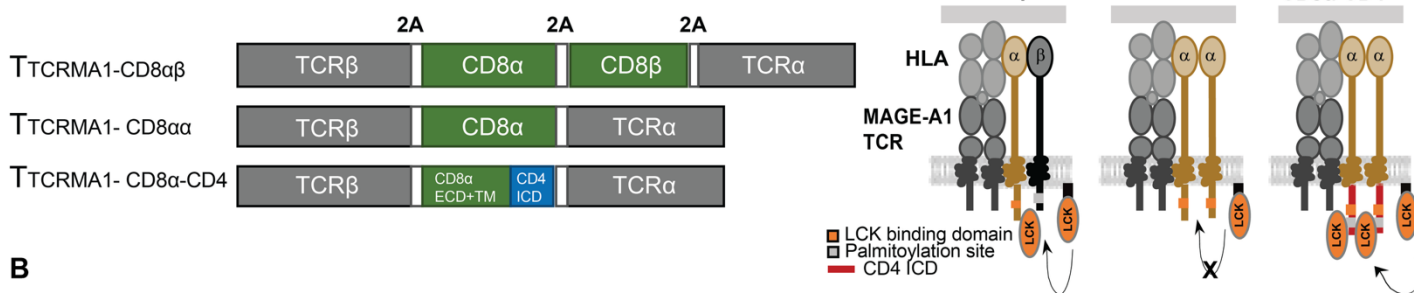
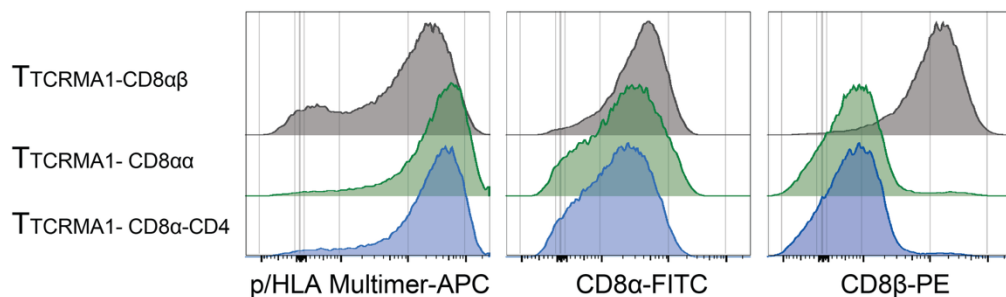
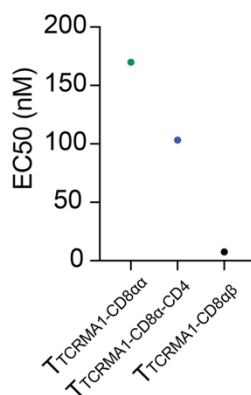
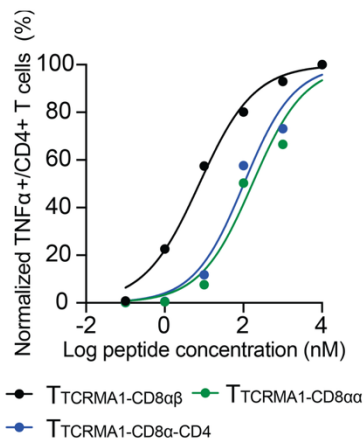
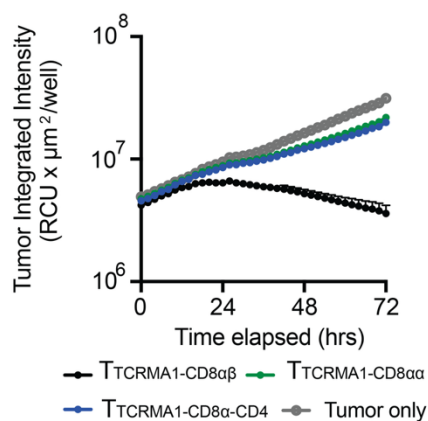
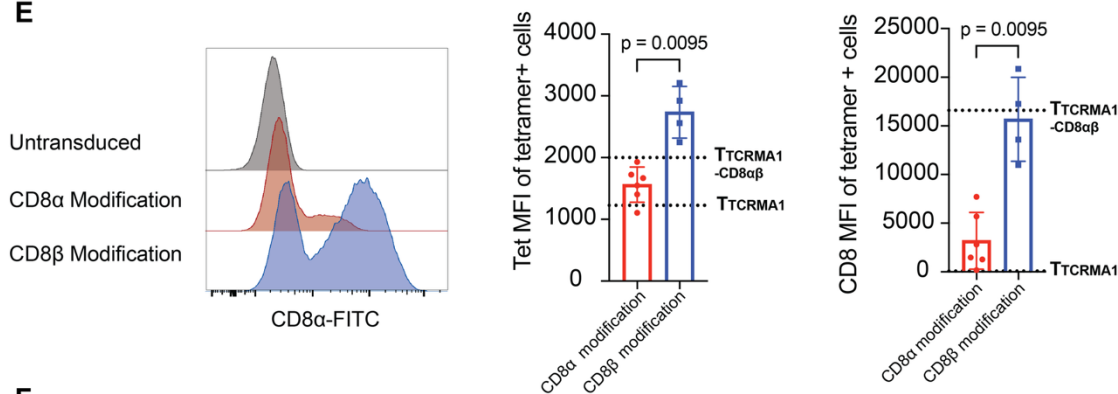
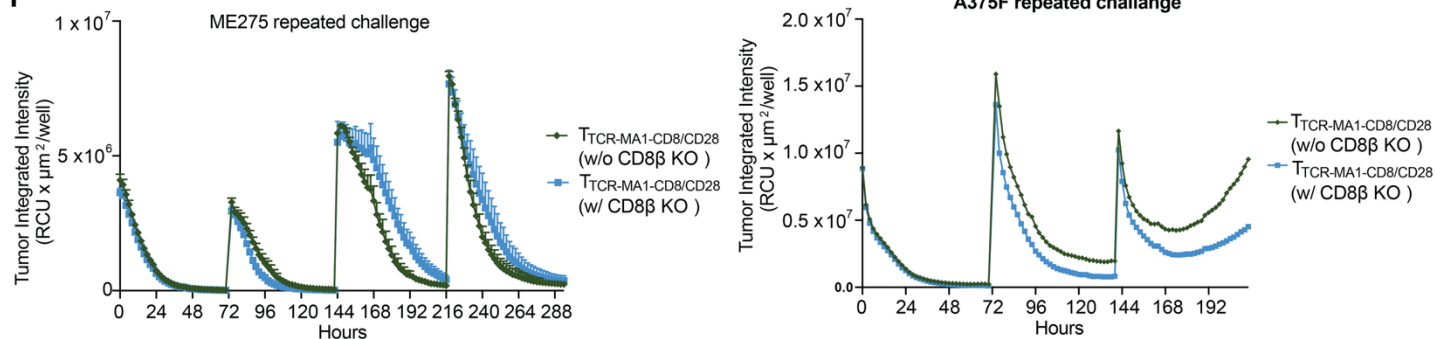
(A) Surface expression of CD28 (left) in CD4⁺ T_{TCR-MA1-CD8 $\alpha\beta$} before injection into *in vivo* models and the bar graph represents expression of surface CD28 (right) CD4⁺ T_{TCR-MA1-CD8 $\alpha\beta$} with different CCRs. (n=6 wells). (B-C) UMAP plot showing the two-dimensional distribution of the different cell states identified in the lung cancer (B) and melanoma (C) scRNAseq datasets based on differential gene expression.

(D) Expression of surface 41BB-L, CD86, CD80 of A375F and ME275 tumor cell lines used in this study. Histogram plots show representative staining (red) and isotype control (blue).



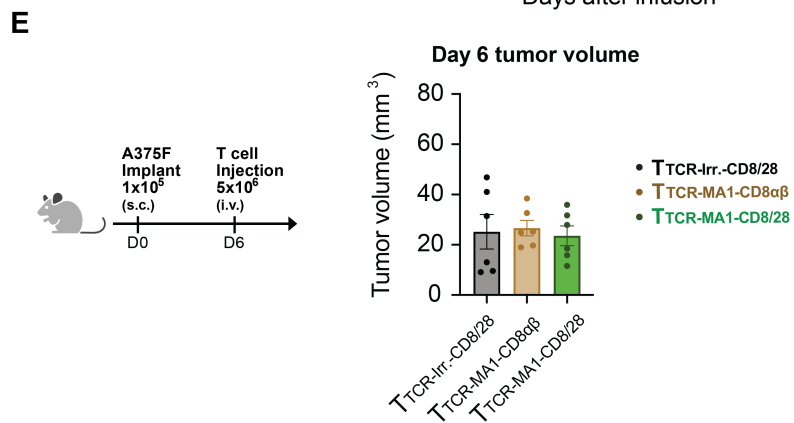
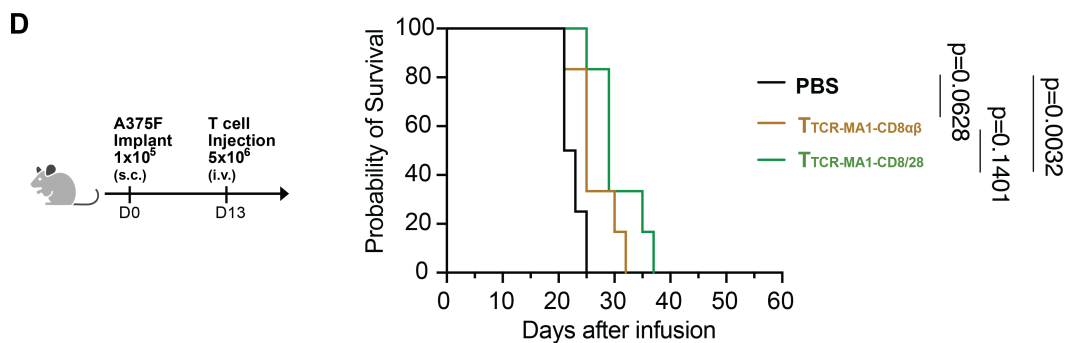
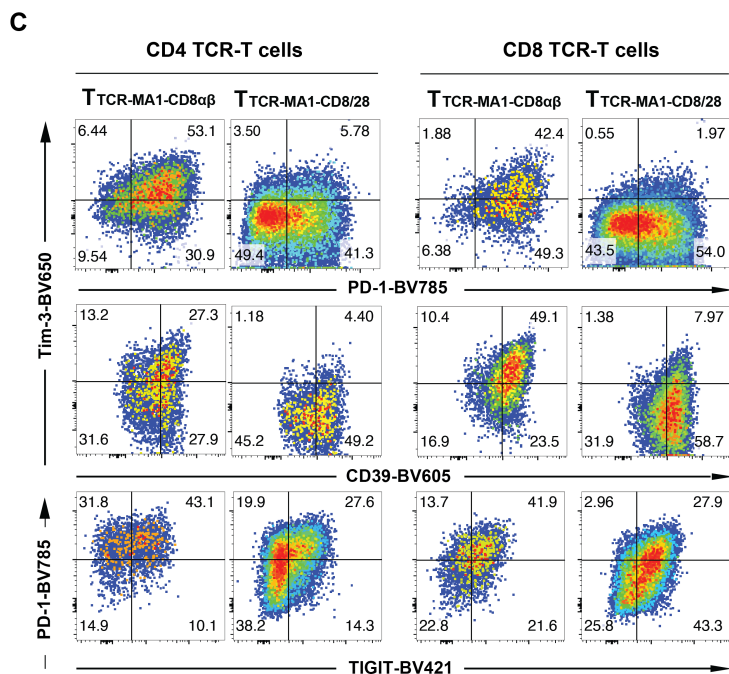
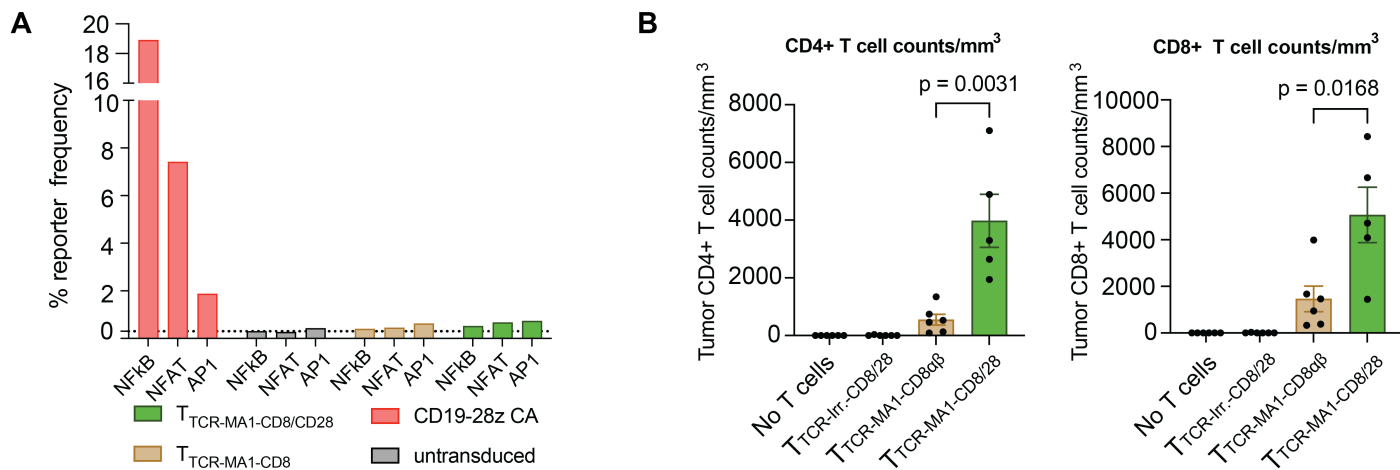
Supplementary Fig. 7: TCR signaling dampened upon TCR intracellular modification.

(A) Schematic of construct design. Image created in BioRender. Tang, A. (2026) <https://BioRender.com/6wg9k7f>; **(B)** Chimeric TCRs are expressed on the cell surface identified by p/HLA multimer. CD8⁺ T cells were transduced with the modified TCR_{C4} constructs and p/HLA multimer binding was analyzed by flow cytometry. **(C)** TCR transduced CD8⁺ T cells were stimulated with cognate peptide at 1uM and IL-2 and IFN γ levels were analyzed by flow cytometry.

A**B****C****D****E****F**

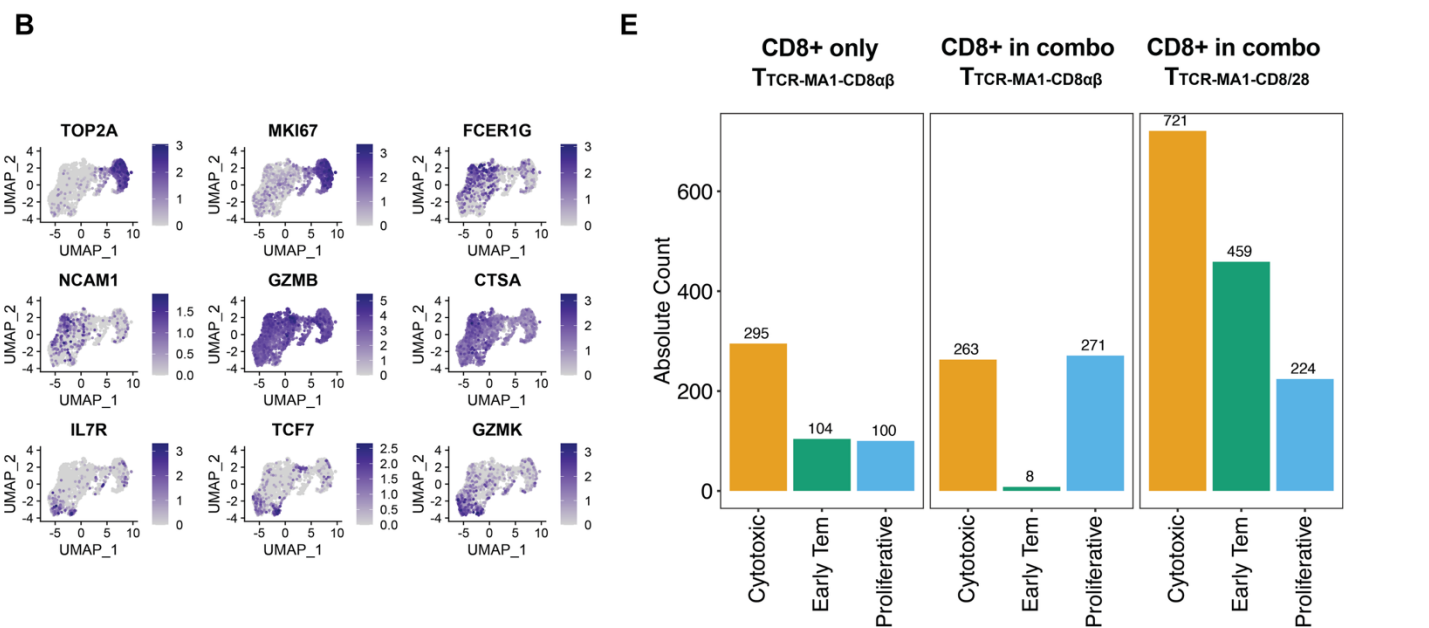
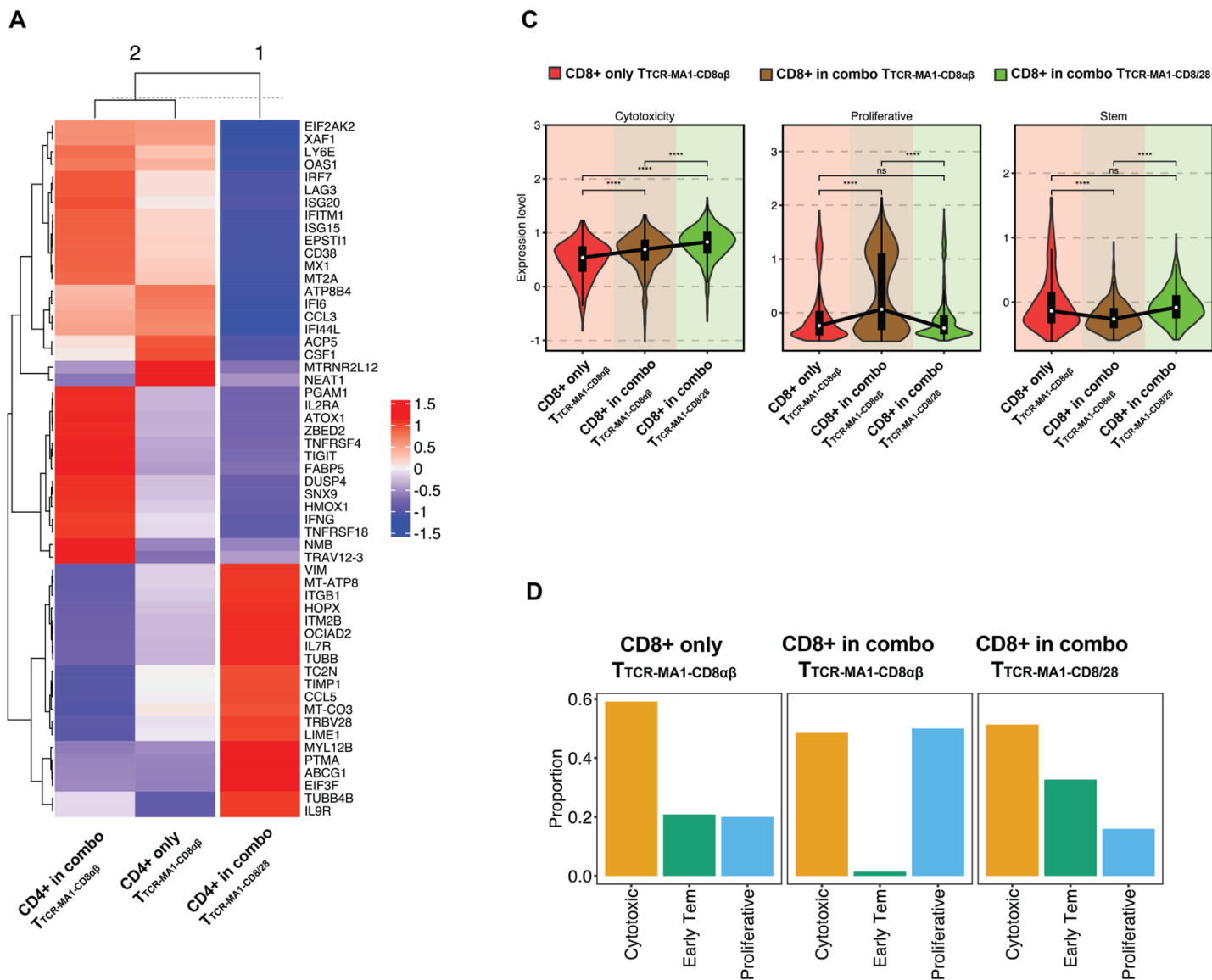
Supplementary Fig. 8: CD8 β chain is essential for MAGE-specific CD4⁺ TCR-T cells function.

(A) (left) Schema of the multi-cistronic vector designs used to express TCR-CD8 $\alpha\beta$, TCR with CD8 α only (TCR-CD8 $\alpha\alpha$), and CD8 α with additional CD4 intracellular domain (TCR-CD8 α -CD4), (right) schema representing the three construct's LCK binding domain and palmitoylation sites. Image created in BioRender. Tang, A. (2026) <https://BioRender.com/6wg9k7f> **(B)** Expression of p/HLA multimer (left), CD8 α (middle), and CD8 β (right panel) on the indicated constructs. **(C)** Dose response curves of TNF α expression by CD4⁺ T cells transduced with the indicated constructs exposed to decreasing concentrations of MAGE A1₂₇₈₋₈₆ (left), mean half-maximal effective concentrations required to induce 50% TNF α expression (EC50) (right). **(D)** Growth kinetics for the A375F cell line in a live tumor visualization assay in the absence (gray line) or presence of CD4⁺ transduced with T_{TCR-MA1} with CD8 $\alpha\beta$ (black line), CD8 α (CD8 $\alpha\alpha$ dimer, green line) and CD8 α -CD4 (blue line). An E:T ratio of 5:1 was used for all conditions. Graph represents mean $\pm$ SD. (n=3 wells) **(E)** (left) Surface expression of CD8 on Jurkat cells untransduced (top) or modified to express CD8 α with intracellular CD28 (middle), and CD8 β with intracellular CD28 (bottom) with T_{TCR-MA1}. (right) MFI of p/HLA multimer (left) and CD8 (right) on Jurkat cells transduced to express T_{TCR-MA1} and CD8 α intracellular domain-modified (red, n=6) or CD8 β intracellular domain-modified (blue, n=4) constructs. Graph represents mean $\pm$ SD. The indicated p value was calculated by Mann-Whitney U test. Dashed lines represent the MFI of T_{TCR-MA1} alone or T_{TCR-MA1} with unmodified CD8 $\alpha\beta$. (n=4-6 wells/group). **(F)** Growth kinetics of the ME275 (left) and A375F (right) cell lines in the presence of the indicated CCR constructs T_{TCR-MA1}-CD8/CD28 without CRISPR KO of endogenous CD8 β , and T_{TCR-MA1}-CD8/CD28 with CRISPR KO of endogenous CD8 β . An E:T ratio of 5:1 was used. The mean mean $\pm$ SD of triplicate wells is shown. (n=3 wells/group).



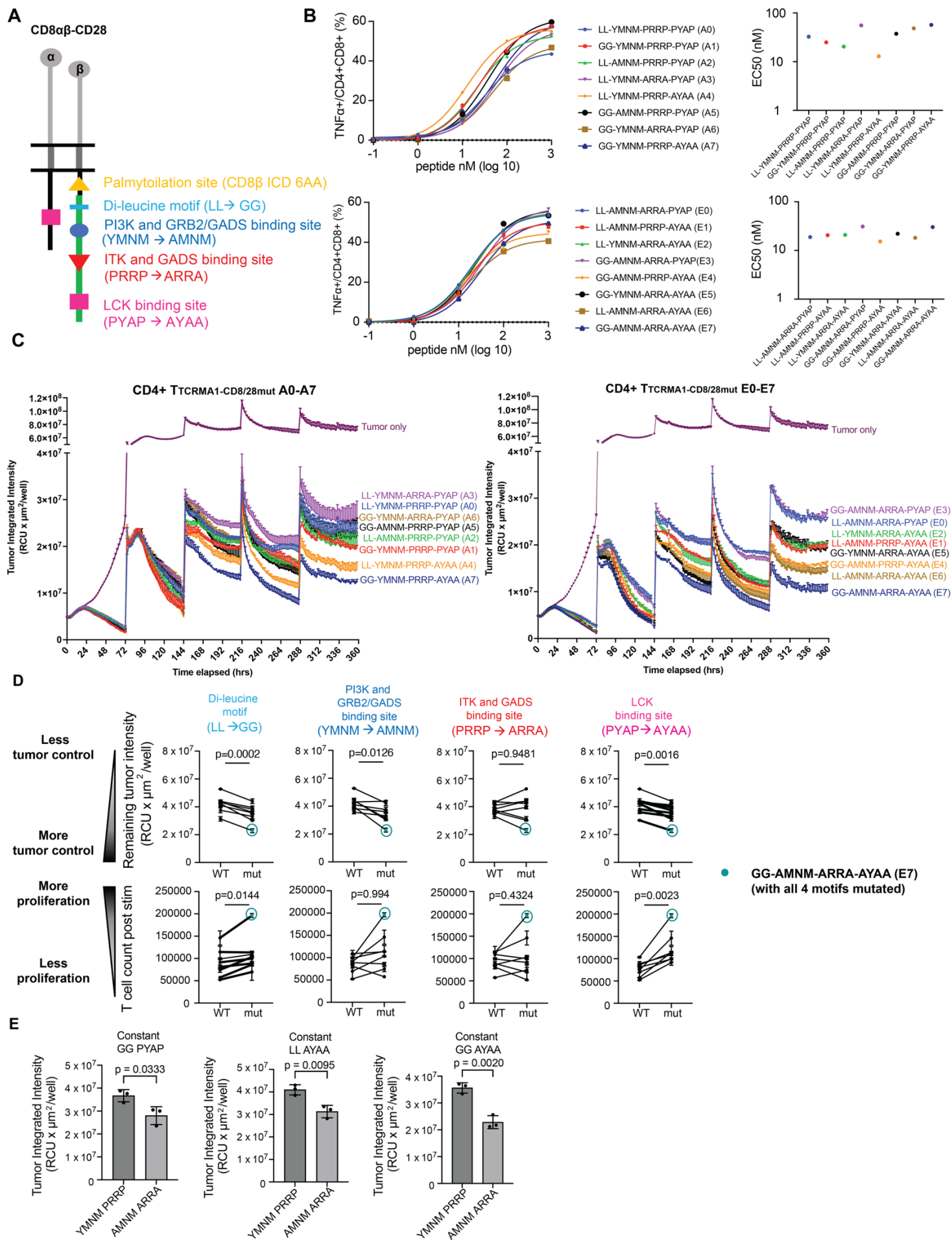
Supplementary Fig. 9: CD8/CD28 CCR enhances tumor control *in vivo* with no tonic signaling observed *in vitro*.

(A) Tonic signaling was assessed by flow cytometry analysis of NFAT, NF- κ B and AP1 expression across the T_{TCR-MA1} constructs, using triple reporter Jurkat cells expressing NF- κ B, AP1 and NFAT reporter constructs. **(B)** Ratio of CD4⁺ T cell and CD8⁺ T cell counts to tumor cells from A375F tumors engrafted in NSG mice infused with either no T cells, T_{TCR-Irr.-CD8/CD28}, T_{TCR-MA1-CD8 $\alpha\beta$} or T_{TCR-MA1-CD8/CD28}, analyzed from IHC images. Analysis was performed using HALO Link software. Graph represents mean $\pm$ SEM. p value was determined by unpaired two-tailed t-test. (n=3 mice/group, 2 tumors/mouse). **(C)** Representative flow plot showing co-expression of PD-1 (x-axis) and Tim-3 (y-axis) (top row panels), co-expression of CD39 (x-axis) and Tim-3 (y-axis) (middle row panels), and co-expression of TIGIT (x-axis) and PD-1 (y-axis) (bottom row panels) in CD4⁺ (left 2 panels) and CD8⁺ (right 2 panels) TILs identified in tumor between T_{TCR-MA1-CD8 $\alpha\beta$} and T_{TCR-MA1-CD8/CD28}. Single cell suspensions were obtained at sacrifice, 8 days after T cell infusion with same experimental groups from **(Fig. 4A)**. **(D)** Schematic (left) of NSG mice engrafted with 1×10^5 cells A375F and subsequently infused with total 5×10^6 CD4⁺ and CD8⁺ T_{TCR-MA1-CD8 $\alpha\beta$} CCR cells (T_{TCR-Irr.-CD8/CD28}, T_{TCR-MA1-CD8 $\alpha\beta$} , T_{TCR-MA1-CD8/CD28}; 1:1 ratio of combined CD4⁺ and CD8⁺ T_{TCR-MA1-CD8 $\alpha\beta$}) 13 days post engraftment. Survival analysis (right) was carried out when tumor burden reached 500mm³, and assessed tumor progression occurring 37 days vs. 32 days vs. 25 days post T cell transfer, respectively; Kaplan-Meier test for analysis of survival applied (left). p values were determined using a log-rank test. (n=4-6 mice/group). **(E)** Schematic (left) and tumor volume (right) of 1×10^5 A375F tumors engrafted into NSG mice before subsequent transfer 5×10^6 CD4⁺ and CD8⁺ T_{TCR-MA1-CD8 $\alpha\beta$} CCR cells equal numbers of T cells (T_{Irr.-CD8/28}, T_{TCR-MA1-CD8 $\alpha\beta$} , T_{TCR-MA1-CD8/28}; 1:1 ratio of combined CD4⁺ and CD8⁺ T_{TCR-MA1-CD8 $\alpha\beta$}) 6 days after engraftment. Graph represents mean $\pm$ SEM (n=6 mice/group). Panel D,E created in BioRender. Tang, A. (2026) <https://BioRender.com/6wg9k7f>.



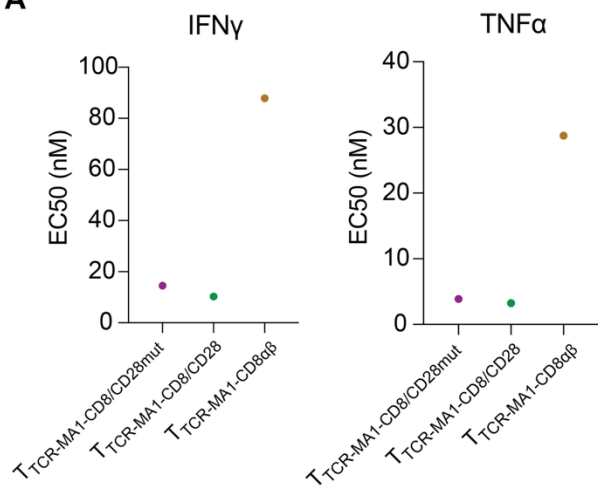
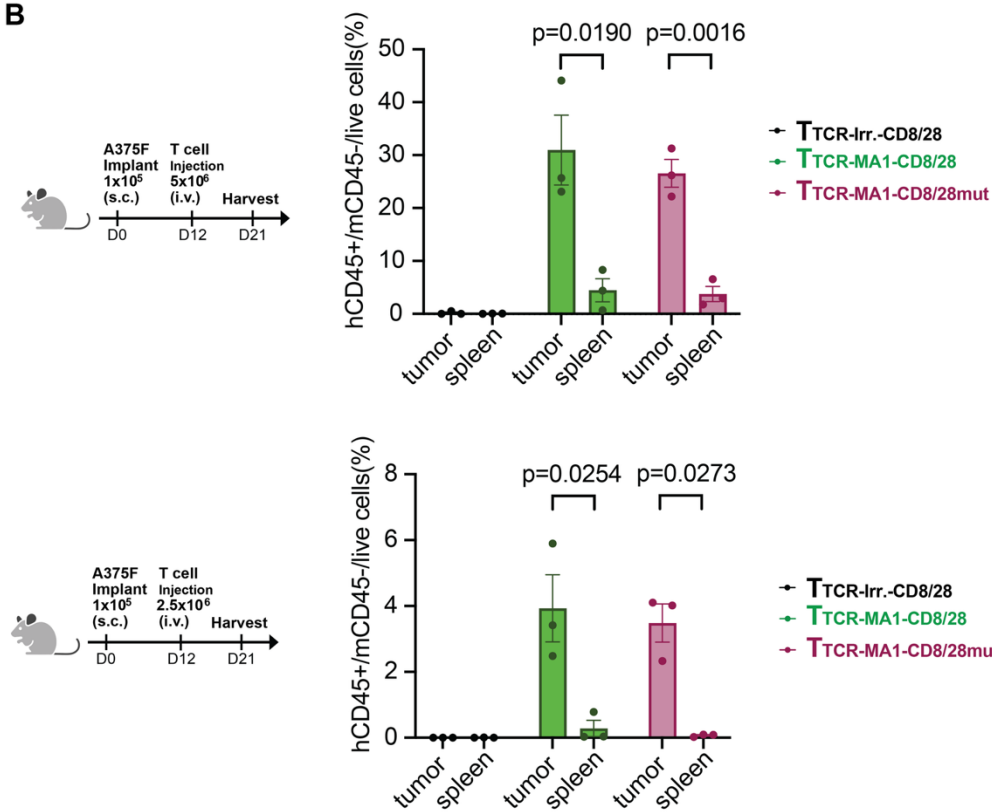
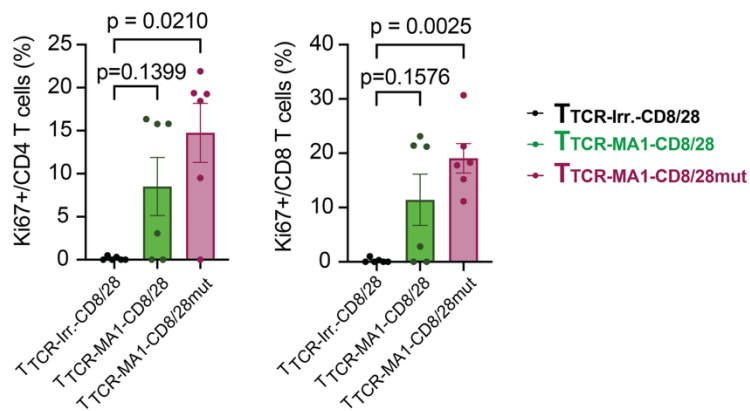
Supplementary Fig. 10 CD4⁺ CD8/CD28 co-receptor TCR-T cells associated with self-renewal genes *in vivo*.

(A) Heatmap showing the top 20 genes with the most significant differential expression identified within each murine group. The dendrogram on the left displays the similarity between the groups, which were determined through unsupervised clustering based on gene co-expression patterns. The top dendrogram shows the relationships between the different groups based on their gene expression patterns. **(B)** UMAP visualization of CD8⁺ T cells, with color intensity indicating expression of selected marker genes. **(C)** Violin plots displaying the expression levels (y-axis) of stem-like (*TCF7*, *SELL*, *IL7R*, *LTB*, *LEF1*, *NELL2*), proliferation (*MKI67*, *TOP2A*, *PCNA*), and cytotoxicity (*KLRG1*, *KLRD1*, *NKG7*, *GZMB*, *GNLY*, *PRF1*, *GZMA*, *GZMM*, *CSTA*, *CSTW*) markers across CD8⁺ T-cell transcriptional states (x-axis). Statistical significance was assessed using two-sided Wilcoxon rank sum test. Boxplots within violins show the median, interquartile range (box; 25th–75th percentiles), and whiskers extending to 1.5× IQR. Asterisks indicate the following thresholds of significance: **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001. **(D)** Bar graph colored by the annotated T cells indicating the proportional (y-axis) abundance across the annotated subsets (x-axis) for the three groups. **(E)** Bar graph colored by the annotated T cells indicating the absolute (y-axis) abundance across the annotated subsets (x-axis) for the three groups.



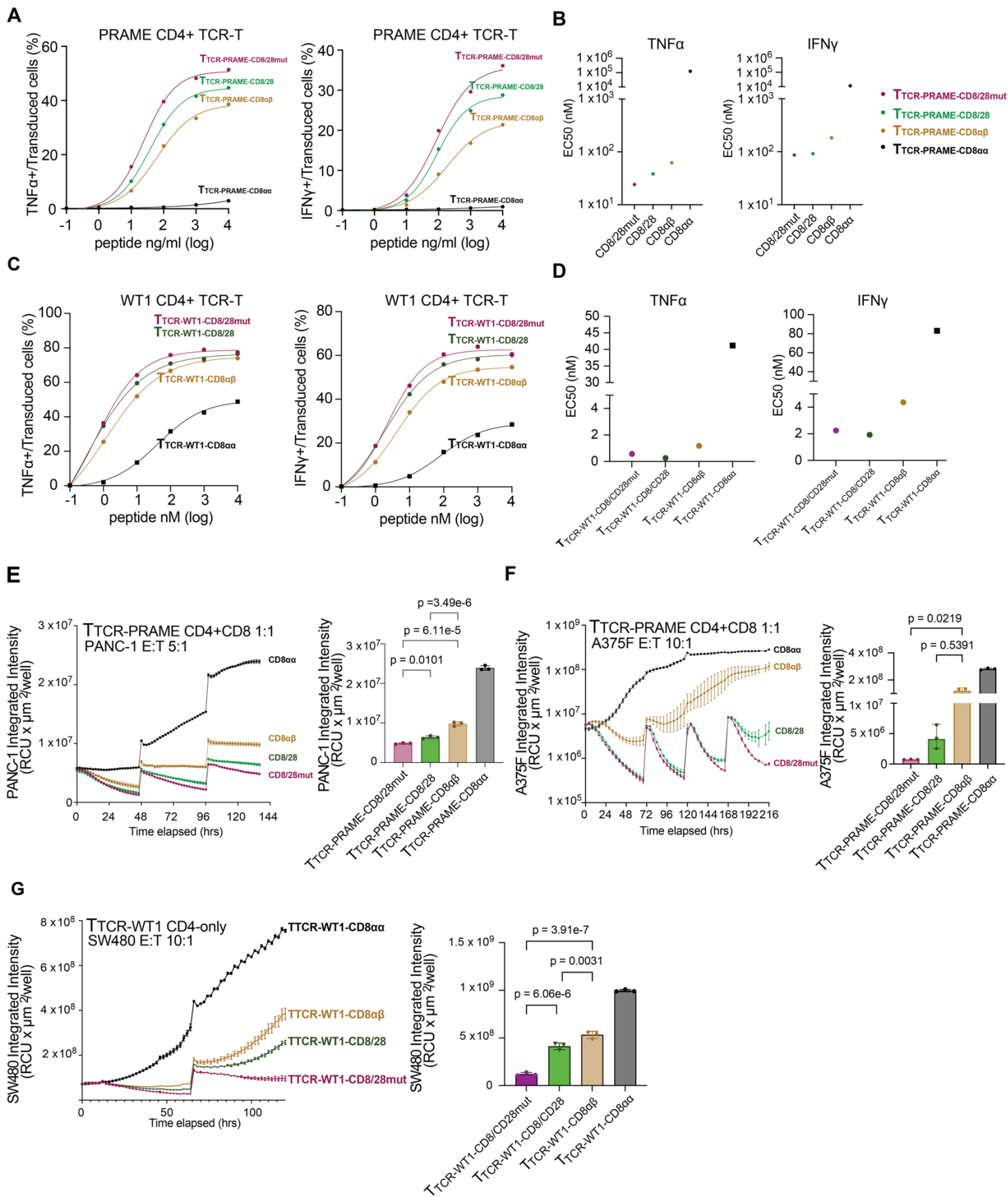
Supplementary Fig. 11: CD8 $\alpha\beta$ -CD28 motif mutation discovery.

(A) Schematic showing the specific protein binding site and corresponding motif mutation. **(B)** Dose response curve of CD4⁺ T cells transduced with 16 mutant CD8/CD28 constructs [A0-A7 constructs (top) and E0-E7 construct (bottom)], represented by TNF α levels to decreasing cognate peptide overnight stimulation., with EC₅₀ values of the individual conditions (right). Constructs labels A0-7 and E0-7 corresponding to Table 7. **(C)** Tumor cell survival of ME275 incubated with 16 CD8/CD28mut CD4⁺ CCR (E:T 5:1) measured using real-time cytotoxic serial killing assay on the Incucyte (S3). Constructs labels A0-7 and E0-7 corresponding to Table 7. Graph represents mean $\pm$ SD. (n=3 wells/group). **(D)** Final tumor intensity (top) and final T cell count (bottom) of the A375F cells incubated with 16 mutant constructs and grouped by their specific mutation site. Graph represents mean $\pm$ SD. The indicated p values were calculated by paired t-test. (n=3 wells/group). **(E)** Final tumor integrated intensity of A375F cells incubated with T cells with either YMNM/ PRRP wild type or double mutation AMNM/ARRA with the CD28 constant motif of GG/PYAP (left), LL/AYAA (middle), and GG/AYAA (right). Graph represents mean $\pm$ SD. p values were determined by unpaired t-test. (n=3 wells/group).

A**B****C**

Supplementary Fig. 12: CD8/CD28 and CD8/CD28mut CCR T cells persisted in spleens and tumors *in vivo*.

(A) EC50 of CD4⁺ T_{TCR-MA1-CD8 $\alpha\beta$} , T_{TCR-MA1-CD8/CD28}, and T_{TCR-MA1-CD8/CD28mut} constructs represented by IFN γ and TNF α cytokine levels. **(B)** Schematic (left) and T cell counts in spleens and tumors (right) of A375F tumors engrafted NSG mice with subsequent transfer of 5×10^6 or 2.5×10^6 equal numbers of T cells (T_{TCR-Irr-CD8/28}, T_{TCR-MA1-CD8/28}, T_{TCR-MA1-CD8/28mut} 1:1 ratio of combined CD4⁺ and CD8⁺) 12 days after implantation. Spleens and tumors were collected at 8 days after T cell infusion and T cells were identified in flow cytometry analysis. Graph represents mean $\pm$ SEM. p values determined with unpaired two-tailed t-test. (n=3 mice per group). Image created in BioRender. Tang, A. (2026) <https://BioRender.com/6wg9k7f>. **(C)** Immunohistochemistry analysis of Ki-67 expression in CD4⁺ or CD8⁺ T cells between T_{TCR-Irr-CD8/28}, T_{TCR-MA1-CD8/28} and T_{TCR-MA1-CD8/28mut} groups from **(Fig. 6D)**. p value determined with Brown-Forsythe and Welch ANOVA test with Dunnett's multiple comparison test. (n=3 mice/group, 2 tumors per mouse).



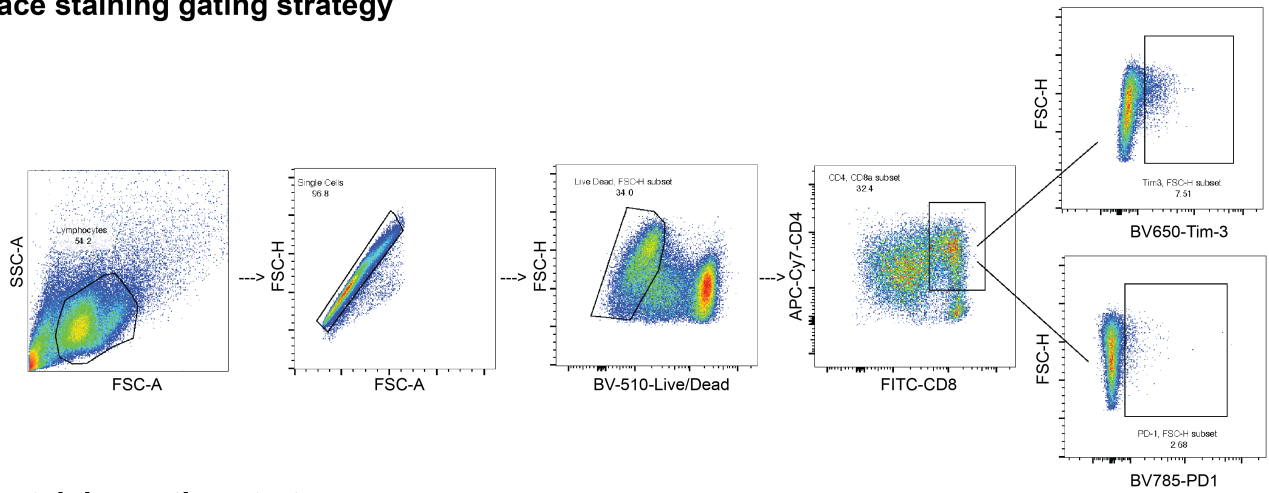
Supplementary Fig. 13: CD8/CD28 CCR integrated into WT1, and PRAME TCR.

(A) Dose response curves showing IFN γ and TNF α levels in CD4⁺ T_{TCR-PRAME-CD8 $\alpha\alpha$} , T_{TCR-PRAME-CD8 $\alpha\beta$} , T_{TCR-PRAME-CD8/CD28}, and T_{TCR-PRAME-CD8/CD28mut} cells incubated with decreasing concentrations of cognate peptide overnight. **(B)** Mean EC₅₀ of IFN γ and TNF α cytokine expression for CD4⁺ T_{TCR-PRAME-CD8 $\alpha\alpha$} , T_{TCR-PRAME-CD8 $\alpha\beta$} , T_{TCR-PRAME-CD8/CD28}, and T_{TCR-PRAME-CD8/CD28mut} cells from previous dose response curve **(A)**. **(C)** Dose response curve showing IFN γ and TNF α levels in CD4⁺ T_{TCR-WT1-CD8 $\alpha\alpha$} , T_{TCR-WT1-CD8 $\alpha\beta$} , T_{TCR-WT1-CD8/CD28}, and T_{TCR-WT1-CD8/CD28mut} cells incubated with decreasing concentrations of cognate peptide overnight. **(D)** Mean EC₅₀ of IFN γ and TNF α cytokine expression for CD4⁺ T_{TCR-WT1-CD8 $\alpha\alpha$} , T_{TCR-WT1-CD8 $\alpha\beta$} , T_{TCR-WT1-CD8/CD28}, and T_{TCR-WT1-CD8/CD28mut}. **(E)** (left) Growth kinetics of the PANC1 cell line in the presence of CD4⁺ and CD8⁺ with the indicated CCR constructs (T_{TCR-PRAME-CD8 $\alpha\alpha$} , T_{TCR-PRAME-CD8 $\alpha\beta$} , T_{TCR-PRAME-CD8/CD28}, and T_{TCR-PRAME-CD8/CD28mut}) at a ratio of E:T 5:1. (right) Final tumor integrated intensity of Incucyte killing assay was analyzed. Graph represents mean $\pm$ SD. p values were determined with one-way ANOVA with Tukey's multiple comparison test. (n=3 wells/group). **(F)** (left) Growth kinetics of the A375F cell line in the presence of CD4⁺ and CD8⁺ with the indicated CCR constructs (T_{TCR-PRAME-CD8 $\alpha\alpha$} , T_{TCR-PRAME-CD8 $\alpha\beta$} , T_{TCR-PRAME-CD8/CD28}, and T_{TCR-PRAME-CD8/CD28mut}) at a ratio of E:T 10:1. (right) Final tumor integrated intensity of Incucyte killing assay was analyzed. Graph represents mean $\pm$ SD. p values were determined by one-way ANOVA with Tukey's multiple comparison test. (n=3 wells/group). **(G)** (left) Growth kinetics of SW480 cell line in the presence of CD4⁺ with the indicated CCR constructs (T_{TCR-WT1-CD8 $\alpha\alpha$} , T_{TCR-WT1-CD8 $\alpha\beta$} , T_{TCR-WT1-CD8/CD28}, and T_{TCR-WT1-CD8/CD28mut}) at a ratio of E:T 10:1. Final tumor integrated intensity of Incucyte killing assay was analyzed. Graph represents mean $\pm$ SD. p values were determined by one-way ANOVA with Tukey's multiple comparison test. (n=3 wells/group).

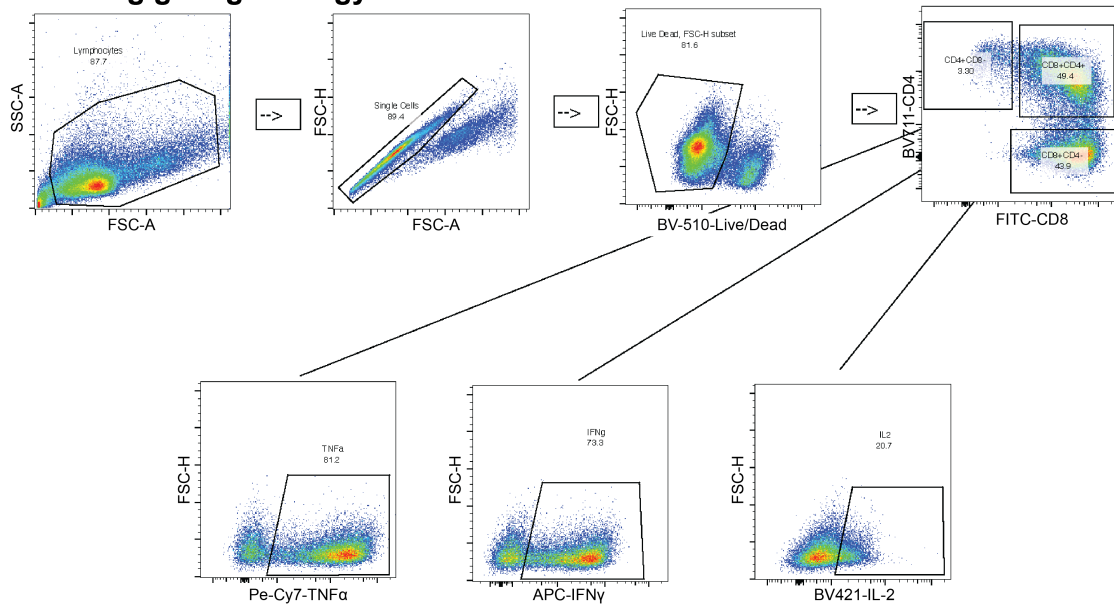
Supplementary Fig. 14: Bulk-seq data reveals major transcriptional differences between T_{TCR-MA1-CD8αβ}, T_{TCR-MA1-CD8/CD28}, and T_{TCR-MA1-CD8/CD28mut}.

(A) Intracellular expression of (p)-ERK(top) and (p)-AKT(bottom) across CD4⁺ T cells constructs (T_{TCR-MA1-CD8αβ}, T_{TCR-MA1-CD8/CD28}, and T_{TCR-MA1-CD8/CD28mut}) stimulated with 1uM of cognate peptide[loaded on TAP-deficient T2 B lymphoblastoid cell lines (T2-BLCL)] at three different time point. Time course (left) and fold change (right) compared to CD8αβ. The mean and standard deviation (SD) of triplicate wells from 2 independent experiment is shown. p value determined by one-way ANOVA with Tukey's multiple comparison test. n= 6 wells/group, from 2 separate experiments). **(B)** Heatmap showing the expression levels of differentially expressed genes across constructs (T_{TCR-MA1-CD8αβ}, T_{TCR-MA1-CD8/CD28}, T_{TCR-MA1-CD8/CD28mut}) and stimulations (unstimulated vs. stimulated) in the rlog-transformed data. Rows represent genes, and columns represent individual samples (3 biological replicates per condition). Expression values are color-coded from low (blue) to high (red) based on z-scores. The dendrograms on the axes indicate hierarchical clustering of genes and samples.

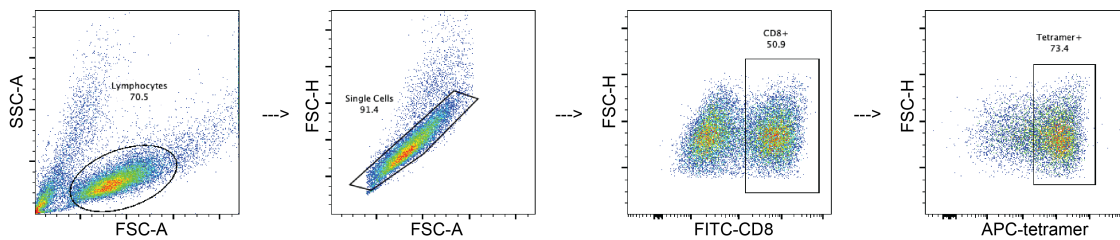
A. Surface staining gating strategy



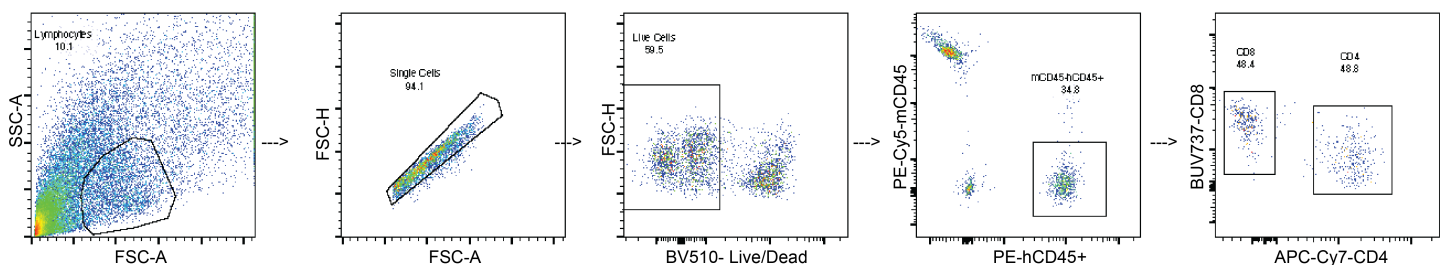
B. ICS staining gating strategy



C. T cell generation FACS sort strategy



D. *In vivo* T cell identification gating strategy



Supplementary Fig 15: FACS gating and sorting strategy (A) Gating strategy used to identify CD4 and CD8 T_{TCR-MA1-CD8αβ} cells with exhaustion associated markers for in vitro figures presented on Fig 7H and common gating strategy for identifying CD4 and CD8 T_{TCR-MA1-CD8αβ} with or without CCR presented on Fig. 1D, 2A, 3B, 3E. (B) Gating strategy used to identify CD4 and CD8 T_{TCR-MA1-CD8αβ} cells with cytokine markers for in vitro figures presented on Fig. 1B, 2G, 3C, 3D, 4H, 6B, 7I) . (C) Gating strategy used to sort out transduced CD4 and CD8 T_{TCR-MA1-CD8αβ} cells with or without CCR followed by rapid expansion protocol for in vitro and in vivo downstream assays presented on Fig 3B. (D) Gating strategy used to identify CD4 and CD8 T_{TCR-MA1-CD8αβ} cells with or without CCR from tumor single cell suspension obtained from *in vivo* tumor models presented on Fig. 2F, 2G, 4G, 4H.

Supplementary Table 1. Human cancer cell lines

Cell Line	HLA status	MAGE A1 status	Cancer Type
ME275	HLA-A2 ⁺	MAGE-A1 ⁺	Melanoma
A375	HLA-A2 ⁺	MAGE-A1 ⁺	Melanoma
A375F	HLA-A2 ⁺ overexpressed	MAGE-A1 ⁺ overexpressed	Melanoma
SK-MEL37	HLA-A2 ⁺	MAGE-A1 ⁺	Melanoma
PANC-1	HLA-A2 ⁺	PRAME ⁺	Pancreatic cancer
SW-480	HLA-A2 ⁺ overexpressed	WT1 ⁺	colon adenocarcinoma

Supplementary Table 2. Adverse events experienced following CD4+ CD8+ T_{TCR-MAI-CD8αβ} infusion product in a participant with non-small cell lung cancer.

BODY SYSTEM (CTC/CTCAE CATEGORY)	EVENT	# Participants Affected	Maximal Grade	Maximal Attribution
Cardiac disorders	Sinus tachycardia	1	1	Possible
Gastrointestinal Disorders	Bloating	1	1	Not related
General disorders and administration site conditions	Fatigue	1	2	Possible
Metabolism and nutrition disorders	Hyperglycemia	1	1	Not related
	Hyperphosphatemia	1	1	Unlikely
Musculoskeletal and connective tissue disorders	Generalized muscle weakness	1	2	Not related
Nervous system disorders	Dizziness	1	1	Unlikely
Psychiatric disorders	Insomnia	1	1	Unlikely
Respiratory, thoracic and mediastinal disorders	Hoarseness	1	1	Not related
skin and subcutaneous tissue disorders	Dry skin	1	1	Not related

Supplementary Table 3. Curated genes for costimulatory ligands on patient scRNA seq dataset.

Costim	Inhibitory
CD80	CD276
CD86	CD274
CD40	PDCD1LG2
TNFRSF4	LGALS9
ICOSLG	TGFB1
TNFSF9	VTCN1

Supplementary Table 4. CD8 α chain modification vs CD8 β chain modification.

	Name	CCR molecule	Trans- gene ECD	Trans- gene 1 TM	Trans- gene 1 ICD	Trans- gene 2 ECD	Trans- gene 2 TM	Trans- gene 2 ICD	CD8a modified	CD8b modified
With CD8a chain modified	C	CD8a*28CD8b*28	CD8a	CD8a	CD28	CD8b	CD8b	CD28	Yes	Yes
	F	CD8a*28mtCD8b*28mt	CD8a	CD8a	CD28 partial mutant	CD8b	CD8b	CD28 partial mutant	Yes	Yes
	N	CD8a_bSTALK_28tm_28IC	CD8a-CD8bstalk	CD28	CD28	x	x	x	Yes	N/A
	O	CD8a_bSTALK_28Tm_28IC_aIC	CD8a-CD8bstalk	CD28	CD28 + CD8a	x	x	x	Yes	N/A
	P	CD8a_bSTALK_aTm_aIC	CD8a-CD8bstalk	CD8a	CD8a	x	x	x	Yes	N/A
	T	CD8-28 full mutant	CD8a	CD8a	CD28 full mutant	CD8b	CD8b	CD28 full mutant	Yes	Yes
With CD8b chain modified	A	CD8aCD8b6*28	CD8a	CD8a	CD8a	CD8b	CD8b	6 AA CD8b +CD28	No	Yes
	E	CD8aCD8b*28mt	CD8a	CD8a	CD8a	CD8b	CD8b	6 AA CD8b + CD28 full mutant	No	Yes
	G	CD8a_CD8-CD28	CD8a	CD8a	CD8a	CD8b	CD8b	6 AA CD8b +CD28 wt	No	Yes
	H	CD8a_CD8_41bb	CD8a	CD8a	CD8a	CD8b	CD8b	6 AA CD8b +41BB	No	Yes

Supplementary Table 5. CD8αβ chimeric co-receptor constructs.

Identifier	Name	Transgene 1 design			Transgene 2 design			CD8a modified	CD8b modified
		extracellular	tm	intracellular	extracellular	tm	intracellular		
CD8/CD28mut	CD8a_CD8b ECD-CD28mut ICD	CD8a	CD8a	CD8a	CD8b	CD8b	6AA CD8b + CD28 full mutant	No	Yes
CD8/CD28	CD8a_CD8b ECD-CD28 ICD	CD8a	CD8a	CD8a	CD8b	CD8b	6 AA CD8b +CD28wt	No	Yes
CD8/41BB	CD8a_CD8b ECD_41bb ICD	CD8a	CD8a	CD8a	CD8b	CD8b	6 AA CD8b +41BB	No	Yes
CD8/ICOS	CD8a_CD8b ECD_ICOS	CD8a	CD8a	CD8a	CD8b	CD8b	6 AA CD8b +ICOS	No	Yes
CD8/OX40	CD8a_CD8b ECD_OX40 ICD	CD8a	CD8a	CD8a	CD8b	CD8b	6 AA CD8b +OX40	No	Yes
CD8/GITR	CD8a_CD8b ECD_GITR ICD	CD8a	CD8a	CD8a	CD8b	CD8b	6 AA CD8b +GITR	No	Yes
CD8ab	TCRM0_CD8ab	CD8a	CD8a	CD8a	CD8b	CD8b	CD8b	No	No
TCR-MA1	TCR alone							N/A	N/A
Irr.CD8ab	Irrelevant TCR + CD8ab	CD8a	CD8a	CD8a	CD8b	CD8b	CD8b	No	No
Irr-CD8/CD28	Irrelevant TCR + CD8a_CD8b-CD28	CD8a	CD8a	CD8a	CD8b	CD8b	6 AA CD8b +CD28wt	No	Yes
Irr.-CD8/CD28mut	Irrelevant TCR + CD8a_CD8b ECD-CD28mut ICD	CD8a	CD8a	CD8a	CD8b	CD8b	6AA CD8b + CD28 full mutant	No	Yes

Supplementary Table 6. CD8-based costimulatory molecule library.

Identifier	Transgene 1	Transgene 2			
		Extra-cellular	Tm	intracellular	Intracellular domain sequence
CD8/CD28 mut	CD8a	CD8b	CD8b	6 AA CD8b +CD28GG mut	HLCCRRRSKRSRGGHSDAMNMTA RRAGPTRKHYQAYAAPRDFAAAYRS
CD8/CD28	CD8a	CD8b	CD8b	6 AA CD8b +CD28wt	HLCCRRRSKRSRLLHSDYMNMTPR RPGPTRKHYQPYAPPRDFAAAYRS
CD8/41BB	CD8a	CD8b	CD8b	6AA CD8b +4-1BB	HLCCRRKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEGGCEL
CD8/ICOS	CD8a	CD8b	CD8b	6AA CD8b +ICOS	HLCCRRKYSSSVHDPNGEYMFMR VNTAKKSRLTDVT
CD8/OX40	CD8a	CD8b	CD8b	6AA CD8b +OX40	HLCCRRALYLLRRDQRLPPDAHKPP GGGSFRTPIQEEQADAHSTLAKI
CD8/GITR	CD8a	CD8b	CD8b	6AA CD8b +GITR	HLCCRRQLGLHIWQLRSQCMWPRE TQLLLEVPPSTEDARSCQFPEEERGE RSAEEKGRLGDLWV
CD8ab	CD8a	CD8b	CD8b	CD8b	HLCCRRRRARLRFMKQFYK

Supplementary Table 7. CD28 motif mutation variation.

no.	CD28 Chimeric co-receptor	Di-leucine motif	PI3K and GRB2/GADS binding site	ITK and GADS binding site	LCK binding site
A0	CD8 α β -CD28 wt (TCR _{MA1} -CD8/CD28)	LL	YMNМ	PRRP	PYAP
A1	CD8 α β -CD28 m1-1	GG	YMNМ	PRRP	PYAP
A2	CD8 α β -CD28 m1-2	LL	AMNM	PRRP	PYAP
A3	CD8 α β -CD28 m1-3	LL	YMNМ	ARRA	PYAP
A4	CD8 α β -CD28 m1-4	LL	YMNМ	PRRP	AYAA
A5	CD8 α β -CD28 m2-1	GG	AMNM	PRRP	PYAP
A6	CD8 α β -CD28 m2-2	GG	YMNМ	ARRA	PYAP
A7	CD8 α β -CD28 m2-3	GG	YMNМ	PRRP	AYAA
E0	CD8 α β -CD28 m2-4	LL	AMNM	ARRA	PYAP
E1	CD8 α β -CD28 m2-5	LL	AMNM	PRRP	AYAA
E2	CD8 α β -CD28 m2-6	LL	YMNМ	ARRA	AYAA
E3	CD8 α β -CD28 m3-1	GG	AMNM	ARRA	PYAP
E4	CD8 α β -CD28 m3-2	GG	AMNM	PRRP	AYAA
E5	CD8 α β -CD28 m3-3	GG	YMNМ	ARRA	AYAA
E6	CD8 α β -CD28 m3-4	LL	AMNM	ARRA	AYAA
E7	CD8 α β -CD28 mut (TCR _{MA1} -CD8/CD28 _{mut})	GG	AMNM	ARRA	AYAA

Supplementary Table 8. Proliferation and Exhaustion gene list for bulk RNA seq dataset.

Proliferation	Exhaustion
MKI67	PDCD1
PCNA*	LAG3
MCM2*	HAVCR2
MCM3	CTLA4*
MCM4	TIGIT*
MCM5*	CD160
MCM6*	BTLA
MCM7	ENTPD1
CCND1	TOX
CCND2	PDCD2
CCND3	
CDK1	
CDK2	
CDK4	
CDK6	
CDC6	
CDC45	
TOP2A	
AURKA	
AURKB	
TUBB	
* significantly differentially expressed	

Supplementary Note 1. Clinical trial protocol

Protocol 10420/RG1007463

**FRED HUTCH
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE**

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: 08/04/2020

Previous date: 04/24/2020

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FHCRC IRB Approval
02/11/2021
Document Released Date

IND number: TBD

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: 10420	Product Names: FH-MagIC TCR-T
Title of Study: <i>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</i>	
Sponsor: Fred Hutch Cancer Research Center	Phase of Development: I
IND Number: Pending	
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, 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 of 2+ or 3+ by immunohistochemistry for MAGE-A1 antigen 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. 5. 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. 6. For cohorts receiving treatment in combination with atezolizumab, 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. 7. Patient must be HLA type HLA-A*02:01. 8. Patient must be 18 years of age or older. 9. Patient must be capable of understanding and providing a written informed consent.	

10. Patient must be willing to comply with reproductive requirements.
11. ECOG performance status of 0, 1, or 2.
12. Patient must be willing to undergo serial tumor biopsies.
13. Patients must be at least three weeks from last systemic treatment for metastatic disease.
14. 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. Patients with grade 3 or higher immune-mediated toxicity to any prior PD-L1 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 $\geq$ 3 allergic reaction related to the TCR-T cell infusion
- Grade $\geq$ 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 $\geq$ 3 neurotoxicity of greater than 7 days duration
- Grade $\geq$ 3 neurotoxicity that does not revert to Grade 1 or baseline within 28 days
- Grade $\geq$ 3 seizures that do not resolve to < grade 3 within 3 days
- Grade $\geq$ 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

TABLE OF CONTENTS

1.0	BACKGROUND	9
2.0	STUDY PURPOSE AND RATIONALE.....	10
2.1	Rationale for Investigation of FH-MagIC TCR-T.....	11
2.1.1	Scientific basis for development of TCR against MAGE-A1	11
2.1.2	Target epitope: HLA-A02 restricted “KVLEYVIKV” MAGE-A1 Epitope.....	12
2.1.3	Clinical experience to date with cell therapies for TNBC and NSCLC.....	12
2.1.4	Benefits of a transgenic approach	12
2.1.5	Safety of TCR transgenic T cells for other cancers	13
2.1.6	Rationale for isolating a TCR from the peripheral repertoire of HLA matched healthy donors.....	13
2.1.7	Why include transgenic CD4+ T cells in addition to transgenic CD8+ T cells?.....	13
2.1.8	TCR-MagIC is a highly potent natural TCR that effectively lyses cell lines expressing MAGE-A1	14
2.1.9	Evaluation of FH-MagIC TCR-T potential for off-target activity.....	15
2.1.10	Evaluation of FH-MagIC TCR-T potential for cross- and allo-reactivity	17
2.1.11	Why use a third generation lentiviral vector to transduce TCR-MagIC into autologous T cells?	19
2.1.12	Why transduce CD62L+ (“young” but well differentiated) CD8+ T Cells?.....	20
2.2	FH-MagIC TCR-T Dose Rationale.....	21
2.3	Rationale for Lymphodepleting Chemotherapy.....	21
3.0	STUDY OBJECTIVES	22
3.1	Primary Objectives	22
3.2	Secondary Objectives	22
3.3	Exploratory Objectives	23
3.4	Projected Target Accrual	23
4.0	STUDY DESIGN AND INVESTIGATIONAL PLAN	23
4.1	Overall Study Design: Phase I/II single arm dose escalation non-masked interventional study	23
4.2	Protocol Enrollment	25
4.3	Staggering of Patients within each Dose Level.....	26
5.0	STUDY POPULATION	26
5.1	Inclusion Criteria.....	26
5.2	Exclusion Criteria	28
5.3	Reproductive Potential and Contraception Requirements	29
6.0	TREATMENT PLAN	30
6.1	Leukapheresis.....	30
6.2	Lymphodepletion	30
6.3	FH-MagIC TCR-T Treatment.....	30
6.3.1	FH-MagIC TCR-T Combination Therapy.....	31
6.3.2	Preparation of Cell Product.....	31
6.3.3	T Cell Infusions.....	32
6.3.4	Acute infusion reactions.....	32
6.3.5	Guidelines for the determination of 1 or 2 cycles of T cell therapy.....	32
6.4	Recommended Supportive Care, Additional Treatment, and Monitoring.....	33
7.0	POTENTIAL RISKS AND TOXICITY MONITORING	33

7.1	Acute Toxicity Associated with T-Cell Infusion.....	34
7.2	Acute Toxicity Associated with Atezolizumab or Other PD1-Axis Inhibitor Infusion	34
7.3	Cytokine Release Syndrome	36
7.4	Neurologic Toxicity	37
7.5	Tumor Lysis Syndrome	37
7.6	Macrophage Activation Syndrome.....	37
7.7	Adrenal Toxicity	38
7.8	Immune-Mediated Adverse Events.....	38
7.9	Persistent Uncontrolled T-cell Proliferation.....	38
7.10	Replication-Competent Lentivirus.....	38
7.11	Management of Other Toxicities.....	39
8.0	INFORMED CONSENT OF SUBJECT AND DONOR.....	39
8.1	Patient screening.....	39
8.2	Treatment.....	39
9.0	SUBJECT REGISTRATION.....	39
10.0	CLINICAL AND LABORATORY EVALUATIONS	40
10.1	Screening Evaluations.....	40
10.2	Pre-treatment Evaluations	40
10.3	Evaluations prior to leukapheresis.....	41
10.4	Evaluations prior to LD	41
10.5	Evaluations prior to T cell Infusion	41
10.6	Evaluations following T cell infusion	42
10.7	Response Assessment	44
10.8	Participant discontinuation of active treatment.....	44
10.9	Long-term follow-up.....	45
11.0	ADVERSE EVENT REPORTING	45
11.1	Adverse Event Definitions	45
11.2	Monitoring and Recording Adverse Events.....	46
11.3	Grading of the Severity of an Adverse Event	46
11.4	Attribution of Adverse Event.....	46
11.5	Adverse Event Reporting Period	47
11.6	Dose-limiting Toxicities to Guide Dose Modification	48
11.7	Adverse Event Reporting Requirements	48
11.7.1	Reporting to IRB.....	48
11.7.2	Reporting to Sponsor	48
11.7.3	Institution-Sponsor Reporting Requirements	49
11.7.4	Dose Assessment Committee.....	49
12.0	STATISTICAL CONSIDERATIONS	50
12.1	Type of Study.....	50
12.2	Safety Assessment.....	50
12.2.1	Guidelines for Dose Modification.....	50
12.3	Efficacy Assessment.....	51
12.3.1	Definition of response	51
12.3.2	Definition of success and power of success	51
12.4	Secondary and exploratory analyses.....	51

12.5	Sample Size and Accrual	51
13.0	DATA AND SAFETY MONITORING PLAN	51
13.1	Overall Scope of Monitoring Activities.....	51
13.2	Monitoring the Progress of Trial and Safety of Participants	52
14.0	DATA MANAGEMENT/CONFIDENTIALITY	52
15.0	TERMINATION OF STUDY	53
16.0	REFERENCES.....	54
APPENDIX A: Schedule of Evaluations		59
APPENDIX B: Research Sample Checklist.....		63
APPENDIX C: Karnofsky Performance Status Scale		67
APPENDIX D: ECOG Performance Status Scale		68
APPENDIX E: CRS Grading Criteria (CTCAE v. 5.0)		69
APPENDIX F: Recommended Management of CRS		70
APPENDIX G: Long Term Follow-Up		73
APPENDIX H: Dose Adjustment Committee.....		74
APPENDIX I: MAGE-A1-SPECIFIC IMMUNOHISTOCHEMISTRY (IHC) ASSAY		75

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 trial⁴ 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 the trial is ongoing and data is being acquired, infusion of the first T cell dose corresponding to $\sim 5 \times 10^8$ 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**).

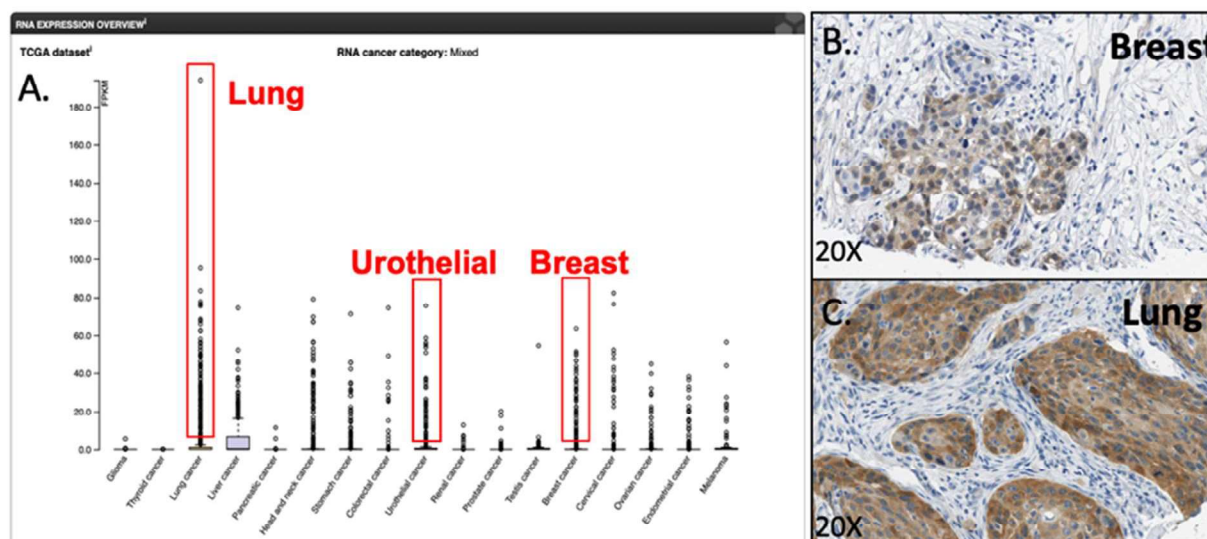


Figure 1: Somatic MAGE-A1 protein expression is limited to malignant cells, reducing chance of on-target off-tissue toxicity. A] MAGE-A1 expression (RNA FPKM) from the cancer genome atlas¹⁹⁻²¹ (TCGA) for various malignancies. MAGE-A1 positive tumor cells (Brown) as determined by IHC using monoclonal 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-A1₂₇₈₋₂₈₇ 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 tumors⁴⁰. An alternative is to confer function to CD4⁺ T cells through expression of a high-affinity Class I TCR (TCR_{Class-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).

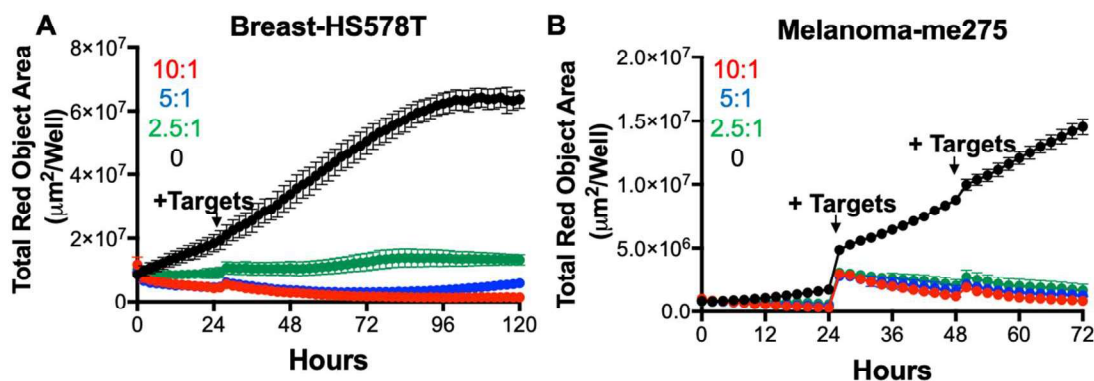


Figure 2: FH-MagIc TCR-T specifically kill HLA-A2 MAGEA1 positive tumor cell lines. [A,B] *In vitro* cytotoxic activity of FH-MagIc TCR-T cells at decreasing E:T ratios generated from three healthy donors measured by dynamic killing of red labeled [A] HS578T breast cell line with a single re-challenge 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+ myeloma⁴⁵ to test the *in vivo* efficacy of FH-MagIc TCR-T. Briefly, mice were engrafted with 2×10^6 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, 15×10^6 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.

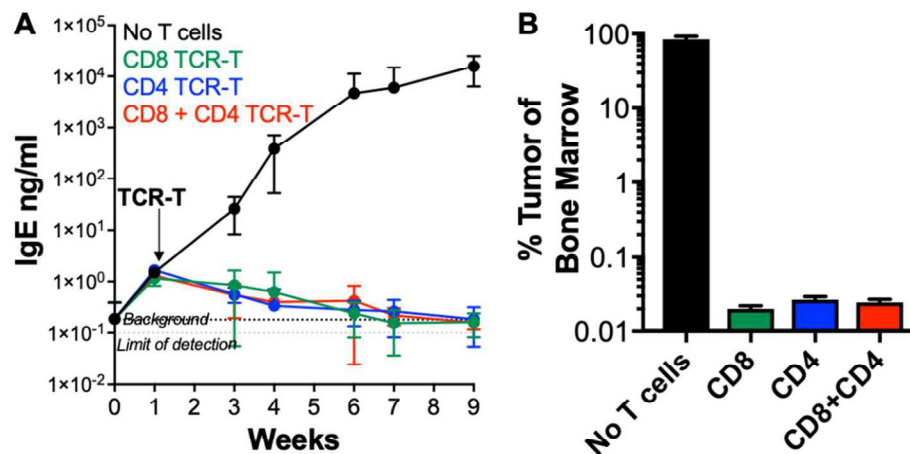


Figure 3: FH-MagIc TCR-T controls HLA-A2+ MAGEA1+ U266 xenograft in vivo. [A] IgE levels over time in the serum (ng/ml) of U266 (iv injected) tumor bearing NSG mice with and without FH-MagIc TCR-T cells transfer. [B] Tumor burden in the bone marrow 9 weeks post U266 engraftment in FH-MagIc TCR-T cell 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-A1₂₇₈₋₂₈₇ [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 4.0⁴⁶ (**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 untransduced 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 GFP 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.

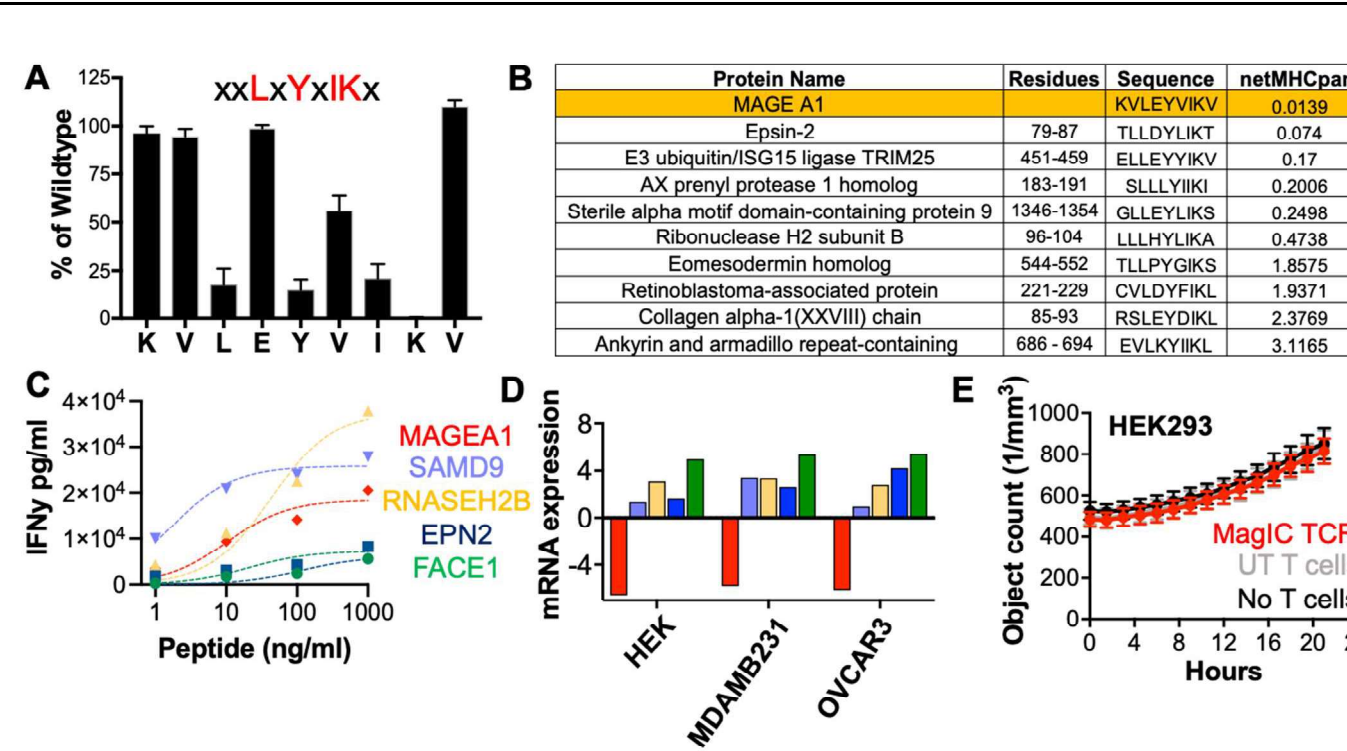


Figure 4: In Silico analysis of MagIC TCR-T cross-reactivity. **A:** Alanine scan identifies critical residues (in red) for peptide-MHC recognition. **B:** Peptides identified by *in silico* analyses and rank on HLA-A2 binding scoring system. **C:** Reactivity of MagIC TCR-T to titrated amounts of exogenously 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 IFN γ 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 γ (Figure 5).

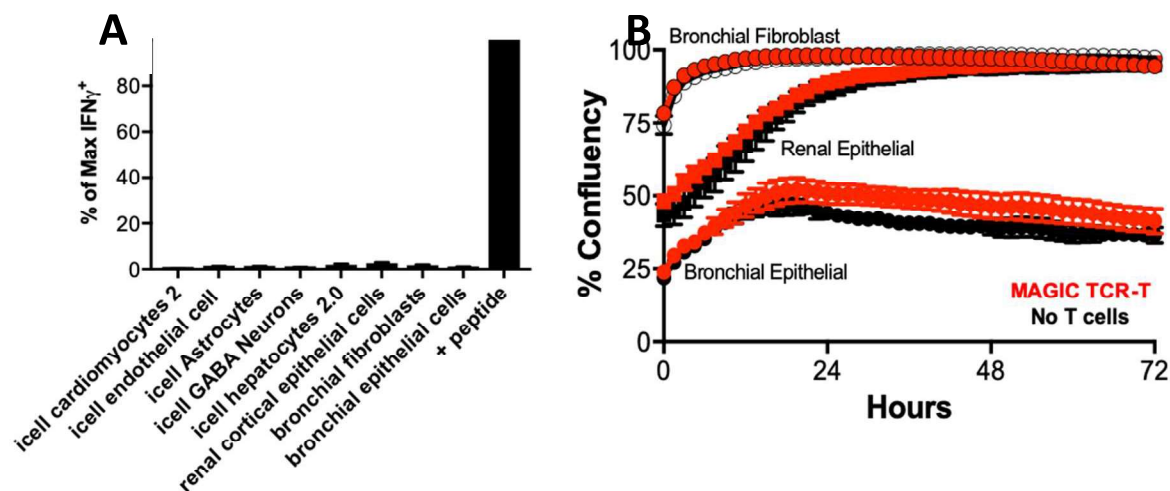


Figure 5: MagIC TCR-T does not recognize self-peptides presented by HLA-A2*01 on healthy human cell lines. **A:** Cytokine production by MagIC TCR-T after co-culture of HLA-A2*01 iPS derived human cell lines or harvested normal human cell lines (determined by ICS or ELISA). Independent experiments performed 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	

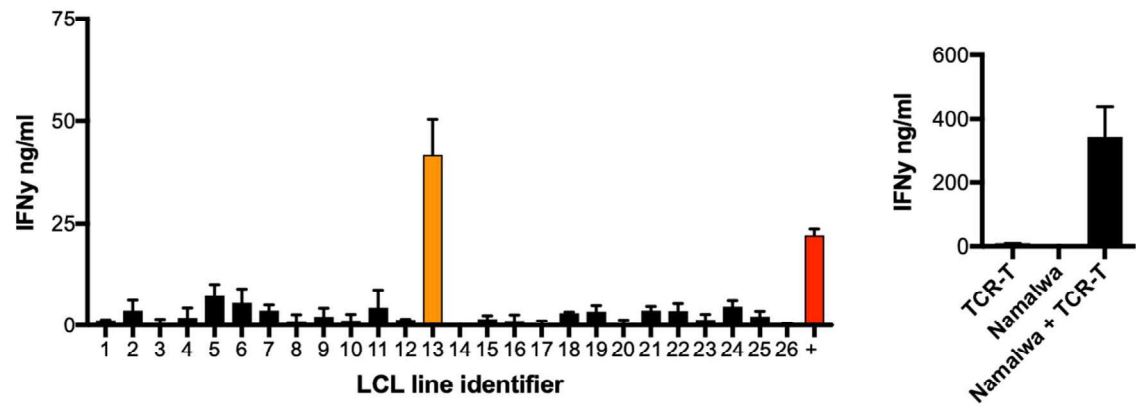


Figure 6: FH-MagIc TCR-T Recognition of HLA-B49*01. **A:** Cytokine production by FH-MagIc TCR-T after co-culture with a panel of B-LCL cell lines (determined by ICS or ELISA). Independent experiments (n=2). **B:** Cytokine production by FH-MagIc TCR-T after co-culture of an HLA-B49+ tumor cell line 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 melanoma⁵¹ 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 (T_N, T_M). T_M 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 T_{CM} provide enhanced protective immunity from in vivo challenge compared to T_{EM}. Although T_{CM} cells expand and also differentiate into effector cells in response to in vitro stimulation, adoptive transfer studies in non-human primates revealed that T_{CM}-derived effectors had been imprinted and retained some of the beneficial properties of their parent T_{CM} 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 lymphocyte 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 (1×10^9) and eventually 5 billion (5×10^9) 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 efficacy⁵⁹⁻⁶¹. 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 cells⁶².

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/m²/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 ($300 \text{ mg/m}^2/\text{day}$ for 3 days) and fludarabine ($30 \text{ mg/m}^2/\text{day}$ 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 ($25 \text{ mg/m}^2/\text{day}$ 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 $\leq 35\%$).
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. 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

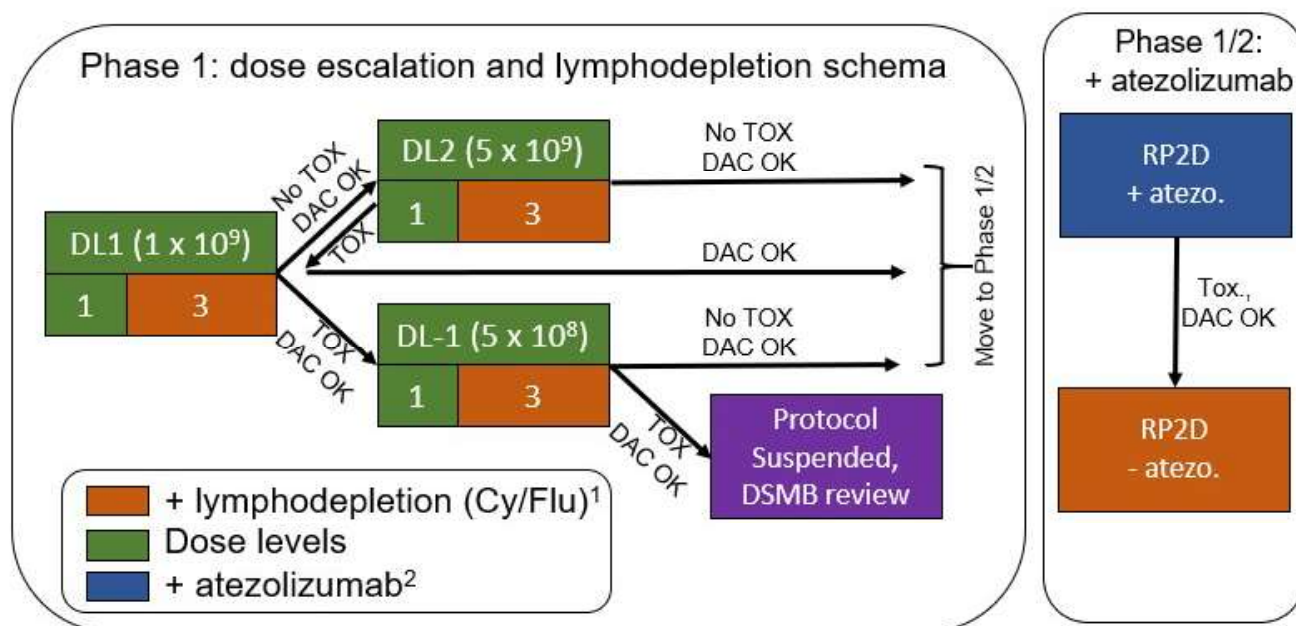
4.0 STUDY DESIGN AND INVESTIGATIONAL PLAN

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. Atezolizumab will be provided as standard of care in those patients enrolled on to the Phase 1/2 portion of the trial (Figure 6). 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 1×10^9 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 5×10^9 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

$\times 10^8$ CD3+ cells (DL-1) with the first patient receiving cellular therapy only and the next three patients receiving lymphodepletion followed by cellular therapy. The RP2D is targeted to be 5×10^9 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 (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/m² and fludarabine (Flu) 30mg/m² 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 and expression of MAGE-A1:** 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 FHCRC/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. Baseline tissue will be stained to confirm MAGE-A1 expression.
- 2) **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 (for example diagnostic CT chest/abdomen/pelvis and imaging of the affected extremity as appropriate), brain imaging (MRI or CT scan) must be obtained within 45 days of prior to start of first planned FH-MAGEA1-A2TCR infusion. MRI can be substituted for CT in patients unable to have CT contrast.
- 3) **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.
- 4) **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.

- 5) **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 through a molecular approach in a clinical laboratory licensed for HLA typing.
- 6) **Life expectancy must be anticipated to be >3 months at trial entry**
- 7) **18 years or older**
- 8) **Capable of understanding and providing a written informed consent**
- 9) **If fertile, willingness to comply with reproductive requirements:** Outlined in section 5.3.
- 10) **ECOG performance status of 0, 1, or 2:** Please see Appendix D for ECOG performance status.
- 11) **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.
- 12) **Participants must be at least three weeks from last systemic treatment:** 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 of treatment.
- 13) **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 FEV₁ ≥ 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 $\geq 35\%$. 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 allogeneic 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

Following confirmation of the eligibility based on HLA typing and MAGE-A1 expression on the tumor, patients will undergo 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. 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 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 Lymphodepletion

Lymphodepletion will comprise of cyclophosphamide (Cy) 300mg/m² and fludarabine (Flu) 30mg/m² 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.

6.3 FH-MagIC TCR-T Treatment

Patients will receive either 1 or 2 infusions of FH-MagIC TCR-T starting at 1x10⁹ (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 2nd-4th 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.

6.3.1 FH-MagIC TCR-T Combination Therapy

6.3.1.1 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 of 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 is atezolizumab as it is standard of care for all three clinical indications. However, if the commercial supplier of the checkpoint inhibitor agrees to only provide an alternative PD1-axis inhibitor to atezolizumab, substitution is permitted.

6.3.2 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. It is anticipated to take approximately 9-14 days to manufacture a product in most cases.

The formulated cell dose refers to TCR+ CD3+ T cells determined by binding to HLA2-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 $\geq 10\%$ of the scheduled CD3+ transgenic T cell dose.

6.3.3 T Cell Infusions

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

Patients deemed not appropriate for T cell administration may receive a T cell infusion outside the specified window if they subsequently resolve the clinical and/or laboratory concerns which deemed them inappropriate within 60 days of the planned initial infusion.

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.

6.3.4 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.

6.3.5 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). 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.

6.4 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 infusional 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 $> 38.2^{\circ}\text{C}$ may be managed with acetaminophen 650 mg PO every 4 to 6 hrs. All subjects who develop fever $> 38.2^{\circ}\text{C}$ 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 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.	Stop the infusion immediately and disconnect infusion tubing from the participant. 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 lymphohistiocytosis⁷⁰.

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 adrenocorticotrophic 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.

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 FHCRC 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

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
- 3) Confirmation of diagnosis by internal pathology review of initial or subsequent biopsy or other pathologic material at the Fred Hutch/SCCA/UWMC
- 4) Baseline pulse oximetry and documentation of O₂ saturation on room air
- 5) Baseline 12-lead EKG
- 6) Patients 60 years or older: Echocardiogram or MUGA scan within 60 days of enrollment.
- 7) Baseline imaging (generally CT chest/abdomen/pelvis (and if present affected extremity), MRI brain if history of brain metastasis or neurologic symptoms, CT neck if prior cervical adenopathy, MRI or PET may be substituted as clinical judgement dictates). Imaging must be obtained within 45 days of prior to start of first planned FH-MagIC TCR-T infusion

Research samples:

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

10.3 Evaluations prior to leukapheresis

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

10.4 Evaluations prior to LD

- 1) Blood draw for laboratory studies:
 - a. CBC, differential, platelet count
 - b. Renal/Hepatic Function Panel
 - c. Magnesium and Lactate Dehydrogenase (LDH)

10.5 Evaluations prior to T cell Infusion

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

- 1) Interval history and physical exam and ECOG performance status
- 2) Blood draw for laboratory studies:
 - a. CBC, differential, platelet count
 - b. Renal/Hepatic Function Panel
 - c. Magnesium and Lactate Dehydrogenase (LDH)

d. Uric acid

- 3) Serum pregnancy test within 72 hours of infusion
- 4) Research Labs:
 - a. A 30 ml blood sample (in EDTA [lavender top] tube) for research should be sent to the Specimen Processing/Research Cell Bank lab
 - b. A 10 ml blood sample (serum separator tube) should be sent to the Specimen Processing/Research Cell Bank lab

10.6 Evaluations following T cell infusion

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

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

on approximately days 1, 3, 7, 14, 21, and 28 during the first month after each T cell infusion, and also at 2, 3, 6, 9, and 12 months after the T cell infusion

iii. If patients become febrile, develop signs of cytokine release syndrome, or cytokine assessment is clinically appropriate at times other than those indicated, we may collect additional research samples at additional timepoints (optional).

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

a. Serum storage for measurement of serum cytokine levels

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

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

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

c. Evaluation of migration of adoptively transferred TCR T cells

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

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

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

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

Whole exome sequencing and RNA Seq: For patients in whom endogenous immune responses are detected, more detailed evaluation may be performed to characterize T cell responses by predicting which tumor mutations are likely to

be immunogenic and then evaluating peripheral blood T cells for reactivity to these epitopes. To identify mutations and determine the patient's HLA alleles, whole exome sequencing and RNA Seq of baseline tumor as well as whole exome sequencing of normal tissue may be performed (optional).

e. RCL testing

RCL testing, as per FDA guidelines.

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

10.7 Response Assessment

Participants will have evaluation of tumor burden at baseline (physical exam with tumor measurements, cross-sectional imaging as detailed above, RECIST 1.1 and imRECIST reads of cross-sectional imaging), at approximately 12 weeks after first T cell infusion, at 12 weeks after second infusion (if given), and then at discretion of treating oncologist (suggested frequency every 3-6 months). These assessments have a window of ± 7 calendar days. Imaging should also be performed if suspicion of progression. Imaging will be performed on a clinical basis with RECIST 1.1/imRECIST reads on a research basis. For skin/cutaneous tumors, representative/target tumors should be photographed at key response assessment/measurement time points.

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

10.8 Participant discontinuation of active treatment

A patient will no longer be able to receive active treatment on study (T cells, atezolizumab) for any of the reasons listed below. In this scenario (unless withdrawal of consent or death), they will transition to the LTFU phase of study:

- Progressive disease or development of new metastasis requiring urgent change in treatment following second T cell infusion. Patients can be considered for chemotherapy/radiation for progression between the 1st and second infusions and remain on study if considered appropriate by the PI.
- The participant withdraws consent
- Patient death
- Occurrence of pregnancy
- Participation in another therapeutic trial during the treatment duration of this trial
- Occurrence of an exclusion criterion, which is clinically relevant and affects the subject's safety, if discontinuation is considered necessary by the Investigator or Sponsor

- A patient will no longer be eligible to receive additional therapy if the PI or designee determines that additional T cell infusions are not in the best interest of the patient
- Occurrence of any non-pre-existing grade 3 or higher AEs / repetitive Grade 2 AEDRs that are deemed related to the treatment, except for the exceptions detailed in section 12.
- Cytokine release syndrome requiring pressors for >24 hours or tocilizumab

10.9 Long-term follow-up

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

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

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

11.0 ADVERSE EVENT REPORTING

11.1 Adverse Event Definitions

- **Adverse Event**

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

- **Serious Adverse Event**

A serious adverse event (SAE) is defined as an untoward medical occurrence that results in any of the following outcomes:

1. Death.
2. Life-threatening situation (i.e., with an immediate risk of death from the event as it occurred but not including an event that, had it occurred in a more serious form, might have caused death).
3. In-patient hospitalization or prolongation of existing hospitalization. Inpatient hospitalization comprises formal admission to a hospital for medical reasons, for any length of time, whether or not hospitalization extends overnight. However, hospital admissions for administration of the study drug, procedures required by the study protocol, or tumor-related diagnostic procedures are not considered serious.
4. Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.

5. Congenital anomaly/birth defect.
6. An important medical event that requires intervention to prevent one of the above outcomes.

- **Unexpected Adverse Event**

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

11.2 Monitoring and Recording Adverse Events

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

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

11.3 Grading of the Severity of an Adverse Event

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

11.4 Attribution of Adverse Event

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

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

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

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

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

11.5 Adverse Event Reporting Period

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

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

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

- Disease progression or relapse.
- Hospitalization to facilitate infusions is not considered an AE. Any AE requiring prolongation of this hospitalization will be recorded and patient to applicable SAE reporting.
- Hospitalization to facilitate monitoring/due to the absence of caregiver/any other reason that is not related to T cells. Medical or surgical procedures in and of themselves, including those that require hospitalization (e.g., surgery, endoscopy, biopsy procedures) are not considered AEs. However, an event or condition requiring such procedures may be an AE.
- Hospitalization that is otherwise unrelated to adverse events.

11.6 Dose-limiting Toxicities to Guide Dose Modification

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

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

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

11.7 Adverse Event Reporting Requirements

11.7.1 Reporting to IRB

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

11.7.2 Reporting to Sponsor

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

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

PI to IND Sponsor Reporting Requirements for Adverse Events

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

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

11.7.3 Institution-Sponsor Reporting Requirements

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

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

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

11.7.4 Dose Assessment Committee

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

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

12.0 STATISTICAL CONSIDERATIONS

12.1 Type of Study

Our study is a phase I/II, non-blinded interventional trial. Our target sample size is 15-18 patients (8-12 per year/1.5-2 years). This enrollment target is chosen for three reasons: it is achievable from an enrollment standpoint (our clinic sees hundreds of metastatic TNBC, urothelial or NSCLC patients potentially eligible per year), it is logistically possible from a funding and cell processing facility standpoint, and it allows >80% power to observe a statistically significant (at one-sided 0.05 level) efficacy signal that is clinically meaningful (see below). Patients will be treated at three potential dose levels (from 1×10^9 up to 5×10^9 TCR-MagIC transgenic CD3+s).

12.2 Safety Assessment

12.2.1 Guidelines for Dose Modification

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

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

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

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

12.3 Efficacy Assessment

12.3.1 Definition of response

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

12.3.2 Definition of success and power of success

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

12.4 Secondary and exploratory analyses

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

12.5 Sample Size and Accrual

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

13.0 DATA AND SAFETY MONITORING PLAN

13.1 Overall Scope of Monitoring Activities

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

determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

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

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

13.2 Monitoring the Progress of Trial and Safety of Participants

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

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

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

14.0 DATA MANAGEMENT/CONFIDENTIALITY

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

The investigator will ensure that data collected conform to all established guidelines for coding collection, key entry and verification. Each patient is assigned a unique patient number to assure patient confidentiality. Information forwarded to the FDA, NIH, NCI or other agencies about patients on this protocol refers to patients by a coded identifier and not by name. Patients will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents. Additional clinical data may be made available from the Fred Hutch core database which is managed and verified independent of the research group.

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

15.0 TERMINATION OF STUDY

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

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

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APPENDIX A: Schedule of Evaluations

Assessment	Screening	Pretreatment Evaluation	Lymphodepleting Chemotherapy	T cell Infusion (Day 0)	1	3	7	14	21	28	*see Note
Informed consent/HIPAA	X	X									
HLA typing	X ^a										
MAGE A1 evaluation	X										
I/E criteria		X									
Medical history ^a		X			X		X	X	X	X	
Physical exam		X		X	X		X	X	X	X	
Height/weight ^b		X		X							
ECOG performance status		X		X							
Pregnancy test		X		X ^e							
Vitals including O ₂ sat				X ^c							
Brain imaging ^h		X									
12-lead ECG		X									
MUGA/ECHO ^d		X									

Protocol 10420
RG1007463

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

Protocol 10420
RG1007463

Assessment	Screening	Pretreatment Evaluation	Lymphodepleting Chemotherapy	T cell Infusion (Day 0)	1	3	7	14	21	28	*see Note
CBC, differential, platelet count		X	X	X	X	X	X	X	X	X	Concurrent with restaging as clinically indicated Concurrent with T-cell persistence evaluation
Renal, hepatic function with LDH, Mg		X	X	X	X	X	X	X	X	X	Concurrent with restaging as clinically indicated
Uric acid		X		X	X	X	X				
Serum ferritin		X									As clinically indicated
CRP		X									As clinically indicated
IL-6		X									As clinically indicated
DIC panel without platelets		X									As clinically indicated
Quantitative IgG ^f		X									Approximately monthly
Virology panel		X									
ABO blood typing; antibody screen		X									
G6PD screening		X									
T cell persistence		X		X	X	X	X	X	X	X	Approx. 6 weeks and 2, 3, 6, 9, and 12 months after the T cell infusion and at the time of tumor relapse
T cell cellular immune response		X								X	Approx 2, 3, 6, 9 and 12 months after the T cell infusion
RCL testing by VSVG qPCR		X									Approximately 3, 6, and 12 months after the last T cell infusion
EDTA plasma archive		X								X	Approximately at 1, 2, 3, 4, 5, 6, 9 and 12 months after the last T cell infusion

	Screening	Pretreatment Evaluation	Lymphodepleting Chemotherapy	T cell Infusion (Day 0)	1	3	7	14	21	28	
Assessment											*see Note
PBMC archive								X		X	Approximately at 3, 6, 9 and 12 months after the last T cell infusion
Serum archive											Approximately at 3, 6, 9 and 12 months after the last T cell infusion
Citrate plasma archive		X		X	X	X	X	X	X	X	Approximately at 3, 6, 9 and 12 months after the last T cell infusion
Tumor Biopsy		X						X			Prior to enrollment, 2 weeks after and 12 weeks after the first T cell infusion.

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

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

^b Height required only at Pretreatment Evaluation .

^c Vital signs and O₂ saturation being recorded before, approximately **every 15 minutes during**, at completion of the infusion, and hourly after the infusion for at least 2 hours after infusion.

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

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

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

^g Pregnancy test within 72 hours of cell infusion

^h if history of brain metastasis or neurologic symptoms

ⁱ Imaging must be obtained within 45 days of enrollment

APPENDIX B: Research Sample Checklist

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

RECIPIENT RESEARCH EVALUATIONS BEFORE T CELL INFUSION

SAMPLE	TIME	TEST	TUBE	VOL. (Approx)	LAB
Blood	At Enrollment	T cell persistence and functional studies, epitope spreading, scRNAseq, clonality	Lavender top	60 mL	Research Cell Bank (RCB)
Blood	At Enrollment	Cytokine levels	Serum separator	20 ml	RCB
Blood	At Enrollment	RCL Testing	Lavender top	10 ml	RCB
Tumor	At Enrollment	Single cell RNA sequencing	RPML	3 cores	Chapuis D3-235
Tumor	At Enrollment	T cell localization and tumor microenvironment characteristics	Formalin	1 core; in addition to clinical core	Chapuis D3-235
Blood	Day 0	Cytokine levels (research)	Serum separator	10 ml	RCB
Blood	Day 0	T cell baseline persistence, function	Lavender top	30 ml	RCB

RECIPIENT RESEARCH EVALUATIONS AFTER T CELL INFUSION

SAMPLE	TIME	TEST	TUBE	VOL. (Approx)	LAB
Blood	Day 1	Cytokine levels (research)	Serum separator	10 ml	RCB
Blood	Day 1	T cell persistence, phenotype	Lavender top	40 ml	RCB
Blood	Day 3	Cytokine levels (research)	Serum separator	10 ml	RCB

SAMPLE	TIME	TEST	TUBE	VOL. (Approx)	LAB
Blood	Day 3	T cell persistence	Lavender top	20 ml	RCB
Blood	Day 7	Cytokine levels (research)	Serum separator	10 ml	RCB
Blood	Day 7	T cell persistence	Lavender top)	20 ml	RCB
Blood	Day 14	Cytokine levels (research)	Serum separator	20 ml	RCB
Blood	Day 14	T cell persistence and functional studies, epitope spreading, scRNAseq, clonality	Lavender top	60 ml	RCB
Blood	Day 21	Cytokine levels (research)	Serum separator	10 ml	RCB
Blood	Day 21	T cell persistence	Lavender top	30 ml	RCB
Blood	Day 28	Cytokine levels (research)	Serum separator	10 ml	RCB
Blood	Day 28	T cell persistence, immunophenotype	Lavender top	40 ml	RCB
Blood	Day 56	Cytokine levels (research)	Serum separator	10 ml	RCB
Blood	Day 56	T cell persistence, immunophenotype	Lavender top	40 ml	RCB
Blood	Day 84	T cell persistence, epitope spreading, scRNAseq, immunophenotype, clonality, RCL	Lavender top	60 ml	RCB
Blood	Day 84	Cytokines, research serologies	Serum separator	20 ml	RCB
If second infusion, repeat pattern above/please refer to study schedule					
Blood	Day 180	T cell persistence, epitope spreading, immunophenotype, clonality, RCL	Lavender top	60 ml	RCB
Blood	Day 180	Cytokines, research serologies	Serum separator	20 ml	RCB

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

SAMPLE	TIME	TEST	TUBE	VOL. (Approx)	LAB
Tumor	Clinical event	Single cell RNA sequencing	RPMI	3 cores	Chapuis D3-235
Tumor	Clinical event	T cell localization and tumor microenvironment characteristics	Formalin	1 core; in addition to clinical core	Chapuis D3-235

APPENDIX C: KARNOFSKY PERFORMANCE STATUS SCALE

KARNOFSKY PERFORMANCE STATUS SCALE

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

APPENDIX D: ECOG Performance Status Scale

ECOG Performance Status Scale

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

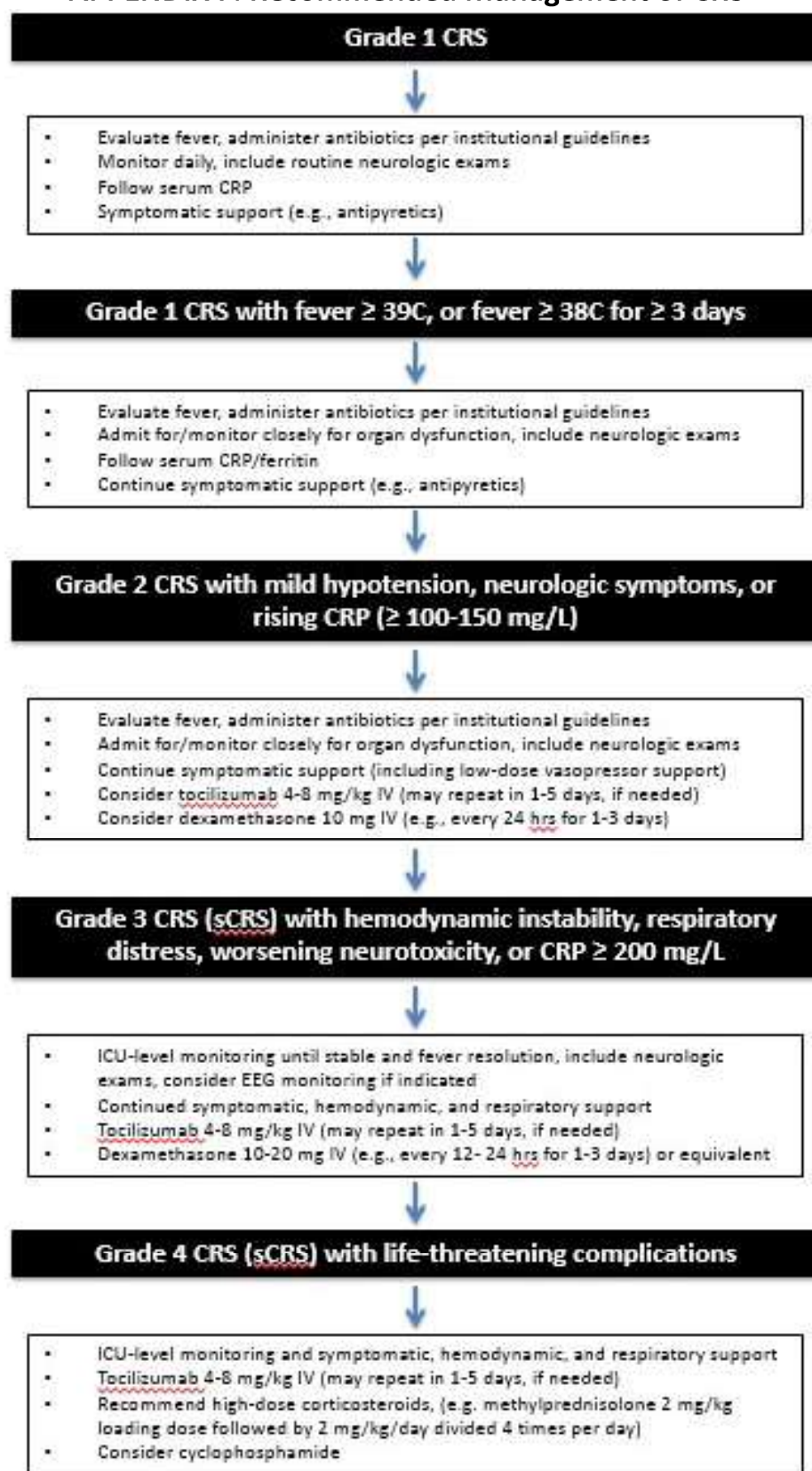
APPENDIX E: CRS Grading Criteria (CTCAE v. 5.0)

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

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

^a Organ toxicity excludes neurotoxicity, which will be evaluated separately

APPENDIX F: Recommended Management of CRS



ASTCT CRS Consensus Grading

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

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

*Fever is defined as temperature ≥38°C not attributable to any other cause. In patients who have CRS then receive antipyretic or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

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

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

ASTCT ICANS Consensus Grading for Adults

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

ICANS grade is determined by the most severe event (ICE score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause: for example, a patient with an ICE score of 3 who has a generalized seizure is classified as grade 3 ICANS.

N/A indicates not applicable

* A patient with an ICE score of 0 may be classified as grade 3 ICANS if awake with global aphasia, but a patient with an ICE score of 0 may be classified as grade 4 ICANS if unarousable.

† Depressed level of consciousness should be attributable to no other cause (e.g. No sedating medications)

‡ Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading.

§ Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE v 5.0

APPENDIX G: Long Term Follow-Up

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

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

Years 1 - 15:

1. Recommendation that patients undergo at least annual history and physical examination with their primary physician:
 - Adverse event screening guidance for the primary physician in the form of a gene therapy LTFU-directed screening survey may be available.
 - A request for the study team to be notified of all new malignancies and unexpected illnesses.
 - The primary physician may be provided with a blood draw courier kit to enable samples to be returned to the Chapuis Lab for archival purposes, and for analysis for transgene and vector persistence, and RCL, as dictated by studies of transferred T cell persistence.
 - If the patient develops a late recurrence, request for up to 50 cc of research blood as well as leftover or archival biopsy/surgical tissue, in order to evaluate for mechanisms of late recurrence (may be collected remotely with a courier kit)
2. Annual phone call survey or questionnaire to the participant to screen for adverse events.
3. Offer the opportunity to return to FHCRC for an annual LTFU clinic visit.
4. Compliance with 21 CFR 312.32 in adverse event reporting.
5. Research bloodwork: 30 cc of blood annually for persistence and RCL monitoring

APPENDIX H: DOSE ADJUSTMENT COMMITTEE

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

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

Ted Gooley, PhD (Biostatistician)

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

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

1. Description of the assay

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

2. Application of assay results to the clinical trial

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

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

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

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

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

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

5. Risk Analysis

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

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