Official Title	ATTAC-MCC: Phase I/II study of Autologous CD8+ and CD4+ Transgenic T cells expressing high affinity MCPyV-specific TCRs combined with Avelumab and Class I MHC-upregulation in patients with metastatic MCC refractory to PD-1 axis blockade
NCT Number	NCT03747484
Document Type	Protocol and Statistical Analysis Plan
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FRED HUTCHINSON CANCER CENTER

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Protocol Number: IRB 9845; Consortium RG1003611

Current Version: Version 15 - 02/06/2023

Prior Version(s): Version 14 – 11/01/2022

IND Number: FDA IND 18931

Investigational Agent: FH-MCVA2TCR: Autologous CD8+ and CD4+ T cells transduced with transgenic TCR A2-MCC1, interferon gamma

Study Regimen: Interferon gamma, FH-MCVA2TCR, and Avelumab or pembrolizumab

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Signature Page:
Investigator Statement:

I have carefully read Protocol 9845 entitled ATTAC-MCC: Phase I/II study of Autologous CD8+ and CD4+ Transgenic T cells expressing high affinity MCPyV-specific TCRs combined with Avelumab and Class I MHC -upregulation in patients with metastatic MCC refractory to PD-1 axis blockade ("Protocol") version date: 02/06/2023.

I agree to carry out my responsibilities in accordance with the Protocol, applicable laws and regulations (including 21 CFR Part 312), Good Clinical Practice: Consolidated Guidance (ICH-E6), and applicable policies of Fred Hutch.

Principal Investigator:

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Signature:_____ Date: _____

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PROTOCOL SYNOPSIS

Protocol: 9845	**RG:** 1003611	**Product Name:** FH-MCVA2TCR
Title of Study: *ATTAC-MCC: Phase I/II study of Autologous CD8+ and CD4+ Transgenic T cells expressing high affinity MCPyV-specific TCRs combined with Avelumab and Class I MHC - upregulation in patients with metastatic MCC refractory to PD-1 axis blockade*		
Sponsor: Fred Hutch		**Phase of Development:** I/II

Study Objectives:

Primary: Safety, Efficacy

Secondary/Exploratory: T cell persistence, T cell phenotype, T cell function, T cell localization into tumor, epitope spreading of T cell responses, recurrence-free survival, overall survival

Study Purpose and Rationale:

Metastatic Merkel cell carcinoma is an aggressive skin cancer caused by a virus. There are currently no effective treatments for individuals whose cancer progresses on or after treatment with PD-1 axis immunotherapies. We have developed an engineered autologous T cell therapy targeting Merkel cell polyomavirus. In a phase I/II trial, we seek to determine the safety and potential efficacy of this T cell therapy combined with interferon gamma, in addition to standard treatments (anti-PDL1/avelumab) for patients whose virus-associated metastatic/unresectable MCC tumors are resistant to PD-1 axis treatments.

Study Population:

Individuals that are HLA-A02+ with metastatic Merkel cell polyomavirus associated Merkel cell carcinoma whose disease has progressed on or after treatment with a PD-1 axis checkpoint inhibitor

Test Product:

- FH-MCVA2TCR: Autologous CD8+ and CD4+ T cells transduced with transgenic TCR A2-MCC1.
- Interferon gamma

Additional Interventions (not test product, given as standard of care):

- Avelumab
- Pembrolizumab (as an alternative to avelumab)

Safety Assessments: Ongoing; with evaluation of part I/II transition after three patients have been treated

DLT Definition:

DLTs will be assessed for 28 days following the infusion of the TCR transgenic T cell product. The following events will be considered DLTs if they are attributed as at least possibly related to TCR T

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cell administration. Grading will be done in accordance with NCI CTCAE version 5.0 unless otherwise specified. 1. Grade 3 allergic reaction or other event that leads to discontinuation of TCR T cell infusion 2. Grade 3 autoimmune reactions 3. Grade 3 neurotoxicity that does not resolve to grade 1 or less within 7 days 4. Grade 3 CRS that does not resolve to < grade 3 within 7 days. 5. Grade 4 organ toxicity not pre-existing or due to the underlying malignancy and occurring within 28 days of study product infusion. 6. Any other toxicity not meeting above criteria that is deemed by the PI to represent a DLT.
Efficacy Assessments: At completion of study
Statistical Methods: Phase I/II interventional trial, not masked, dose-escalation. Goal grade 3+ treatment related toxicity rate of <40%; likelihood of stoppage if true rate 25% is 0.05. Response determination by RECIST 1.1 with goal response rate statistically >5%; power to detect this is true response rate is 20% is 0.84. All secondary and exploratory endpoints exploratory.
Sample size: up to 16 participants

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1. BACKGROUND

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

Merkel cell carcinoma (MCC) is a highly aggressive skin cancer, with an incidence that has doubled since the year 2000 to over 2500 cases/year in the US.[1] Over one-third of patients will develop widespread disease, and survival in these patients has been historically poor with a 5-year survival rate of <10%.[2] MCC is very immune sensitive, with better immune responses being associated with better disease outcomes.[3] This is due to the strong antigenicity (visibility to the immune system) of the cancer-causing viral (Merkel cell polyomavirus; MCPyV) oncoproteins expressed in most MCC tumors.[4,5] Hence, immune therapies, particularly those targeting the viral oncoproteins may be effective for MCC treatment. Indeed, recent international studies led out of Fred Hutchinson Cancer Center (Fred Hutch) have demonstrated that immune checkpoint inhibitors (ICIs) targeting the PD-1 axis (including pembrolizumab/Keytruda and avelumab/Bavencio) have efficacy, with one third to one half of the treated patients experiencing disease shrinkage and a small minority of patients (<10%) experiencing complete response.[6,7] These demonstrate the potential for immunotherapy in MCC. However, for the majority of patients these treatments alone are ineffective and additional treatments are needed. We hypothesize that the patients who do not respond to checkpoint therapy alone have insufficient quantity of and/or poor-quality antiviral (and thus antitumor) T cells. Thus, we believe adding T cell therapies to the standard of care ICI avelumab [7-12] and administration of interferon gamma to facilitate T cell expansion (and increase the visibility of the tumor to the immune system) may be able to rescue patients who do not respond to an ICI alone.

In our previous Fred Hutch protocol 9245, we have combined avelumab, class I HLA upregulation, and autologous bulk expanded antiviral T cells in patients with advanced MCC.[13] Seven patients have received T cells as of October, 2018, with no unexpected toxicities. Encouragingly, three patients had durable complete responses (CRs) and a fourth patient had a non-durable partial response (PR). ICI have only recently become available for treatment of MCC, and treated patients on 9245 represent a mix of those with and without prior immune checkpoint inhibitor (ICI exposure). However, due to availability, accessibility, safety and cost considerations for the current protocol 9845/ATTAC we will focus on patients in second-line (failure of ICI monotherapy) where the clinical need is greatest and where there are no available FDA-approved treatments.

Although previous protocols had success with bulk expanded/endogenous T cells, generation of these endogenous T cells is an expensive and slow process. In addition, it is difficult to achieve the required dose as patient-derived MCPyV-specific T cells are poorly avid and are rare or undetectable.[5] Therefore, in the current proposed ATTAC regimen we are switching to a transgenic approach, which will allow us to deliver T cells to more patients, more cheaply, in greater numbers, and of higher quality (higher avidity). Similar transgenic T cells with a different target protein have been delivered to individuals with other malignancies including non-small cell lung cancer, mesothelioma, and acute myeloid leukemia.[14]

The present protocol, Protocol 9845: The ATTAC-MCC trial, is a phase I/II trial of the ATTAC regimen in patients with advanced MCC that has progressed on or after treatment with immune checkpoint (PD-1 or PD-L1 inhibitor) therapy. The ATTAC regimen is a combination of the FH-MCVA2TCR autologous transgenic CD8+ and CD4+ T cells carrying the transgenic TCR A2-MCC1 targeting the MCPyV,

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avelumab or pembrolizumab (PD-L1 and PD-1 inhibitors approved by the FDA for treatment of Merkel Cell Carcinoma), and class I MHC upregulation in the tumor tissue with interferon gamma. In part I (until safety demonstrated in 3 consecutive participants), participants will receive a lower dose of T cells for first infusion (5-fold dose reduction from target dose). If adequate safety profile is observed, we will proceed to part II; if adverse safety signal is seen, we will re-design trial in consultation with our DSMB and restart part I. In part II, participants will receive full dose of T cells for both infusions. We anticipate enrolling up to 16 HLA-A02+ participants with Merkel cell polyomavirus positive advanced MCC over 2 years.

We hope that study participation will provide direct benefits to the treated patients. This study will also benefit the research field for immunotherapy development for MCC and cancer more generally. Merkel cell carcinoma is an ideal cancer in which to develop immunotherapies due to the target of the viral oncoprotein which is immunodominant. This allows us to give T cells that are more on-target (with less risk of immune related adverse events; iRAEs) and also to more closely monitor immune responses to the cancer. If treatment is successful, as we are hopeful based on our early success with similar approaches as above, similar regimens could be expanded to other malignancies. If treatment is unsuccessful, our sophisticated immunological monitoring of the infused cells, native immune cells, and tumor tissues will allow for a cutting edge, comprehensive understanding of the mechanisms for tumor immune evasion that will provide essential data for developing new and better immunotherapies for MCC.

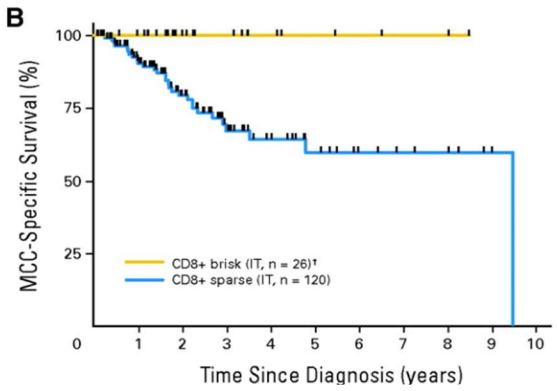

Figure 1: Improved MCC-Specific Survival in Patients with Brisk CD8+ T cell Infiltrate

2. STUDY PURPOSE AND RATIONALE

2.1 Rationale for Investigation of FH-MCVA2TCR

2.1.1 Scientific basis for T cell therapy targeting MCPyV in MCC

MCC is a highly immune sensitive tumor. Patients with immune suppression have very poor outcomes[3] whereas those with brisk T cell responses have excellent outcomes[15](**Figure 1**). Furthermore, immunotherapy is effective in MCC, with up to 50% of cases responding to PD-1 axis blockade (**Figure 2**).[6,7,10]

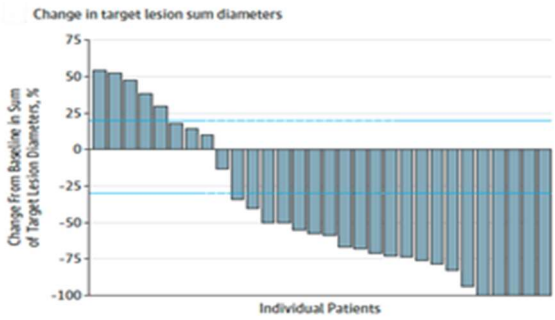

Figure 2: Response to first line avelumab (anti-PD-L1) in metastatic MCC[10]

The majority of MCC tumors (>80%) are caused by the Merkel cell polyomavirus (MCPyV). [16] The wild-type virus is a frequent skin commensal on healthy adults,[17-19] but it has been well demonstrated to play a causative role in virus-positive MCCs (VP-MCC). To become tumorigenic, MCPyV must undergo non-homologous recombination with the host cell genome and acquire an inactivating

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secondary mutation to block viral DNA replication, which would otherwise lead to host cell apoptosis (**Figure 3**).[16] The integrated virus is thus not transmissible (analogous to human papilloma virus in most cervical carcinomas). With no DNA replication capacity, integrated MCPyV is resistant to standard antiviral agents. However, viral early proteins (T-antigens) remain highly expressed and promote cell cycle progression through multiple mechanisms including inhibition of the tumor suppressor Retinoblastoma protein.[20]

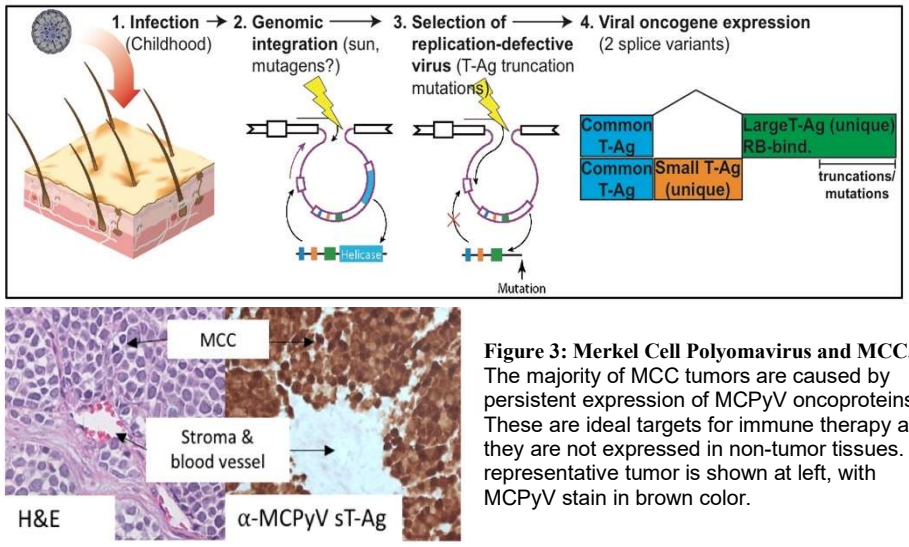

Figure 3: Merkel Cell Polyomavirus and MCC. The majority of MCC tumors are caused by persistent expression of MCPyV oncoproteins. These are ideal targets for immune therapy as they are not expressed in non-tumor tissues. A representative tumor is shown at left, with MCPyV stain in brown color.

In MCPyV-positive MCC tumors, the tumor cells are addicted to high expression of the viral oncoproteins ("T-Antigens")(**Figure 3**) and these antigens thus cannot be readily lost.[20] Importantly, the viral gene expression is only limited to the tumor tissue and is not present in healthy tissues, making these proteins a highly specific target for immunotherapies. In addition, these antigens are recognized by the immune system[21] and are immunodominant.[22-24] Patients with improved CD8+ T cell immune responses to the T antigen (as defined by quantity of T cells, number of reactive clones, and increased avidity of reactive T cells) have improved disease specific outcomes including survival.[5] Altogether, these antigens are compelling targets for tumor-specific immunotherapy.

2.1.2 Target epitope: "KLLEIAPNC" MCPyV Epitope

Effective T cell responses depend on the binding of the T cell receptor on the T cell surface to the class I HLA bound to viral peptide on the tumor cell surface. T cell receptor-based therapy is thus necessarily restricted to a single HLA type. For purposes of having the best data for safety and efficacy in the setting of a small trial, we will focus on a single epitope (T cell target) within the MCPyV-- the "KLL epitope." This epitope (KLLEIAPNC) is in the shared/common portions of the MCPyV oncoproteins (effectively doubling its expression within any given cell), is well conserved across MCC tumors,[25] and is not present in the human proteome by BLASTp search. It is restricted by HLA*A0201 which is the most common HLA allele detectable in MCC patients and is found in >59% of virus positive cases (**Table 1**).

Onco-protein	Epitope	AA 1-3	HLA Allele	% Pts	Infused in pts?
Common T Ag	cT_{15-23}	KLL	A*0201	59%	Yes
	cT_{20-30}	APN	B*0702	11%	Yes
	cT_{32-40}	AAF	A*0301	24%	No
	cT_{32-40}	AAF	A*1101	16%	No
	cT_{45-53}	KGG	B*3503	4%	No
Small T Ag	sT_{83-91}	FPW	B*3502	10%	Yes
	sT_{89-101}	KFK	B*3701	6%	No
Large T Ag	LT_{77-85}	DEV	B*1801	6%	No
	LT_{92-101}	EWW	A*2402	21%	Yes
	LT_{95-103}	RSG	A*0301	24%	No
	$LT_{137-145}$	HSQ	A*0101	26%	No

Table 1: Epitopes in MCPyV T antigens. Class I HLA restricted epitopes in MCPyV oncoproteins are listed, with AA1-3 (first three amino acids). HLA restrictions and percent of VP-MCC patients (n=127) carrying allele listed. KLL (top row) is the target of TCR A2-MCC1 and was selected as it is the highest frequency target among VP-MCC patients and has been effectively targeted with endogenous cell therapy (detailed in 2.1.3)

2.1.3 Clinical experience with T cells targeting MCPyV in MCC:

A total of 12 patients have been treated on three separate trials with autologous *ex vivo* expanded T cells, class I HLA upregulation with intralesional interferon beta and/or single fraction radiation).[13,26]

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	Patient	Targeted Epitope(s)	HLA restrict.	HLA upreg. Method	Grade III-IV AE or II-IV CRS	Best response	Response duration (months)
Double therapy (HLA ↑ + T cells)	2558-1	EWW	A*2402	IFN/XRT	Lymphopenia	PD	
	2586-1	KLL	A*0201	IFN		PD	
	2586-2	EWW & KLL	A*2402, A*0201	XRT	Lymphopenia	PD	
	2586-3	EWW	A*2402	IFN/XRT	CRS (gr. 2)	CR	13
	2586-4	FPW	B*3502	XRT	CRS (gr. 2)	PD*	
Triple Tx (HLA ↑+ T cells + anti-PDL1)	9245-1	EWW & APN	A*2402, B*0702	XRT	Lymphopenia	CR	24, ongoing
	9245-2	KLL	A*0201	IFN	Lymphopenia	CR	24, ongoing
	9245-3	KLL & FPW	A*0201, B*35	IFN/XRT	Lymphopenia, CRS (gr.	CR	18
	9245-5	KLL	A*0201	XRT	Lymphopenia	PR	3
	9245-6	KLL	A*0201	IFN		PD	
	9245-7	EWW	A*2401	XRT		PD	
	9245-8	KLL	A*0201	XRT	Treatment Ongoing		

Table 2: Results of endogenous cell therapy to date in MCC. Results of double therapy protocols (2558/2586) and triple therapy protocols (9245) are shown. Patient 2586-4 developed a subsequent late partial response lasting 22 months when immune checkpoint inhibitors were restarted after completing protocol treatment, disease had previously been immunotherapy refractory.

Seven of these patients were additionally treated with avelumab, closely mimicking the proposed regimen (which differs in its use of transgenic T cells). In these trials (**Table 1**), Grade 3-4 T cell-related adverse events were as anticipated in both groups, and included transient lymphopenia and modest cytokine release syndrome lasting<48 hours, manageable on the general ward with supportive care alone. The safety profile was similar between patients who received avelumab and those that did not. There have been no treatment related deaths nor sustained grade 3 or IV toxicities.

T cell persistence: Infused MCPyV specific autologous endogenous T cells persisted for years after initial infusion as confirmed by tetramer staining and demonstrated an effector memory (T_{EM}) phenotype during sustained remission (**Figure 4**).

Efficacy: Responses to T cell therapy to date for MCC are outlined in **Table 2**. In brief, in patients receiving endogenous T cells and HLA-upregulation, there was one objective response of 5 patients treated (ORR 20%). In patients receiving endogenous T cells, HLA upregulation and avelumab, there were 4 objective responses of 6 evaluable patients (ORR 67%). Patients had received between 0-3+ lines of prior systemic therapy prior to T cell therapy trial enrollment. Example responding patient shown in **Figure 5**.

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2.1.4 Rationale for Transgenic T cell approach

A transgenic approach offers following advantages over the prior endogenous T cell therapy approaches: time, potency, dose, number of patients, and cost. These are further detailed:

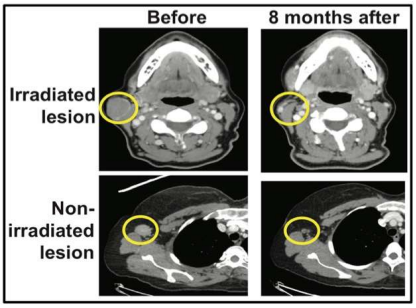

Figure. 5: MCC regression after TT. CT scans of irradiated and non-irradiated lesions are shown before and 8 mos after therapy began for Pt 9245-1, who achieved a biopsy-proven CR, ongoing at 24 mos

a) **Time to T cell product**: On our previous *ex vivo* expanded T cell trial for MCC patients, the time to T cell product production ranged from 49-80 days. Given MCC's rapid growth pattern, this delay is significant. A transgenic approach allows for T cells to be produced in less than one-quarter the time, approximately 10 days.

b) **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 virus-expressing tumor cells) varies greatly between patients. Indeed, for MCC, there is a 3-order of magnitude range of antiviral T cell avidity. Furthermore, patients with the best outcomes (thus least likely to need our treatments) have the best/most avid T cells, and the patients with advanced disease generally have worse T cells.[5] A highly avid T cell offers the best opportunity to "stick to" and kill a cell that has weak expression of the class I HLA genes, such as MCC tumor cells that are infected with MCPyV.[27] By transgenically transferring a highly avid T-cell receptor specifically targeting MCPyV oncoproteins, we can improve the ability of transferred T cells to respond to tumor.

c) **Ability to deliver a larger number of transgenic T cells:** Most endogenous cell products for MCC have not hit targeted dose and have instead ranged from 0.25-14% of targeted dose. As dose delivered is directly related to T cell persistence (which is thought to be essential for long term tumor control for T cell therapy,[28] it is important to reach as close to target dose as possible. By starting with a larger number of cells and transducing/gene-modifying them, target doses are more achievable.

d) **Ability to reach patients that are not otherwise targetable:** In a study of 69 virus positive, HLA-A02 positive patients with MCC, only 14% had detectable T cells targeting the MCPyV in their peripheral blood.[5] The other 86% of patients would be not be treatable by *ex vivo* expansion but would be treatable by transgenic approaches.

e) **Reduced treatment costs:** The ability to generate more potent T cell products in a relatively short-time and in a large scale will also ultimately reduce the cost associated with treatment.

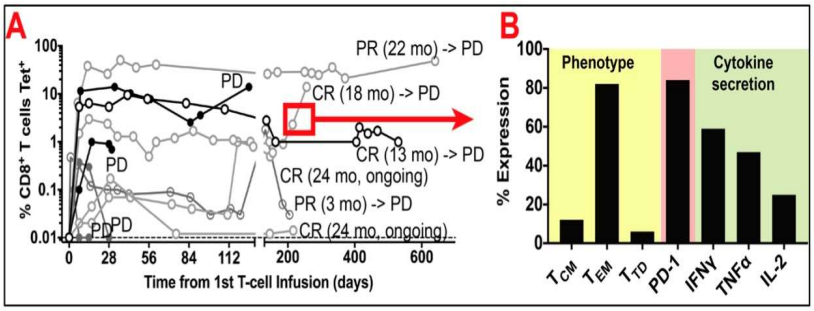

Figure 4: (A) persistence of MCPyV-specific T cells. %Tet$^+$ CD8$^+$ in peripheral blood (y-axis) after the first infusion (x-axis). The limit of detection (0.01%) is indicated. **(B) Phenotype and function of transferred cells.** Analysis of Pt 9245-3, on day 203 after transfer, showing % expression of T_{CM} (RA$^-$,R7$^+$), T_{EM} (RA$^-$,R7$^-$) and T_{TD} (RA$^+$, R7$^-$) (yellow), PD-1 (red) and %Tet$^+$ cells producing IFNγ, TNFα and IL-2 (green) in response to cognate antigen.

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2.1.5 Transgenic TCR vs. Transgenic CAR

Given the unqualified success of gene-modified CAR T cells in the treatment of lymphoid leukemias,[29] we carefully considered gene modified T cell therapy for MCC. T cell receptors (TCRs) and chimeric antigen receptors (CARs) have their distinct advantages and disadvantages for engineering therapeutic T cells.[30,31] We opted to design our MCC therapy with TCRs as the MCPyV T antigens have nuclear expression. We opted to design our MCC therapy with TCRs as the MCPyV T-Ag antigens have nuclear expression. Such intracellular targets are inaccessible to a CAR, whereas MCPyV peptide epitopes can be processed and presented to a TCR via HLA Class I. Additionally, naturally occurring TCRs, even high affinity TCRs, having survived negative selection during normal development in a human thymus that deletes self-reactive TCRs, further reducing the likelihood for an autologous T cell expressing such a TCR to mediate off-target toxicity.[32,33] Other groups are pursuing high-affinity TCRs for MCPyV, which have been shown to be effective in mouse models,[34] supporting the general approach. However, these TCRs were identified in mice, and such artificially derived TCRs have been associated with toxicity in other disease contexts.[35] Thus, we believe by exploiting the *in vivo* safety screen provided by the human thymus, we will have a safer naturally-isolated TCR product for our trials.

2.1.6 Safety of TCR transgenic T cells for other cancers

All prior MCPyV targeting T cell trials have been with non-transgenic T cell product. However**,** we have experience in preparing and delivering transgenic T cells expressing natural human transgenic TCRs isolated using similar approaches and in the same clinical vector in other disease processes. In clinical trial # NCT01640301,[36] we have treated 24 acute myeloid leukemia patients post allogeneic stem cell transplant with transgenic CD8+ T cells targeting the WT1 antigen. Apart from expected grade 1-2 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.[37]

2.1.7 TCR A2-MCC1 is a highly potent natural TCR that effectively lyses MCC cell lines

FH-MCVA2TCR cells are autologous CD8+ and CD4+ T cells gene modified to carry the TCR A2-MCC1, a high affinity T cell receptor identified from the natural repertoire from a healthy individual. For an effective TCR for clinical translation, we sought several features, most notably high-affinity (to generate highly avid T cells better able to stick to and recognize MCC tumors that have weak antigen presentation), safety (as further detailed below), and ability to recognize MCPyV variants. We screened 20 TCRs and identified the best candidates that were isolated from three approaches: the highest affinity TCR from traditional cloning from MCC TIL (TCR A2-Trad), the highest affinity TCR from an endogenous cell therapy product that eradicated an MCC tumor (TCR A2 9245-3), and a screen of the peripheral repertoire of 10 healthy HLA-A2 blood donors (TCR A2-MCC1) (**Figure 6**). Although both TCR A2 9245-3 and TCR A2-MCC1 demonstrated much higher affinity than those identified by standard cloning (**Figure 6E**), TCR A2-MCC1 was chosen for clinical translation. This is because TCR A2-MCC1 exhibited superior killing of MCC cell lines *in vitro* (**Figure 6F**) and recognized a frequent variant MCPyV sequence KLLEISPNC[25] in addition to the standard sequence KLLEIAPNC obviating the need for tumor DNA sequencing to confirm MCPyV epitope presence.

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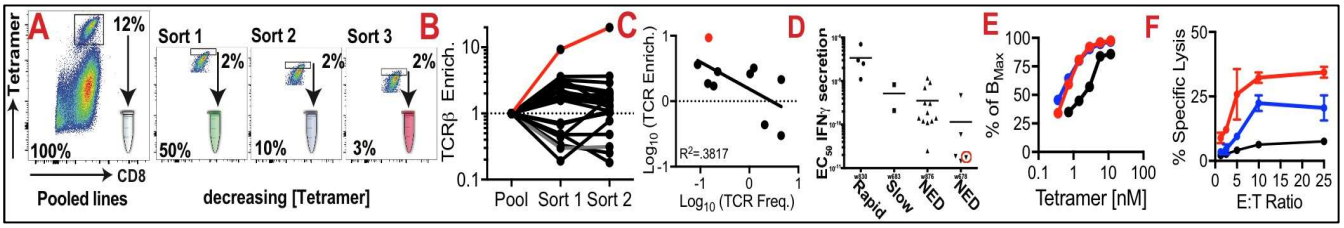


Figure 6. High-throughput TCR isolation. (A) The top 2% of cells are selected, using serial sorts at decreasing [tet.]. **(B)** Ratios (sort:pooled line frequency) identify clonotypes enriched with decreasing tetramer concentrations (red line –$TCR_{A2\text{-}MCC1}$). **(C)** TCR enrichment ratio (y-axis) inversely correlates with clonotype frequency in total pool. **(D)** EC_{50} of IFNγ secretion to decreasing [peptide] for T cell clones isolated from MCC pts with different clinical outcomes (adapted from [17]). Clinical outcomes: Rapid- rapid progression, Slow - slow progression, NED - no evidence of disease. Circle identifies $T_{A2\text{-}trad.}$ **(E)** Tet binding (affinity) and **(F)** Specific lysis of MCC Waga cell line by $TCR_{A2\text{-}trad.}$(black), $TCR_{A2\text{-}9245\text{-}3}$ (blue) and $TCR_{A2\text{-}MCC1}$ (red).

2.1.8 Evaluation of potential for off-target activity and cross or allo-reactivity of TCR A2-MCC1

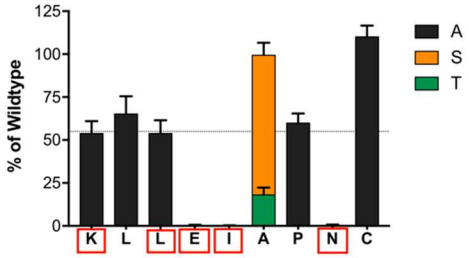
An alanine scan[38] of target peptide recognition performed for $T_{A2\text{-}MCC1}$ identified four residues *not* critical for binding to the TCR (**Figure 7**). A BLAST search of the human genome for peptides with allowed substitutions at each non-critical residue identified 10 potentially recognizable self-peptides,[39] none of which elicited responses. In addition, $T_{A2\text{-}MCC1}$ elicited no response to six HLA-$A2^+$ normal tissue cells and a panel of HLA-mismatched lymphoblastoid cells, indicating lack of cross- and allo-reactivity, respectively (**Table 3**).

	Cell line	% max (IFNγ+)
Eval. Tissue Cross-Reactivity	Cardiomyocytes	0.2
	Endothelial cell	1.1
	Hepatocytes	0.1
	Astrocytes	0.2
	GABA Neurons	0.4
	Renal epithelial cells	1.4
Evaluate HLA AlloReactivity	LCL1 (A2/A30 B7/B44 C7)	1.6
	LCL2 (A2/A11 B15/B44 C3/C5)	0.5
	LCL3 (A24/A32 B35/B40 C2/C4)	0.9
	LCL4 (A2 B44 C5/C7)	0.1
	LCL5 (A1/A2 B7/B15 C1/C7)	0.3
	LCL6 (A3/A68 B13/B40 C2/C6)	1.0
	LCL7 (A1/A68 B44/B55 C3/C5)	0.3
	LCL8 (A2/A33 B7/B15 C3/C7)	0.4
	LCL9 (A1/A2 B8/B55 C3/C7)	0.1
+ Cntrl	+ 'KLL' epitope peptide	100.0

Table 3: $T_{A2\text{-}MCC1}$ cross- and allo-reactivity.

2.1.9 Rationale for using a third-generation lentiviral vector

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.[40]



Gene Name	AA residues	AA sequence	SYFPEITHI	T1/2	netMHCpan rank
		KLLEIAPNC	16	263.78	1.20%
TNMD	173 - 181	KiLEIcdNv	23	248.81	0.40%
ATP4B	92 - 100	KgLEIvyNv	20	55.44	3.50%
CC168	1794 - 1802	KaLEIqlNl	20	15.75	5.50%
CLIP1	805 - 813	KhLEIekNa	8	0.18	60%
UBTFL1	59 - 67	KwLEIscNl	15	0.16	11%
STAB2	1263 - 1271	KvLEIqkNr	8	0.07	41%
TM9S3	159 - 167	KkLEIgfNg	5	0.03	60%
B4GN2	360 - 368	KpLEIkdNh	5	0.01	95%
PRKX	350 - 358	KdLEIfkNf	4	0.01	55%
CC178	454 - 462	KkLEIdiNk	5	0.00	60%

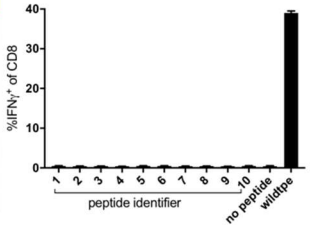


Figure 7: Alanine scan of TCRA2-MCC1 reveals absence of cross reactivity. Left panel: results of alanine scan reveals non-critical residues. Center panel: Results of BLAST search identifies 10 potentially cross-reactive peptides in human proteome requiring further evaluation. Right panel: absence of detectable cross-reactivity to any of the 10 identified peptides.

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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.[41]

Insertional oncogenesis was not observed in the long-term results from three clinical trials evaluating γ-RV-engineered T cells for patients with HIV.[42] 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.[43] The MSGV1-based γ-RV was also used to transduce autologous T cells to express an NY-ESO-1-specific TCR that was infused in 17 patients with metastatic synovial sarcoma or melanoma[43] 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).[44] 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.[45] 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.[46] *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.[47]

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.[29]

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 MCC. The potential toxicities are listed in the protocol consent form and will be discussed 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 13**.

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2.1.10 Rationale for substrate T cell of 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 MCC 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,[48,49] and we can choose the T cell type used for adoptive cell therapy.

Conventional CD8+ T cells can be divided into naïve and antigen-experienced, memory T cell subtypes (TN, TM). TM cells can be further divided into T_{CM} and effector memory T cell (T_{EM}) subsets, which have distinct transcriptional programs associated with defined characteristics, including homing, phenotype, and function. When the 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.[50]

Studies of transferred purified CD8+ T cell subsets in a murine virus infection model revealed that transferred TCM provide enhanced protective immunity from *in vivo* challenge compared to T_{EM}. Although TCM cells expand and also differentiate into effector cells in response to *in vitro* stimulation, adoptive transfer studies in non-human primates revealed that TCM–derived effectors had been imprinted and retained some of the beneficial properties of their parent TCM cell, in particular the capacity for self-renewal, which translates into improved *in vivo* persistence and response to antigen challenge.[49]

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

2.1.11 Rationale for inclusion of 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.[51] 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 we showed long ago in an FBL murine leukemia model.[52] More recently this has been shown with therapeutic CD19-CAR T cells,[53,54] 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.[38,55] $CD4^+$ T cells expressing Class II-restricted TCRs exhibit direct cytolytic activity in metastatic melanoma and have had anti-tumor activity against human cholangiocarcinoma.[56,57] However, Class II expression is rare in solid tumors[58] and absent in MCC. An alternative might be to confer

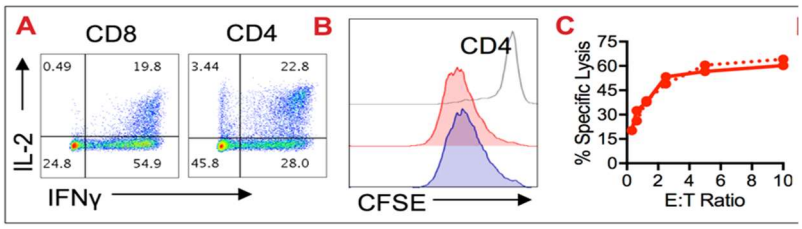

Figure. 8. $TCR_{A2\text{-}MCC1}$ engages $CD4^+$ T cell effector mechanisms. (A) Cytokine production after cognate antigen encounter of $T_{A2\text{-}MCC1}$ $CD4^+$ and $CD8^+$ T cells. **(B)** CFSE dilution of proliferating $tCD4^+$ day 5 post stimulation (red=$TCR_{A2\text{-}MCC1}$, blue= $TCR_{A2\text{-}9245\text{-}3}$). **(C)** Specific lysis by $T_{A2\text{-}MCC1}$ $CD4^+$ (dashed line) and $CD8^+$ T cells (solid line) of MCPyV expressing fibroblasts.

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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.[59]

TCR A2-MCC1 can engage CD4+ T cells even when transduced in absence of CD8+ coreceptor. CD4+ T cells carrying TCR A2-MCC1 can produce beneficial cytokines (**Figure 8A**), proliferate (**Figure 8B**), and kill MCPyV oncoprotein expressing cells (**Figure 8C)**. Therefore, FH-MCVA2TCR will include both CD8+ and CD4+ T cells transduced to carry TCR A2-MCC1.

2.1.12 FH-MCVA2TCR Dose Rationale

Our prior endogenous cell therapy trials targeted an infused dose of 10 billion (10^{10}) MCPyV-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 the 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 goal dose is 1 billion (10^9) MCPyV-specific TCR transgenic CD8+ T cells for the initial low dose part I infusion, and 5 billion ($5x10^9$) MCPyV-specific TCR transgenic CD8+ T cells for the second dose infusion or part 2 infusions. CD8+ and CD4+ cells are co-cultured and transgenic CD4+ cells will also be included. The dosing will be based on CD8+ T cells for consistency in formulation. Please see **Section 6.4** for additional dosing information. This dose was selected for several reasons: it is achievable in an approximately 2 week production, it is similar to dosing for TCR transgenic T cells for other disease processes, and it is similar to doses given to 3 previous patients on previous versions of this trial with no toxicity attributed to the transferred T cells. All patients included in this study will be adult and are thus expected to have similar body surface areas, and thus we have selected a flat rather than weight-based dosing; this approach is used with dosing with that of other transgenic T cell products for adults including FDA-approved CAR-T therapies (for example, Kymriah).

2.1.13 Rationale for omission of Lymphodepleting Chemotherapy prior to T cell infusion

Chemotherapy lymphodepletion, typically with fludarabine and cyclophosphamide, is frequently used for transgenic T cell immunotherapy trials, in order to induce a favorable cytokine environment for T cell engraftment and promote expansion and persistence.[60,61] We have elected to omit this chemotherapy conditioning for several reasons.

a. Safety: Cytokine-mediated toxicity of transgenic CAR-T therapies is increased in the context of lymphodepletion,[61] and it is plausible that such toxicities could extend to transgenic TCR therapy. It is also possible that depletion of endogenous T cell responses to the tumor could lead to faster progression of disease. One subject received lymphodepleting chemotherapy in a previous version of this trial, and concerns for increased rapidity of disease progression following lymphodepletion compared to before were noted.
b. Non-necessity: T cell persistence has been demonstrated for patients with MCC out to years without preceding lymphodepletion (**Figure 4**), and a patient treated on an earlier version of this trial achieved a clinical objective response without lymphodepleting chemotherapy. Two patients

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who received lymphodepleting chemotherapy had inferior expansion and persistence of transgenic T cells in an earlier version of this trial.

c. Potential to abrogate epitope spreading: Epitope spreading has been observed in multiple patients (**Figure 9**) and we believe will be essential to combat immunotherapy escape by antigen loss, a common mechanism of escape for TCR and CAR-T cell therapies.[29] Chemotherapy has potential to reduce epitope spreading by depleting endogenous T cells.

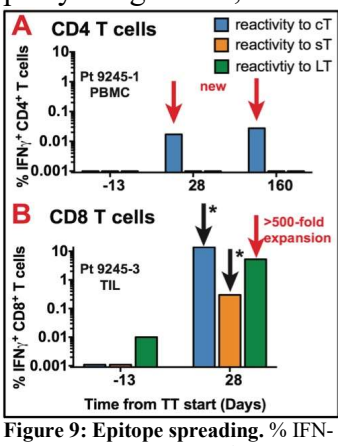

Figure 9: Epitope spreading. % IFN-gamma secreting **(A)** $CD4^+$, Patient 9245-1 peripheral blood mononuclear cells (PBMCs) and **(B)** $CD8^+$ Patient 9245- 3 TIL, exposed to cT, sT and LT peptide pools. Black arrows indicate infused $CD8^+$ specificities; red arrows indicate expanded/new reactivities.

2.2 Rationale for inclusion of subcutaneous interferon gamma

2.2.1 Scientific basis

As our therapy utilizes a transgenic TCR, we are dependent on antigen presentation by the primary tumor through class I MHC. We and others have shown that approximately 80% of MCC tumors downregulate MHC-I presenting an obstacle to T cell efficacy, and this is further true of tumors that have escaped immunotherapy.[27,62] In 5 patients previously treated in an earlier version of this clinical trial, the only patient with a meaningful clinical response was the single patient with robust baseline class I MHC expression. Furthermore, that patient had loss of class I MHC expression in a single resistant lesion. Despite the small number of patients, this suggests that downregulation of class I MHC may be a key mechanism of resistance to transgenic TCR T cell therapy. However, this class I downregulation is typically reversible with one of several interventions, which include radiation treatment, epigenetic modifiers, chemotherapy, and cytokine treatments.[27] Earlier versions of this trial included either single fraction radiation to a subset of lesions (n=3 patients) or chemotherapy treatment (n=2 patients) without clinical efficacy in patients without baseline class I MHC expression. This indicated a different strategy was necessary to efficiently upregulate class I MHC in tumors.

2.2.2 Interferon Gamma- Preclinical results

In order to identify molecules that might be effective at clinically meaningful upregulation of class I MHC, a merkel cell line WAGA was treated with multiple either FDA-approved or investigational small

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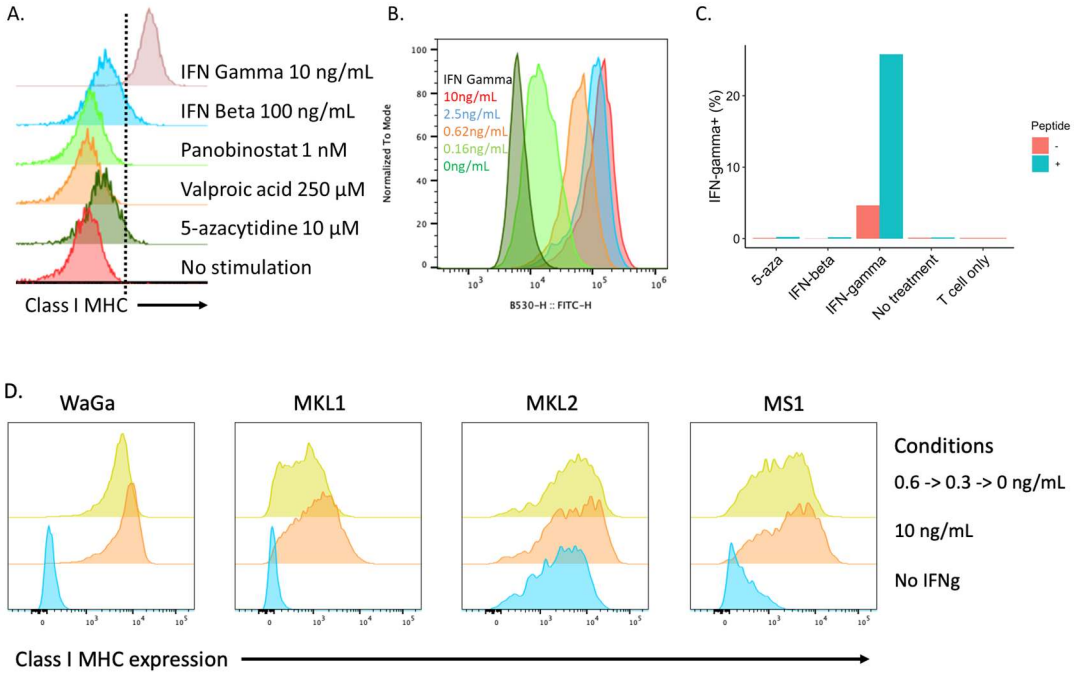

Figure 10: HLA upregulation by interferon gamma in Merkel cell carcinoma cell lines
WAGA merkel cell line was incubated with the indicated agents for 48 hours and Class I MHC expression was measured by flow cytometry (A-B) and recognition of cells by the TCR-T cells was measured with and without addition of exogenous peptide (C). D. Each of 4 indicated Merkel cell lines were incubated with either a stable, high concentration of IFNg (10ng/ml) or mimicking of in vivo pharmacokinetics with 0.6ng/ml for 6 hours, followed by 0.3ng/ml for 6 hours, followed by washout and class I MHC expression was measured after 48 hours by flow cytometry

molecule agents thought to have class I upregulating effects, and interferon gamma showed the most robust upregulation of class I MHC on these cells. We found that the most dramatic effects on class I upregulation were seen with interferon gamma (**Figure 10A**), and these effects were seen at concentrations that could realistically be achieved *in vivo* with standard dosing (**Figure 10B**). Importantly, interferon gamma not only upregulated class I MHC but also allowed for recognition of endogenous antigen in Merkel cells in vitro (**Figure 10C**). Importantly, mimicking conditions that would be seen in vivo with standard dosing of interferon gamma (Peak concentration 0.6ng/ml, half life of 6 hours) but using 0.6ng/ml for 6 hours and 0.3ng/ml for 6 hours followed by washout showed robust upregulation of class I MHC across 4 different Merkel cell carcinoma cell lines (**Figure 10D**).

2.2.3 Interferon gamma- safety and clinical results

Interferon gamma is FDA-approved for the treatment of chronic granulomatous disease at a dosage of 50mcg/m2 given three times weekly, and is generally tolerated with the known common (>10%) side effects of fever, headache, chills, fatigue, rash, diarrhea, vomiting and injection site reactions. The peak concentration of interferon gamma occurs in 7 hours and is 0.6ng/ml in plasma, with a half-life of 6 hours. Importantly this concentration is associated with significant class I MHC upregulation in Merkel cell carcinoma cells in vitro (**Figure 10B**). Use of subcutaneous interferon gamma in cancer patients has been explored in a previously reported phase 0 trial conducted in sarcoma patients.[63] In this trial, administration of interferon gamma was well tolerated and resulted in upregulation of class I MHC in patient tumors and increases in measures of tumor inflammation. Interferon gamma at standard dosing has been given in combination with the PD-1 inhibitor nivolumab in a phase I clinical trial of over 20 patients with no evidence of increased immune related adverse events related to nivolumab (no cases of

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grade III or greater immune toxicity during combination treatment) or potentiation of side effects from interferon gamma (Matthew Zibelman, personal communication, manuscript in preparation).

2.3 Rationale for administration of TCR-transgenic T cells in the context of treatment with avelumab or Pembrolizumab as a standard of care

2.3.1 Avelumab and Pembrolizumab - Scientific basis

Our data from current T cell therapy trials, protocols 2586 and 9245, strongly support inclusion of avelumab. In protocol 2586, combining autologous *ex vivo* expanded anti-MCPyV T cells with HLA upregulation but without immune checkpoint blockade for MCC, we observed only one patient out of four responding. We further noted that the patient who responded had much lower PD-1 expression on their infused T cells, as compared to patients who did not respond. We thus hypothesized efficacy may be augmented by combination treatment (**Figure 5**), combining three immunotherapies ("triple therapy"): T cells, class I MHC upregulation, and PD-L1 blockade with avelumab. Indeed, in protocol 9245 combining T-cells with HLA-upregulation and avelumab, we noted improved response rate with 4 of 6 patients responding, including several durable complete responses. We have noted no worsened toxicity with addition of avelumab.

Table 4: Toxicities of avelumab observed among 88 patients with advanced MCC treated as part of the JAVELIN-200 trial

	Grade 1–2	Grade 3
Fatigue	21 (24%)	0
Infusion-related reaction*	15 (17%)	0
Diarrhoea	8 (9%)	0
Nausea	8 (9%)	0
Asthenia	7 (8%)	0
Rash	6 (7%)	0
Decreased appetite	5 (6%)	0
Maculopapular rash	5 (6%)	0
Blood creatine phosphokinase increase	1 (1%)	1 (1%)
Lymphopenia	0	2 (2%)
Blood cholesterol increase	0	1 (1%)
Aminotransferase increase	0	1 (1%)
Potential immune-mediated treatment-related adverse event†		
Hypothyroidism	3 (3%)	0
Hyperthyroidism	2 (2%)	0
Pneumonitis	1 (1%)	0
Type 1 diabetes mellitus	1 (1%)	0

Multiple ICIs have been studied in MCC, including avelumab (anti-PD-L1), pembrolizumab, and nivolumab (anti-PD-1). All are showing clinical effect, and all would be expected to synergize with T cell therapy. Both Avelumab and Pembrolizumab have been FDA approved as standard of care treatments in MCC, and for this reason, they have been chosen as two alternatives for use in this trial.

2.3.2 Avelumab - Clinical experience & safety

Avelumab (trade name Bavencio) is a recently FDA-approved fully humanized monoclonal antibody targeting PD-L1 with indications for Merkel cell carcinoma and advanced bladder cancer.[7,64] As described above, we believe the addition PD-1 axis blockade to T cell therapy essential due to high PD-1 expression on infused T cells and improved efficacy signal combination therapy. Major side effects include: infusion reactions, immune related adverse events (iRAEs), and other. Infusion reactions are a well-characterized side effect of infusional antibody therapy and occur with 17-22% of patients receiving Bavencio[7]. Consistent with package insert/manufacturer's guidelines, patients will be premedicated with acetaminophen and an H1 blocking antihistamine prior to each infusion, infusions will be given slowly, and patients will be monitored closely for the development of infusion reaction. iRAEs are a well-described complication of any PD-1 axis blocking agent; for avelumab the most commonly reported include pneumonitis, colitis, hepatitis, endocrinopathies, and type 1 diabetes. Thyroid endocrinopathies are the most common iRAE (occurring in up to 6% of patients) and will be closely monitored for with regular blood work according to manufacturer's recommendations.

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Pneumonitis is the most severe reported complication. Among all patients receiving avelumab, pneumonitis is reported per FDA labelling insert as occurring in 1.2% (21/1738) of patients receiving avelumab. There has been one reported case (0.1%) of a patient with Grade 5 pneumonitis resulting in death, one (0.1%) with Grade 4, and five (0.3%) with Grade 3 pneumonitis. Immune-mediated pneumonitis led to permanent discontinuation of avelumab in 0.3% (6/1738) of patients. Finally, other side effects include fatigue, arthralgias, and headache (see table 4). Avelumab has been successfully given with ex vivo expanded T cells in 6 MCC patients, with no increased toxicity seen.[13] As detailed below, avelumab dosing will be given at standard dose level and interval.

Pembrolizumab is a fully humanized anti PD-1 antibody also FDA approved in MCC. It has a similar incidence of immune related adverse events to avelumab in trials of MCC patients and has a lower incidence of infusion reactions than avelumab. It has also been given safely in combination with adoptive transfer of Chimeric antigen receptor modified T cells in several trials[65-67], suggesting it is likely to be safe in combination with adoptive T cell transfer in this trial.

2.4 Rationale for inclusion of biopsies

Serial biopsies are very informative for determining whether T cells are preferentially localizing into the tumor environment and interactions between tumor and microenvironment. These can help to guide future studies. If treatment is effective, features of tumor-enriched T cells can be studied to optimize future T cell therapy products, and TIL can be investigated for epitope spreading. If the treatment is ineffective, study of the tumor microenvironment will allow determination of the reason for treatment failure and whether it is a tumor intrinsic or T cell intrinsic defect. We have developed a suite of tools to optimize information from small biopsies (multiplex immunohistochemistry, single cell RNA sequencing) in order to maximize benefit from tiny clinical specimens and reduce risk to research participants (**Figure 11 and 12).**

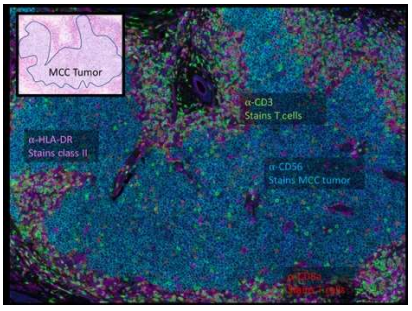

Figure 11: Multispectral IHC. A MCC tumor is shown. Multicolor IHC permits multiple studies to be done on a FFPE slide, maximizing information from precious biopsy specimens.

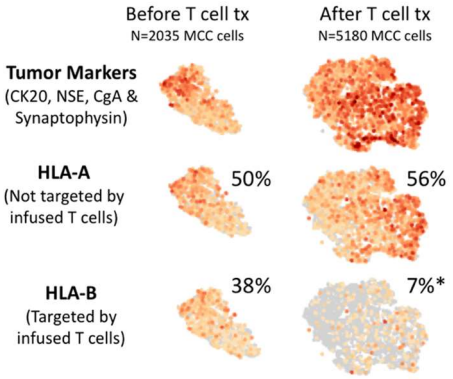

Figure 12: Single Cell RNA sequencing identifies mechanism of T cell escape in an MCC tumor. Expression of the relevant gene is shown by orange color, with darker color indicating increased expression. Each tumor cell is indicated by a dot. This patient was treated with MCPyV targeting T cells restricted to ah HLA-B3502 epitope. After initial 22 month response to treatment the tumor later progressed despite persistence of infused T cells, and the tumor tissue was found to have lost the HLA-B expression necessary for T cell recognition

3. STUDY OBJECTIVES

3.1 Primary Objectives

3.1.1 Safety

To evaluate the safety of adoptive T cell therapy using FH-MCVA2TCR (defined as an observed treatment-related grade 3 or higher toxicity rate consistent with a true rate <= 40%.)

Patients who have metastatic MCC that has progressed beyond treatment with first line PD-1 inhibitors have few therapeutic options and no FDA-approved options. Commonly used treatments in this scenario include platinum-based doublet chemotherapy which has guarded efficacy and a 30 to 40% grade ≥ 3 toxicity rate.[68] In terms of non-chemotherapeutic options, combination ICI immunotherapy (such as CTLA-4/PD-1, ipilimumab + nivolumab) is occasionally used. This combination has not been well studied in MCC but carries a

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55% grade 3-4 toxicity rate in metastatic melanoma.[69] Therefore, a toxicity rate of 40% is acceptably safe in this high-risk population with advanced disease that without treatment is uniformly fatal.

Please see biostatistical section for further details on toxicity assessments.

3.1.2 Efficacy.

To demonstrate clinical activity of FH-MCVA2TCR (defined as an observed response rate that statistically exceeds 5%)

Without treatment, metastatic MCC is near uniformly fatal. Spontaneous regressions have been reported in up to 1% of patients, therefore we will conservatively define a comparison response rate as 5% to allow for this and also for the possibility of very delayed checkpoint inhibitor response (although these have not been reported in MCC).[6,7] There are no FDA-approved or known durably effective treatments for patients with advanced MCC tumors refractory to checkpoint blockade (hence the rationale for pursuing this population). Therefore, success in even a minority of patients would significantly advance the field.

Please see biostatistical section for further details on efficacy assessment and power calculation. In brief, in order to meet this endpoint, at least 3 of 16 patients will need an objective response (best response of partial response or complete response) by RECIST 1.1 criteria.[70]

3.2 Secondary Objectives

3.2.1 Persistence

Persistence of infused transgenic T cells in peripheral blood

3.2.2 Migration

Migration of infused transgenic T cells into tumor tissue

3.2.3 Progression free and overall survival

Determine progression free and overall survival of treated individuals

3.2.4 Determination of preferred dose of FH-MCVA2TCR

Please see section 4.4 for details on dose escalation

3.2.5 Response rate by immune RECIST

Determine objective response rates by immune related RECIST criteria. Please see Hodi *et al* 2018 for definition of iRRC criteria. [71]

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) to other MCPyV and/or neoantigen epitopes 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.

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3.4 Projected Target Accrual

Table 5: Targeted / Planned Enrollment			
Ethnic Category	**Patient Numbers**		
	Females	**Males**	**Total**
Hispanic or Latino	0	1	1
Not Hispanic or Latino	6	9	15
Ethnic Category Total of All Patients	6	10	16
Racial Category			
American Indian / Alaska Native	0	0	0
Asian	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	0	0	0
White	6	10	16
More than One Race	0	0	0
Racial Categories: Total of All Patients	6	10	16

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 reported for MCC cases in the Surveillance, Epidemiology, and End Results (SEER) cancer registry database.**[1] There is a white predominance due to the well described MCC risk factor of UV exposure.[72]

Sex/Gender
Males 63%
Females 37%

Ethnicity
Hispanic/Latino 5%
Not Hispanic/Latino 95%

Race
American Indian/Alaska Native <1%
Asian <1%
Native Hawaiian/Pacific Islander 1%
Black/African American 1%
White 96%

4. STUDY DESIGN AND INVESTIGATIONAL PLAN

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

The study will be a phase I/II, non-masked (not blinded), interventional study aimed at treating up to 16 HLA*A0201 patients with metastatic MCC that has progressed despite immune checkpoint inhibitor therapy (PD-1 or PD-L1 inhibitor). All patients will receive study interventions.

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Patients may receive the other, standard-of-care portions of the ATTAC regimen in addition to T cells. This includes the PD-L1 inhibitor avelumab. Please see **Section 6/treatment plan** for further details).

Patients will receive up to two T cell infusions. Please **see 6.4.4** for details regarding selection of one versus two cycles of T cell therapy.

Any patient experiencing treatment related grade 3 or 4 toxicity (except for the expected toxicity specifically excepted/outlined in **Section 12.6**) will immediately stop receiving T cell infusions, avelumab, and class I HLA upregulation. They will receive treatment as appropriate for their condition (see **Section 7** and **Section 12**). They will continue to be followed for response/outcome. Patients with infusion reactions to avelumab may receive pembrolizumab for first or subsequent infusions.

As a criterion for enrollment into the trial, participants must agree to biopsies of tumor tissue if medically feasible. A minimum of 4 biopsies will be performed as clinical status allows: prior to interferon gamma administration, 1-2 days prior to T cell infusion, 2 weeks after, and 12 weeks after the first full dose T cell infusions. 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 investigator or treating physician's discretion.

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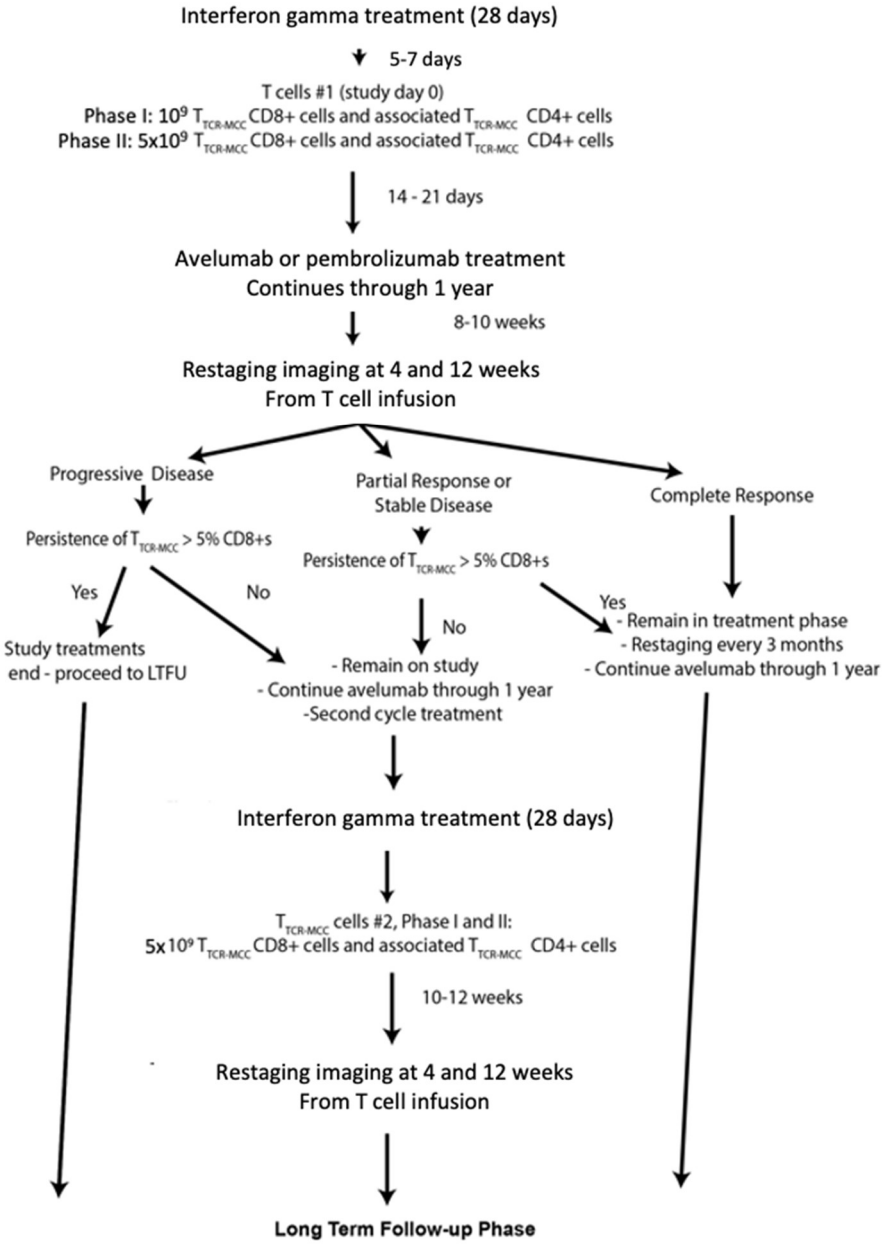
4.2 Schematic



4.3 Protocol Enrollment

Enrollment to study commences after eligibility confirmation and just prior to leukapheresis. Enrollment is expected to take approximately 2 years. Study treatment with initial follow-up is expected to be approximately 1 year. All patients who receive treatment with FH-MCVA2TCR 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 LTFU assessments, is approximately 3.5 years.

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4.4 Staggering and Dose Escalation

At least three patients will be treated on part I. In part I, patients will be treated with a lower-dose first infusion. Each part I patient must complete their dose-limiting-toxicity (DLT) evaluation period (28 days) after their first T cell infusion before the next part I patient may receive their first infusion. This allows for an ample buffer/window to evaluate for potential cytokine release syndrome and/or neurotoxicity, before proceeding to next patient. After three patients have received their first T cell infusion and completed the 28 day DLT evaluation after the lower first dose infusion, with one or fewer patients experiencing grade 3-4 d related adverse events as defined in **Section 12**, the study may move to part II. If two or more of the first three patients have a treatment-related grade 3 or 4 toxicity, or if any of the first three patients experiences a treatment-related death, at that point the trial will be immediately suspended. We will consult with the data and safety monitoring board (DSMB), and a detailed review of the potential causes of toxicity undertaken. We will reconvene with the DSMB and consideration will be given to changing the cell dose or construct if the T cells were implicated. In that scenario, the dose escalation will be restarted and an additional three patients treated before proceeding to the part II portion of the study. Part I will continue until 3 patients are treated at the lower dose level. If the manufacturing process is short of the intended dose targeted for the patient, whatever smaller dose is available from a single manufacturing run will be given.

For the first three patients treated on part II, the staggering interval will continue as for part I, with each treated patient completing their 28-day DLT evaluation period after first T cell infusion before the next part II patient may be treated at that dose. If two or more of the first three part II patients have a treatment-related grade 3 or 4 toxicity, unless a toxicity specifically accepted in **Section 12.5 and 12.6,** or if any of the first three patients experiences a treatment-related death, at that point the trial will be immediately suspended. We will consult with the data and safety monitoring board (DSMB), and a detailed review of the potential causes of toxicity undertaken. We will reconvene with the DSMB and consideration will be given to changing the cell dose to the part I dose level and/or other protocol modifications on guidance of the DSMB. If instead three part II patients have received their first T cell infusion and completed the 28 day DLT evaluation after the lower first dose infusion, with one or fewer patients experiencing grade 3-4 related adverse events as defined in **Section 12**, staggering restrictions will be lifted and there will be no specific staggering requirement.

As patients eligible for second infusion will have tolerated a previous exposure to the same T cell product, between-patient staggering intervals will not apply for second infusion. However, patients will be monitored for a second 28-day DLT period and safety events recorded. Patients previously treated with lower doses of T cells on earlier versions of this trial will be eligible to receive up to an additional 2 doses of TCR transgenic T cells, starting at the dose level for newly entering patients provided that they meet eligibility criteria in **Section 5** before each additional dose.

5. STUDY POPULATION AND ELIGIBILITY

The target study population consists of adult patients with metastatic or unresectable MCPyV-associated Merkel cell carcinoma (VP-MCC) that has progressed on or after prior treatment with a PD-1 axis immune checkpoint inhibitor. Patients must meet all of the inclusion and exclusion criteria to be enrolled in this study.

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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 – Evaluation

Individuals that may be consented to undergo evaluation to determine potential eligibility must have a history of metastatic or unresectable MCC (as documented by medical record), be 18 years of age or older and be capable of understanding and providing informed consent.

5.2 Inclusion Criteria

5.2.1 Metastatic or unresectable VP-MCC

Participants must have metastatic or unresectable, histologically confirmed virus-positive MCC. Confirmation of diagnosis must be or have been performed by internal pathology review of initial or subsequent biopsy or other pathologic material at Fred Hutch.

Approximately 80% of MCCs are caused by MCPyV T Antigens,[16] and the treatment is expected to only be effective in this population. Merkel cell polyomavirus positivity may be established through one of two means: positive Merkel cell polyomavirus T antigen serology (preferred) or immunohistochemistry of a of primary or metastatic MCC tumor lesion (if T antigen seronegative).

MCPyV T Antigen serology will be performed with the AMERK assay run through the University of Washington Medical Center Laboratory Medicine Clinical Immunology Laboratory (https://testguide.labmed.uw.edu/public/view/AMERK).[73,74] Positivity will be defined as a Merkel oncoprotein antibody titer (MSCTT) of >= 75 standard titer units (STU) at any time point from initial diagnosis onward. Patients with negative T antigen serology but MCPyV positive tumor by other methodologies will be considered for additional MCPyV testing as clinically appropriate, as the T antigen serology assays are highly specific [73,75] but incompletely sensitive for MCPyV status.[73] In this case, immunohistochemistry of any tumor lesion will be performed with the CM2B4 Merkel cell polyomavirus T antigen antibody[76]. Expression of MCPyV in at least 10% of tumor cells will be considered positive. CM2B4 staining performed at any point clinically and at any clinical laboratory may be accepted. However, if CM2B4 staining has not been previously performed by a clinical pathology laboratory as part of MCC diagnostic workup, it will be performed in a clinical diagnostic pathology laboratory at UW/Fred Hutch as per standard staining protocols. Persons are not required to have both CM2B4 positivity and seropositivity; either will be acceptable confirmation of viral status and if one negative and the other positive the patient will remain eligible provided other criteria are met.

5.2.2 Previous treatment with a PD-1 axis inhibitor or contraindication to treatment with a PD-1 axis inhibitor

Patients must have been previously treated with at least one dose of a PD-1 axis inhibitor (e.g., PD-1 or PD-L1 inhibiting monoclonal antibody such as pembrolizumab, nivolumab, avelumab, atezolizumab, durvalumab), developed progression of their MCC tumor on or after treatment, or have biopsy confirmed residual disease following treatment. At least four weeks must have passed between the administration of the first dose of PD-1 axis inhibitor and determination of progression or residual disease. If there is significant clinical concern for pseudoprogression (i.e., progression developed rapidly after checkpoint inhibitor therapy), biopsy must be performed to demonstrate true progression. Patients may have received 1 or more prior systemic regimens for MCC. There is no upper limit on prior

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regimens. Patients may have received prior anti-PD-1/anti-PD-L1 in the neoadjuvant or adjuvant setting. Patients are also eligible if they have a contraindication to PD-1/PD-L1 axis blockade such as a history of an autoimmune disease.

5.2.3 HLA type HLA-A*02:01

Participants must be HLA-A*02:01 in order for infused transgenic T cells to recognize antigen-MHC complexes. HLA typing for HLA-A2 should be determined through molecular approaches at a clinical laboratory licensed for HLA testing.

5.2.4 Life expectancy must be anticipated to be >3 months at trial entry

5.2.5 18 years of age or older

Fewer than 0.5% of MCC occur in individuals aged 30 years or younger, thus the protocol includes only adult patients. Participants may be of any gender, race, or ethnicity.

5.2.6 Capable of understanding and providing a written informed consent

5.2.7 If fertile, willingness to comply with reproductive requirements.

These are outlined in detail in **Section 5.4**.

5.2.8 Karnofsky performance status of >= 60%

Please see **Appendix C** for Karnofsky performance status

5.2.9 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 investigator. Similarly, should an investigator determine that a biopsy cannot be performed safely for clinical reasons biopsies may be cancelled or retimed.

5.2.10 Participants must be at least three weeks from last systemic MCC treatment

At least 3 weeks must have passed since any: immunotherapy (for example, T-cell infusions, immunomodulatory agents, interleukins, MCC vaccines, intravenous immunoglobulin, expanded polyclonal TIL or LAK therapy), NK therapy, small molecule or chemotherapy cancer treatment, other investigational agents **or other systemic agents that target MCC.** There is no washout period for radiation.

5.2.11 Acceptable organ function

Acceptable organ function is defined as:

- Renal: serum creatinine < 2 or eGFR > 45
- Hepatic: tBili < 3.0, 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 judgment of the treating physician, patients with FEV1 ≥ 50% of predicted and DLCO (corrected) of ≥ 40% of predicted will be eligible.
- Cardiac: Patients 50 years of age or older are required to have left ventricular ejection fraction (LVEF) evaluation performed within 2 months prior to study treatment. LVEF may be

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established with echocardiogram or MUGA scan and must be ≥ 35%. Cardiac evaluation for other patients is at the discretion of the treating physician.

- Hematologic: ANC > 1000 cells/mm3, ALC > 200 cells/mm3, HCT > 30%, platelet count > 50K.

5.3 Exclusion Criteria

5.3.1 Active autoimmune disease

Active autoimmune disease requiring immunosuppressive therapy is excluded ***unless discussed with the PI.*** **Patients with a history of autoimmune disease that was a contra-indication to PD-1 axis blockade treatment will be eligible, but will not receive PD-1 axis blockade treatment on trial.**

5.3.2 Prior solid organ transplant or allogeneic hematopoietic stem cell transplant.

Kidney transplant 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 likely**. 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 a history of allogeneic stem cell transplant.**

5.3.3 Corticosteroid therapy at a dose equivalent of >10 mg prednisone per day.

5.3.4 Concurrent use of other investigational agents or MCC directed therapies.

5.3.5 Any Medical or psychological condition other than Merkel cell carcinoma that would significantly increase the risk of harm to a subject or interfere with the interpretation of trial endpoints.

5.3.6 Active uncontrolled infection.

HIV positive participants on HAART with a CD4 count > 500 cells/mm^3 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 hepatitis well controlled on medication.

5.3.7 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.

5.3.8 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.

5.3.9 Active treatment for prior immune related adverse event to any immunotherapy.

Participants receiving treatment for prior Immune Related Adverse Events (iRAE) are excluded, with exception of hormone supplementation or corticosteroid therapy at equivalent of up to 10 mg prednisone per day, or immune related adverse events that have resolved to grade 1, unless otherwise approved by

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PI. Patients with Grade 3 or higher Immune related adverse event (iRAE) to any prior PD-1 axis blocking agent will be eligible provided these have resolved to grade 1 or better, and are not requiring treatment greater than prednisone 10mg daily. These patients with prior high grade iRAE will not receive a PD1/PD-L1 axis inhibitor on the trial**.**

iRAEs are persistent T cell mediated inflammatory syndromes caused by PD-1 or PD-L1 inhibitors including colitis, nephritis, pneumonitis, myositis, hepatitis, encephalitis.

5.3.10 Significant underlying neurologic disease.
Study participants must not have significant active underlying neurologic disease, unless approved by PI. Mild neuropathy related to diabetes or prior chemotherapy is acceptable.

5.3.11 Other medical, social, or psychiatric factor that interferes with medical appropriateness and/or ability to comply with study, as determined by PI.

5.4 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)

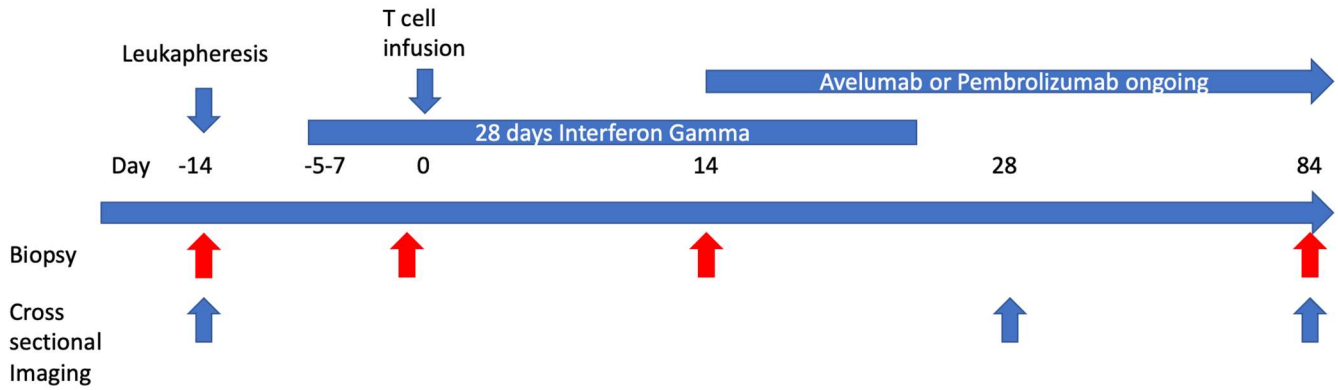
Pregnancy test for females of reproductive potential must be negative within 14 days before leukapheresis.

Female patients with reproductive potential who are not sexually abstinent and male patients who are sexually active with females of reproductive potential must agree to use a suitable method of contraception for the duration of the study (from date of consent through treatment period).

6. TREATMENT PLAN

Please see **Section 4.4** for information on patient staggering and escalation from part I to part II.

6.1 Overall schema



In part I, the first T cell infusion will be up to 1 x 10^9 tetramer positive CD8+ T cells. Although targeted at 1:1, the actual number of transgenic CD4+ T cells will vary between individuals due to interpersonal

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variability (cells are co-cultured). Please see **Section 6.4** for maximum infused transgenic T cells, and **Section 2.1.12** for dose rationale. If patients have progressive disease and tetramer positive T cell persistence below 5% of peripheral CD8+ T cells, a second infusion may be administered at a higher dose of up to 5 x 10^9 tetramer positive CD8+ T cells. In part II, the initial and potential second T cell dose will be up to 5 x 10^9 tetramer positive CD8+ T cells.

Transgenic T cells and interferon gamma are investigational, and avelumab or pembrolizumab are provided as standard of care. Therefore, duration of avelumab or pembrolizumab infusions is provided as a suggestion only and may be shortened or extended on the medical judgement of the treating oncologist.

6.2 Leukapheresis

Patients must be eligible and consented for treatment portion of study prior to leukapheresis because cell product preparation will begin immediately upon leukapheresis in most cases. Following enrollment on the study, a leukapheresis collection will be performed on each patient to obtain peripheral blood mononuclear cells (PBMCs) for the production of the FH-MCVA2TCR investigational product. Leukapheresis will be performed by the Fred Hutch Apheresis Unit using 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-MCVA2TCR cells are manufactured for the prescribed dose, the patient may undergo a second collection procedure.

Patients 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 Fred Hutch or the Cell Therapy Laboratory (CTL) at Fred Hutch for cell selections then transported to the Fred Hutch CPF for study product manufacturing 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.

6.3 Interferon Gamma treatment

Interferon gamma is being administered with the goal of upregulation of class I MHC on Merkel cell carcinoma cells to allow recognition of tumor cells by TCR transgenic T cells. Interferon gamma (Actimmune) will be administered at the FDA-approved dosing (for the indication of chronic granulomatous disease) of 50mcg/m2 given subcutaneously in the abdomen, anterior thigh, or deltoid region three times weekly, starting 5-7 days prior to the T cell infusion and continuing for a total of 4 weeks. Patients will receive premedication with acetaminophen prior to each dose to minimize flu-like symptoms. Doses will be held if creatinine rises above 2.0; creatinine will be monitored by regular comprehensive metabolic panels per **Appendix A.**

Dose reduction or skipping, as clinically required in the event of adverse events is permissible at the discretion of the PI or designee.

6.4 FH-MCVA2TCR Infusions

FH-MCVA2TCR will be administered by IV infusion at Fred Hutch (or at University of Washington Medical Center (UWMC) if the patient is hospitalized). T-cell infusions will be given preferably at least

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48 hours before the next dose of avelumab or pembrolizumab. However, the infusion may be delayed if required for clinical reasons. Patients will receive either 1 or 2 infusions of FH-MCVA2TCR. Infusions will be ideally scheduled for 12 weeks apart; however, they may be given earlier in the case of early disease progression or later in the event of a need for bridging chemotherapy treatment.

Patients in the part I portion will receive a lower dose for the first infusion of up to 10^9 (one billion) TCR A2-MCC1 transgenic CD8+s, and then up to 5 x 10^9 (five billion) TCR A2-MCC1 transgenic CD8+ T cells at the second infusion. Patients in the part II portion will receive a full dose of up to 5 x 10^9 (five billion) TCR A2-MCC1 transgenic CD8+ T cells at both infusions. Infusions will also include CD4+ T cells that will vary naturally between patients as the cells are co-cultured; the total allowable transgenic cell dose (including transgenic CD8+s and CD4+s) will be 10 times higher than the targeted CD8+ dose (e.g., 10^{10} for low dose infusion and 5 x 10^{10} for standard dose infusion respectively).

Patients previously treated on the protocol at lower cell doses than currently available will be eligible to re-enter the protocol to receive up to 2 additional infusions.

T cells will be infused intravenously through a blood component filter set on an electronic infusion device over approximately 60 minutes. The infusion bag will be gently mixed periodically during the infusion. All patients will be observed for 2 hours post infusion and will be released if no AE requiring prolonged hospitalization have occurred.

6.4.1 Preparation of Cell Product

All manufacturing activities are conducted using approved written procedures performed by trained, qualified personnel in accordance with current Good Manufacturing Practices (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.

If a product cannot be formulated to meet a target cell dose, the cell product should be infused at or as close as possible to the total transgenic CD8+ T-cell dose. A product will be considered evaluable for response if it contains ≥10% of the scheduled CD8+ transgenic T cell dose; however, lower dosages of cells may be administered at the discretion of the investigator.

6.4.2 T Cell Infusions

1. TCR-T cells should be administered approximately 5-7 days after the initiation of ***interferon gamma*** A window of 4-8 days is acceptable if a 5-7 day window is not possible for logistical reasons.
2. On the day of scheduled T cell infusion, the patients should undergo a clinical evaluation and a clinical determination for appropriateness to proceed with T cell administration.
3. 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. In these cases, the product will be frozen and thawed on the new day of administration, as per facility policy and procedures.

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4. Patients who are unable to undergo TCR-T cell infusion within the intended window due to manufacturing or release issues may receive the cell infusion outside of this window if the issues can be resolved. Discussion with the protocol PI and notification of IND holder is required.

FH-MCVA2TCR will be provided as a single cell product at the assigned cell dose.

The final product will be prepared and labeled according to standard operating procedure 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 in a cooler with a cool pack. A nurse will then administer the cells using standard/universal institutional precautions for blood borne pathogens.

All patients will be monitored during each T-cell infusion with vital signs and O_2 saturation being recorded (+/- 5 minutes) within 15 minutes prior to the start of infusion, at thirty-minute intervals during, at completion of the infusion, and hourly after the infusion for at least 2 hours after the infusion.

6.4.3 Acute infusion reactions

Acute infusion reactions may occur with administration of FH-MCVA2TCR. Guidelines for the monitoring and treatment of acute infusion reactions are provided in **Sections 7.3 and 7.4**.

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

Each "cycle" of T cell therapy consists of one cycle of interferon gamma plus T cell infusion. Interferon gamma will be given with second infusions unless patient has had a grade 3 or higher toxicity attributed to interferon gamma.

Twelve weeks after first T cell infusion, restaging and evaluation of peripheral blood persistence of infused transgenic T cells will be performed, and patients will be considered for a second cycle (second class I MHC upregulation procedure plus second infusion of transgenic T cells). A second cycle may be given between 6 and 12 weeks after the first cycle in patients who have progressive disease and <5% TCR T cells among CD8 T cells in the blood. Avelumab or pembrolizumab infusions will continue up to the second infusion. Decision-making surrounding a second cycle will be at the discretion of the investigator and will be generally made following the below guidelines:

Individuals with progressive disease and non-persisting T cells (<5% of CD8) may be considered for a second infusion if they meet eligibility criteria for treatment as described in **Section 5** above. Patients with symptomatic progression may receive debulking radiotherapy or chemotherapy for one or more cycles per standard of care between the first and second T cell infusions, but the interval between the end of chemotherapy and the second infusion must be at least 3 weeks. Treatment with avelumab or pembrolizumab will be discontinued during chemotherapy and will be resumed 1 week before the second infusion of T cells.

Individuals with **progressive disease** and persistent T cells (>5% of CD8) will no longer receive additional T cells or avelumab or pembrolizumab on study. They will leave the treatment phase of the study and will transition to long-term follow-up.

Individuals with **complete response** will remain on the treatment phase of the study and will continue to receive avelumab or pembrolizumab to complete a year of treatment. However, they will not receive

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additional T cells, given that they have no lesions with which to target with class I MHC upregulation and also that the risk-benefit profile favors fewer interventions in the setting of complete response. For these patients, T cell product will be cryopreserved, and they may be considered for a second cycle of T cell therapy in the future, at the discretion of the investigator, should their MCC recur.

Individuals with **partial response** or **stable disease** (per RECIST 1.1) will remain on study and will continue to receive avelumab or pembrolizumab to complete a year of treatment. They will have evaluation of the persistence of their transgenic T cell product as well as the suitability of residual disease for class I MHC upregulation. Should they have poor to moderate persistence (for instance <5% TCR A2-MCC1 CD8+s) in the peripheral blood of the transgenic T cells by tetramer stain, a second T cell infusion may be given. If there is a lesion suitable for class I MHC upregulation, they will also receive a second class I MHC upregulation.

6.5 Avelumab or Pembrolizumab – standard of care

Avelumab is a fully human monoclonal antibody of the immunoglobulin (Ig) G1 isotype that selectively binds to PD-L1 and competitively blocks its interaction with PD-1. Compared with anti-PD-1 antibodies that target T cells, Avelumab targets tumor cells and therefore is expected to have fewer side effects, including a lower risk of autoimmune-related safety issues, as blockade of PD-L1 leaves the PD-L2 / PD-1 pathway intact to promote peripheral self-tolerance.[77] In the event of an infusion reaction to avelumab prior to or during the study, the standard of care for the patient may change from avelumab to pembrolizumab. Pembrolizumab is a fully human monoclonal antibody that binds to and inhibits PD-1. Pembrolizumab may have a slightly higher incidence of immune related adverse events in patients with MCC[78]. If the patient has a history of grade 3 or higher immune related adverse event with prior PD-1 axis blockade, or if the patient has a history of a significant autoimmune disease that precluded PD-1 axis blockade treatment, the patient will not be treated with avelumab or pembrolizumab on this study. Patients who experience grade 3 or greater toxicity with ipilimumab containing regimens will be eligible to receive avelumab or pembrolizumab on study if they subsequently tolerated PD-1 axis inhibition without immune adverse event prior to enrollment.

Avelumab (Bavencio) and pembrolizumab (Keytruda) are both FDA-approved for the treatment of MCC.

6.5.1 Provision of avelumab or pembrolizumab

Avelumab will be given as standard of care and will be billed to the study participant's insurance.

Since the initial writing of this protocol, the PD-1 inhibitor pembrolizumab has been FDA approved for the treatment of metastatic Merkel cell carcinoma. For patients with a history of infusion reactions to avelumab, pembrolizumab can be administered with this study in place of avelumab at 200 mg every 3 weeks or at any subsequent FDA approved dosing regimen for this indication, starting 14 days following investigational T cell infusion.

6.5.2 Avelumab or pembrolizumab infusion

Avelumab will be administered as standard of care. Currently, the FDA recommended dosing is 10 mg/kg over the duration of 1 hour (-10/+70 minutes) at an interval of once every 2 weeks. Premedications suggested by package label include pretreatment with H1 blockers (diphenhydramine 12.5mg to 50 mg PO or IV, or equivalent) and acetaminophen PO 500 to 650 mg for first several doses.

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First avelumab dose is intended 14 +/- 3 days after first T cell infusion up to 50 weeks (total of 25 infusions) or longer, as clinically indicated under the standard of care or until Off Study Criteria are met as described in **Section 10**. The first dose of avelumab will be calculated based on the actual weight of the participant determined at study entry. As avelumab is being administered as standard of care, if alternate doses or schedules are approved by the FDA for avelumab for treatment of advanced MCC (for instance q4 week dosing or flat instead of weight-based dosing), these doses and schedules may be substituted at the discretion of the investigator and treating oncologist. Following first avelumab infusion, participants will be observed for 1 hour post infusion for potential infusion-related reactions. Participants will receive avelumab once every 2 weeks for 50 weeks or until the criteria in **Section 11.7** (Off Study Criteria) are met. Modifications of the infusion rate due to infusion-related reactions and management of acute infusion reactions are below as recommended in product label.

The first avelumab infusion after each T cell infusion should be administered at Fred Hutch for monitoring in case of acute adverse event. Subsequent infusions may be given locally by a licensed oncology professional experienced in prescribing immunotherapy. A "Dear Physician" letter detailing the study treatment and providing contact information to the study team including emergency contact information will be provided to local/non-Fred Hutch oncologists.

Monitoring exams and blood work associated with avelumab infusion will be performed clinically at the discretion of the treating oncologist and in keeping with institutional standards for avelumab infusion.

If pembrolizumab is given instead of avelumab, it will be administered over 30 minutes intravenously with similar monitoring as above and without premedications unless at the direction of the clinical team.

Typical practice at Fred Hutch: 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 typically 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. However, different institutions have different standards and policies for avelumab or pembrolizumab monitoring, and labs may need to be more or less often for safety reasons or include additional labs depending on patient's course, therefore the protocol will allow flexibility in the avelumab or pembrolizumab.

Avelumab or pembrolizumab doses may be held, rescheduled or dosages reduced at the discretion of treating oncologist in keeping with standard clinical practice. If immune related adverse event secondary to avelumab or pembrolizumab suspected or confirmed, treating oncologist to discuss management with study team and joint management decision established.

6.5.3 Recommended Supportive Care, Additional Treatment, and Monitoring

Prophylactic treatment/measures are recommended for patients at risk for tumor lysis syndrome (TLS) according to institutional or clinical standards. Supportive care for the management of TLS is detailed in **Section 7**.

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

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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.

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

7. POTENTIAL RISKS AND TOXICITY MONITORING

7.1 Toxicity associated with interferon gamma administration

Administration of interferon gamma can commonly cause fever, headache, chills, fatigue, rash, nausea and vomiting, diarrhea, myalgia, abdominal pain, depression, arthralgias, and local injection site reactions. More rare but serious adverse events include colitis, hypersensitivity reactions, elevations in liver function tests, myocardial infarction, cytopenias, hypokalemia, stevens johnson syndrome and neurologic effects.

7.2 Management of toxicity associated with interferon gamma

Patients will be premedicated with acetaminophen 650 mg prior to each dose.
Mild acute administration reactions should be managed with antipyretics, antihistamines, and anti-emetics as clinically appropriate, and these may be added to prophylaxis for future doses.
Corticosteroids should be avoided. Appropriate emergency medications (e.g., epinephrine, diphenhydramine, etc.) should be readily available during administration and institutional guidelines should be followed for the treatment of anaphylaxis.
Fever and flu like symptoms should be treated with antipyretics as needed.
Local reactions can be managed by choosing alternate sites.
Severe symptoms or those involving hypoxemia or hypotension, particularly following T cell infusion, should be treated as presumptive cytokine release syndrome and treated as below.
Doses may be held or treatment discontinued due to intolerable side effects or grade III or higher adverse events attributable to interferon gamma. Adverse events attributable to T cell infusion (e.g. cytokine release syndrome) will be assumed to be synergistic with interferon gamma, and interferon gamma will be held in these cases.

7.3 Acute Toxicity Associated with T-Cell Infusion (Infusion reaction)

Administration of MCPyV-specific TCRs may cause infusion reactions, including fever, rigors, rash, urticaria, dyspnea, hypotension, and/or nausea. Mild infusion reactions should be managed with antipyretics, antihistamines, and anti-emetics as clinically appropriate. Corticosteroids should be avoided. Appropriate emergency medications (e.g., epinephrine, diphenhydramine, etc.) should be readily available during administration and institutional guidelines should be followed for the treatment of anaphylaxis.

7.4 Management of Acute Infusion Reaction(s)

For infusion reactions or any clinically significant changes from baseline, stop administration of MCPyV-specific TCRs, notify the provider to have the patient assessed and administer appropriate symptomatic treatment.

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- Fever, chills, and temperature elevations > 38.0°C may be managed with acetaminophen 650 mg PO every 4 to 6 hrs. All patients who develop fever > 38.0°C or chills should have a blood culture drawn and the PI or designee should be notified.
- 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.

After the resolution of symptoms, an order may be obtained to resume administration at a reduced rate. If symptoms re-occur, stop the administration, administer symptomatic treatment as ordered and contact the IMTX attending for the final determination to discontinue the infusion.
If an adverse reaction occurred during the IEC infusion or post-monitoring period, the provider will determine in collaboration with the research team if any workup is needed.

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

7.5 Acute Toxicity Associated with Avelumab or Pembrolizumab Infusion

As with all monoclonal antibody therapies, avelumab or pembrolizumab carries a risk of allergic reaction, although infusion reactions are more common with avelumab[10] (20%) than pembrolizumab[78] (2%) in MCC. If hypersensitivity reaction occurs, the participant must be treated according to the best available medical practice. Hypersensitivity reactions associated temporally with infusion of pembrolizumab or avelumab will be attributed to pembrolizumab or avelumab. Reactions not temporally linked with pembrolizumab or avelumab will be attributed to interferon gamma.

Avelumab: special precautions

As infusion reactions have been commonly observed with avelumab, pre-treatment with H1 blockers (diphenhydramine 12.5-50 mg PO, IV, or equivalent), and acetaminophen (500 to 650 mg orally) will be administered 30 to 60 minutes prior to the first avelumab infusion to mitigate infusion-related reactions including hypersensitivity reactions. Pretreatment for subsequent infusions or pembrolizumab infusions will be per treating physician/institutional standard of care.

Infusion of avelumab or pembrolizumab will be stopped in case of Grade ≥ 3 hypersensitivity, inflammatory response, or infusion-related reaction. The treatment recommendations for infusion-related reactions, severe hypersensitivity reactions, and tumor lysis syndrome are as outlined below. These may be substituted with an institution's specific guideline/standard practice for avelumab infusion related reactions.

Avelumab or pembrolizumab: <u>Infusion-related reactions</u>

Symptoms
Fever
Chills
Rigors
Diaphoresis
Headache

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Management

Table 6. Treatment modification for symptoms of infusion-related reactions caused by Avelumab or Pembrolizumab

NCI-CTCAE Grade	Treatment Modification for Avelumab
Grade 1 – mild Mild transient reaction; infusion interruption not indicated; intervention not indicated.	Decrease the Avelumab or Pembrolizumab infusion rate by 50% and monitor closely for any worsening. The total infusion time for Avelumab or Pembrolizumab 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 Avelumab or Pembrolizumab 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. Grade 4: Life-threatening consequences; urgent intervention indicated.	Stop the avelumab or pembrolizumab infusion immediately and disconnect infusion tubing from the participant. Participants who have a reaction associated with avelumab may not receive any further administration of avelumab as part of this study, but they may receive administration of pembrolizumab as part of this study. Participants who have a reaction associated with pembrolizumab may not receive any further administration of pembrolizumab as part of this study. In addition, they may not receive any administration of avelumab as part of this study.”

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

Once the avelumab or pembrolizumab 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 avelumab or pembrolizumab treatment. If a participant experiences a Grade 3 or 4 infusion-related reaction at any time, the participant must discontinue avelumab or pembrolizumab. Patients who experience infusions reactions with avelumab may receive pembrolizumab for future infusions. Patients who experience infusion reactions with pembrolizumab may not receive avelumab for future infusions.

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Management of severe hypersensitivity reactions and flu-like symptoms due to avelumab or pembrolizumab 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

7.6 Cytokine Release Syndrome (CRS)

Administration of transgenic T cells such as FH-MCVA2TCR 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 2019 Lee et al consensus guidelines for immune effector cell will be utilized to quantify T cell associated CRS,[79,80] as detailed in **Appendix E.**

General guidelines for management of CRS are provided in **Appendix F** based on our current institution standard practice**.** Other cytokine-directed therapies may be considered after discussion with the PI and/or immunotherapy attending.

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7.7 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. Neurotoxicity/ICANS will be graded according to 2019 Lee et al consensus guidelines, [79] as detailed in **Appendix E.**

For patients who develop mild neurologic manifestations (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.

7.8 Tumor Lysis Syndrome

Although rarely reported for MCC, FH-MCVA2TCR 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 interferon gamma and continued for as long as the medical team determines appropriate after the infusion, unless there are contraindications. Patients may receive additional hydration for the first 2 weeks after infusion. If TLS develops, as defined by the Cairo Bishop criteria,[81] the Attending Physician will direct patient management with guidance from the study staff. [82] 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.

7.9 Macrophage Activation Syndrome

Macrophage activation syndrome (MAS) is a serious disorder potentially associated with uncontrolled activation and proliferation of T cells and subsequent activation of macrophages. MAS is typically characterized by high-grade, non-remitting fever, cytopenias, and hepatosplenomegaly, and may be difficult to distinguish from CRS. Laboratory abnormalities found in MAS include elevated inflammatory cytokine levels, serum ferritin, soluble IL-2 receptor (sCD25), triglycerides, and decreased circulating NK cells. Other findings include variable levels of transaminases, signs of acute liver failure, coagulopathy, and disseminated intravascular coagulopathy. While there are no definitive diagnostic criteria for MAS, it is typically diagnosed using published criteria for hemophagocytic lymphohistiocytosis.[83]

Patients treated with FH-MCVA2TCR should be monitored for MAS, and cytokine-directed therapy or corticosteroids should be considered as clinically indicated.

7.10 Adrenal Toxicity

Immune-mediated damage to the adrenal gland is a potential toxicity and could be manifested 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.

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

7.11 Immune mediated adverse events

Avelumab and pembrolizumab are associated with a known risk of irAEs. Patients will be closely monitored for potential irAEs,[84,85] 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 avelumab or pembrolizumab will be managed by the Attending Physician in consultation with the study team in accordance with ASCO society guidelines.[86] Both interferon gamma and avelumab/pembrolizumab can cause GI toxicity. More commonly GI toxicity due to pembrolizumab/avelumab will manifest as diarrhea that is persistent. Persistent diarrhea will be attributed to pembrolizumab/avelumab and managed by the attending physician in consultation with the study team in accordance with ASCO society guidelines. Episodic diarrhea or vomiting associated temporally with interferon gamma administration will be attributed to interferon gamma and managed with dose interruption or cessation in cases of grade 3 or higher toxicity.

7.12 Persistent Uncontrolled T-cell Proliferation

Uncontrolled proliferation of TCR modified T cells has not been observed in clinical trials to date.[29] 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.

7.13 Replication-Competent Lentivirus

All patients will be followed in this study for presence of replication-competent lentivirus (RCL) for up to 15 years following the last dose of FH-MCVA2TCR. 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-MCVA2TCR.

7.14 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.

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8. INFORMED CONSENT OF PATIENT

8.1 Patient screening

An evaluation consent, separate from the treatment consent, will be used for the patient to provide consent for records review, pathology review, MCPyV tumor testing (if previously unknown), 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. In this case, evaluation consent discussion may be performed by telephone by trained study personnel, and consent documents exchanged by email, mail or fax.

8.2 Treatment

Patients will be seen at Fred Hutch/UWMC for consideration of treatment options for their disease. 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 will be explained to the patient. Consent from the patient will be obtained using forms approved by the Fred Hutch IRB. A summary of the clinic visits detailing what was covered will be dictated for the medical record.

9. PATIENT REGISTRATION

Eligible patients will be identified and registered into the system by the Clinical Coordinator's Office (CCO; Intake Office) and assigned a Unique Patient Number (UPN). The CCO will register the patient for the protocol through the Data Management Office.

Patients will initially be screened. Enrollment to the therapy portion of the study will occur at the conclusion of the pre-T-cell work-up when data are reviewed for all inclusion and exclusion criteria for therapy by the Immunotherapy attending physician and the patient signs consent for therapy.

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

10. TRAVEL REIMBURSEMENT

Patients who consent to the treatment arm of the study will be eligible for limited travel reimbursement of actual expenses incurred, up to $3,500. Please see consent form for additional details.

11. 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.

11.1 Visits required to be at Fred Hutch/UWMC

Many of the patients with MCC travel >500 miles to receive care at Fred Hutch/UWMC. Although up to two T cell infusions are given, the avelumab is given every 2 weeks for a year (or pembrolizumab every 3 weeks for a year), as a standard of care treatment, using standard of care FDA approved dosing and frequency. Therefore, for interventions surrounding T cell infusions and the first avelumab or pembrolizumab infusion after each T cell infusion, we request care to be at Fred Hutch/UWMC, in order to ensure safety. However, for "maintenance" avelumab or pembrolizumab visits, we will allow patients to receive these infusions with their local/primary oncologist if that is patient preference, except as detailed below.

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Leukapheresis, pre-treatment biopsy, and pre-treatment physical exam, and treatment consenting need be performed through Fred Hutch/UWMC.

For cycle 1 of T cell therapy, the patient is required to receive care at Fred Hutch/UWMC and stay within 30 minutes of Fred Hutch/UWMC from the first dose of interferon gamma (day -5) through T cell infusion and the 28-day dose-limiting toxicity (DLT) period thereafter. This period includes the first (d+14) avelumab or pembrolizumab infusion, if eligible/stable. If the patient shows no signs of T cell mediated toxicity within 17 days of T cell infusion (and 3 days for PD-1/PD-L1 inhibitor infusion), they may return to the care of their treating oncologist with permission of the PI.

Day 28, 42, 56, 70, and 84 avelumab (or day 35, 56 and 77 pembrolizumab) may be given locally or at Fred Hutch and research blood may be shipped/mailed. Day +84/cycle 1 evaluations including research and clinical blood work, exams, imaging, and biopsy should be performed if possible at Fred Hutch or UWMC. Patient must remain within 30 minutes of Fred Hutch/UWMC for second T cell infusion and at least 28 days thereafter, during which avelumab or pembrolizumab is likely to be administered. Subsequent avelumab or pembrolizumab may be given locally. If the patient shows no signs of T cell mediated toxicity within 17 days of T cell infusion (and 3 days for PD-1/PD-L1 inhibitor infusion), they may return to the care of their treating oncologist with permission of the PI.

6 month, 9 month and 12 month imaging and laboratory evaluations are requested to be performed at Fred Hutch; however, they may be performed locally if circumstances preclude travel to Fred Hutch.

11.2 Screening Evaluations

1. Determination of viral status if not previously determined (MCPyV AMERK serology assay and/or testing of archival tumor tissue).
2. HLA Typing (HLA typing will be organized by and billed to study)

11.3 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 screening consent and HIPAA authorization must be signed before any non-standard of care evaluations are performed.

Baseline evaluations include:

1. Medical history and physical examination including prior therapies and response to therapy if known, and Karnofsky performance status.
2. Laboratory tests, including:
   a. CBC, differential, platelet count
   b. Comprehensive metabolic panel
   c. LDH
   d. Fred Hutch (PSBC) Donor Battery Panel (should be performed within 30 days of leukapheresis)
   e. Serum ferritin
   f. Serum CRP
   g. Uric acid

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h. Serum IL-6
i. Prothrombin time (PT), partial thromboplastin time (PTT) (should be performed within 30 days of leukapheresis)
j. Serum pregnancy test for females of childbearing potential within 14 days of planned leukapheresis.

3. Confirmation of diagnosis by internal pathology review of initial or subsequent biopsy or other pathologic material at Fred Hutch.
4. Baseline pulse oximetry and documentation of O_2 saturation on room air
5. Baseline 12-lead EKG
6. Patients age 60 years or older: Echocardiogram or MUGA scan
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-MCVA2TCR infusion
8. For skin/cutaneous tumors, representative/target tumors should be photographed pre treatment for baseline measurements (optional).
9. Clinical documentation of relapsed disease (imaging and/or biopsy).

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 (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 other than bone marrow (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 before both cycles 1 and 2 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: If clinical biopsy performed within 42 days of study enrollment documenting persistent/progressive MCC and research samples not collected, a core needle (6 needle passes) or punch biopsy (1 x 6 mm or 2 x 4 mm) or equivalent sample will be collected for research if clinically feasible. Where possible, research samples will be coordinated with clinical biopsies.

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11.4 Evaluations prior to T cell Infusion

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

1. Interval history and physical exam
2. Research biopsy to evaluate class I MHC upregulation
3. Blood draw for laboratory studies:
    - CBC, differential, and platelet count
    - Comprehensive metabolic panel
    - LDH
    - Uric acid
    - Research Labs: Cytokine levels (research); T cell baseline persistence, function

11.5 Evaluations following T cell infusion

The following evaluations will be performed after the T cell infusion.

1. Record new findings on history and physical exam 1 day after the T cell infusion and at least weekly for 3 weeks.
2. The following laboratory studies at least twice weekly for 2 weeks, then weekly until 4 weeks after the T cell infusion:
    a. CBC, differential, and platelet count
    b. Comprehensive metabolic panel
    c. LDH
    d. Uric acid
3. If patients become 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: between approximately 10 to 16 days following the T cell infusion, all patients with pathologic, palpable or radiologically accessible lymphadenopathy will be asked to undergo a punch, excisional or a core biopsy of an accessible tumor. 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. Patients will also be asked to undergo a biopsy at time of 12-week re-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 (pseudoprogression has been reported at up to 3 months in the JAVELIN trials of avelumab for MCC), and to confirm that there is residual tumor if tumors are shrinking (would not give second T cell infusion if no residual viable tumor). The purpose of the repeat biopsy at time of late relapse would be to confirm that the relapsed lesion represents MCC. These 12-week and relapse biopsies will thus be clinical with additional portion/core for research.
5. Research labs:
    a. 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.
    b. Serum storage for measurement of serum cytokine levels

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i. Serum cytokine levels. Serum should be collected in serum separator tube at volumes indicated. Samples should be sent to the Specimen Processing/Research Cell Bank Lab at Fred Hutch. Serum cytokine levels will be run at selected time points (optional).
ii. If patients become febrile, develop signs of CRS, or cytokine assessment is clinically appropriate at times other than those indicated, we may measure cytokine levels at additional times (optional).

c. Evaluation for persistence and phenotype of **TCR transgenic** T cells
i. PBMC for TCR T cell persistence: Blood samples (EDTA [lavender top] tube) should be obtained on approximately days 1, 3, 7, 14, 21, and 28 during the first month after the T cell infusion, and also at 2, 3, 6, 9, and 12 months after the T cell infusion for analysis of the persistence and phenotype of transferred T cells. Samples should be sent to the Specimen Processing/Research Cell Bank Lab at Fred Hutch. 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).
ii. If patients become febrile, develop possible signs of toxicity, or assessment of TCR T cell persistence is clinically appropriate at times other than those indicated, we may measure the persistence of transferred T cells at additional times.

d. 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).

e. 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. For this purpose, we will collect the following. Additional and/or fewer time points may be performed. 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.

i. Serum anti-tumor immune responses: Serum for humoral immune responses around day 14, and at approximately 3, 6, and 12 months after T cell infusion to the Specimen Processing/Research Cell Bank lab at Fred Hutch. This is for full serologic panel testing for research purposes and is separate from the clinical AMERK MCPyV serology.
ii. PBMC anti-tumor immune responses: (lavender top tubes) for cellular immune responses around day 14, and at approximately 3, 6, and 12 months after T cell infusion to the Specimen Processing/Research Cell Bank lab at Fred Hutch
iii. T-cell repertoire by deep sequencing: Blood (10 ml EDTA [lavender top] tubes) around day 14, and at approximately 3 and 6 months after the T cell infusion to the Specimen Processing/Research Cell Bank lab at Fred Hutch to evaluate the T-cell repertoire by deep sequencing of TCR-β sequence

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

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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).

f. RCL testing
Blood samples should be collected at approximately 3, 6, 9, and 12 months after the TCR T cell infusion and sent to the Specimen Processing Lab for RCL testing, as per FDA guidelines.

11.6 Response Assessment

Participants will have evaluation of tumor burden at baseline (physical exam with tumor measurements, cross-sectional imaging as detailed above, RECIST and imRECIST reads of cross-sectional imaging), at 4 and 12 weeks after first T cell infusion, at 4 and 12 weeks after second infusion (if given), and then at discretion of treating oncologist (suggested frequency every 3-6 months). Patients with progressive disease on 4 week imaging but clinical stability may remain on study given the possibility of delayed clinical responses to immunotherapy. Imaging should also be performed if suspicion of progression. Imaging will be performed on a clinical basis with RECIST/imRECIST reads on a research basis. For skin/cutaneous tumors, representative/target tumors should (optionally) be photographed at key response assessment/measurement time points (day 14, 12 weeks, months 3, 6, 9, and 12 post infusion). Patients without measurable lesions will not be evaluable for response, but will be evaluable for safety endpoints.

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 prior to T cell infusion, 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.

11.7 Participant discontinuation of active treatment

A patient will no longer be able to receive active treatment on study (T cells, avelumab or pembrolizumab, or class I MHC upregulation) for any of the reasons listed below.

- Progressive disease or development of new metastasis requiring urgent change in treatment after the second T cell infusion. Patients with progressive disease on 4 week imaging but clinical stability may remain on study given the possibility of pseudoprogression and delayed clinical responses to immunotherapy.
- 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 his 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 Adverse events deemed related to the treatment, except for expected, transient toxicities associated with avelumab, pembrolizumab, interferon gamma and T cell infusion.

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- Cytokine release syndrome requiring pressors for >24 hours or tocilizumab
- The reasons for premature discontinuation must be recorded on the case report form. The patient may re-enter the study after premature discontinuation only by approval of the PI

Unless there is withdrawal of consent or death, the patient will transition to the LTFU phase of study.

11.8 Long-term follow-up (LTFU)

Enrolled patients who receive FH-MCVA2TCR will be asked to participate in 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. Annual blood work (20 cc lavender top/10 cc serum separator) will be recommended to be used for RCL (replication competent lentivirus; required), persistence, and immune monitoring (optional). Medical records will be requested at least annually to determine long term outcomes. 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.

12. ADVERSE EVENT REPORTING

12.1 Adverse Event Definitions

- **Adverse Event**
  An Adverse Event (AE) is any untoward medical occurrence in a clinical investigation patient 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.

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- **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 12.6** for expected toxicities.

12.2 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, as determined by the PI
- Expectedness of the adverse event based on prior observed and documented adverse events, as determined by the PI
- The outcome of the adverse event

12.3 Grading of the Severity of an Adverse Event

Immune effector cells are recognized to have a unique set of toxicities associated with T cell activation including cytokine release syndrome (CRS) and neurotoxicity/ICANS. In general, TCR-T therapies have not been described to be associated with CRS and neurotoxicity to the same severity and extent that certain CAR-T therapies have. However, given that the underlying mechanism is T cell activation, CRS and neurotoxicity management scales designed for CAR-T are reasonably applied to TCR-T. Therefore, for grading cytokine release syndrome and neurotoxicity associated with TCR-T cells we will utilize the recently published international consensus guidelines: ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells (Lee et al; 2019).[79] Individual components of CRS (e.g. fever, hypotension, hypoxia) will be graded as part of the CRS score, and will not count as separate adverse events, so long as the timing and clinical picture is consistent with cytokine release syndrome.

All other 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.

12.4 Attribution of Adverse Event

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

Definite (must have all 4)	• Has a reasonable temporal relationship to the intervention

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	• Could not have readily been produced by the patient'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 patient'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 patient'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 patient'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.

12.5 Adverse Event Reporting Period -

Adverse events of **grades 3-5** will be monitored and recorded in study-specific case report forms (CRFs) throughout the investigative phases of the study: during and for 48 hours after leukapheresis and then from the start of interferon gamma through day 28 after each T cell infusion. Additionally, grade 1-2 adverse events will be monitored and recorded from the start of T cell infusion through day 28 after each T cell infusion. Adverse events will continue to be monitored and recorded 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 patient withdrawn from the study

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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 for the purpose of facilitating T cell infusion is not considered an AE. Any AE requiring prolongation of this hospitalization will be recorded and patient to applicable SAE reporting.
- 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.

12.6 Expected Grade 3-4 Adverse Events

Avelumab, pembrolizumab, interferon gamma, and T cell infusions each have associated, expected, transient toxicities that will not be considered grounds for therapy discontinuation nor counted as adverse events for determination of treatment safety/dose limiting toxicity. These will however be appropriately logged and reported as with any other grade 3-4 toxicity below.

12.6.1 Expected Adverse Events: T cell infusion

The following toxicities are expected adverse events and will not be counted as dose limiting toxicities

- Grade 3-4 lymphopenia.

Rationale: Lymphopenia is an expected toxicity of T cell therapy, even without lymphodepleting chemotherapy.[87,88] This was also observed in prior trials of endogenous T cells in MCC (**Table 2**), where 6/12 patients had grade 3 or 4 lymphopenia. In most cases, lymphopenia had onset of 24-72 hours after T cell infusion and in all cases lymphopenia had recovered to grade 2 or less by day +28 after T cell infusion.

T cell activation has a known constellation of inflammatory responses that is well defined. This is an expected toxicity of cellular therapy as the immune system activates and seeks to immunologically reject tumor. There are defined guidelines for cytokine release syndrome (CRS) as well as immune-effector cell-associated neurotoxicity syndrome (ICANS).

Therefore, the following toxicities will be evaluated under consensus evaluations for CRS and/or ICANS if suspected due to T cell activation (**Appendix E**)[79] and thus individual toxicities will <u>not</u> be considered DLT unless CRS and/or ICANS is grade III or higher

- Fevers, hypoxia, hypotension: Fevers, hypoxia, hypotension suspected to be related to cytokine release syndrome and occurring within 28 days after T cell infusion will be graded in aggregate under the Lee et al cytokine release syndrome criteria consensus guidelines (**Appendix E**). The individual components will NOT count as DLTs based on CTCAE criteria.
- Tachycardia, flushing, tachypnea: Tachycardia and flushing often accompany CRS as a result of the fevers and inflammation and are included in some grading criteria. Therefore, tachycardia (so long as sinus), flushing, and tachypnea will not individually count as DLTs <u>if suspected to be due to CRS</u> and CRS will instead be graded with Lee criteria, as above.

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- Headache, confusion, seizure, delirium, and encephalopathy will be evaluated under neurotoxicity/ICANS criteria if occurring within 28 days of T cell infusion (**Appendix E**), and ICANS criteria will be used for grading/DLT determination.

12.6.2 Expected Adverse Events: Interferon gamma

Expected toxicities attributable to interferon gamma and considered exceptions to criteria for discontinuation include transient inflammatory responses (Fever, rash, flu like symptoms, **outlined in 7.1**) resulting in Grade 3 or 4 toxicities that return to baseline within 2 weeks.

12.6.3 Expected Adverse Events: Avelumab or pembrolizumab

Expected toxicities attributable to anti-PD-L1 (avelumab) or anti-PD-1 (pembrolizumab) infusions and considered exceptions to criteria for discontinuation include infusion-related reactions with Transient (≤ 24 hours) Grade 3-4 toxicity (outlined in 7.3) that resolved to ≤ Grade 1. Patients with infusion reactions to avelumab will be able to receive pembrolizumab for future infusions. Patients with infusion reactions to pembrolizumab will not be able to receive avelumab for future infusions.

12.7 Adverse Event Reporting Requirements

12.7.1 Reporting to IRB

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

12.7.2 Reporting to Sponsor

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	Contact Information
Serious Adverse Event (SAE)	Fatal or life-threatening	Within 24 hours of research team* awareness	Email notification to Sponsor's Medical Monitor & ISIOC Administrator	Medical Monitor email: mpwerciva@uw.edu ISIOC email: ISIOC@fredhutch.org
	All SAEs	Within 2 business days of research team* awareness	Submit completed Institution-Sponsored IND SAE Reporting Form signed by PI or designated sub-Investigator	ISIOC email: ISIOC@fredhutch.org
Non-serious AE		Per CRF completion guidelines	Record information on appropriate CRFs	N/A

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*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.

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

The information in the Institution-Sponsored IND SAE Reporting Form must match or be reconciled with the information recorded in the adverse events section on the CRF and study database. For example, the same adverse event term should be used on both forms.

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, after reviewing the assessments and supporting documentation provided by the PI. For regulatory reporting purposes, the IND Sponsor will independently determine expectedness relating to the investigational product using safety information specified in the Investigator Brochure, the consent document and the protocol document. 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.

13. STATISTICAL CONSIDERATIONS

13.1 Sample size and Power

Our study is a phase I/II, non-blinded interventional trial. Our target sample size is up to 16 patients (8 per year/2 years). This target is chosen for three reasons: it is achievable from an enrollment standpoint (our clinic sees between 15-20 potentially eligible patients 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 for details). With the eligibility expansion, we expect roughly 12 patients will receive a PD1 checkpoint inhibitor. For some analyses, all 16 patients will be analyzed, yet some analyses will be restricted to patients who receive a PD1 checkpoint inhibitor. See below for the impact of this on statistical power.

13.2 Primary Endpoint: Safety

13.2.1 Definition of treatment related adverse event

For the purposes of statistical analysis and estimation of toxicity rate, a safety event will be any grade 3 or higher adverse event determined to be possibly, probably, or definitely secondary to any of the study treatments, except as expected in **Sections 12.5 and 12.6**. Please see **Section 12.1** for adverse event definition.

13.2.2 Definition of success and probability of suspension

Platinum-based combination chemotherapy is associated with 30 to 40% grade ≥ 3 toxicities[46] and although data are not yet available for MCC, combination checkpoint inhibitors see 45-55% treatment associated grade ≥ 3 toxicity in patients with metastatic melanoma.[69] To avoid exceeding these toxicity

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rates, the treatment will be considered to have an acceptable safety profile if the observed toxicity rate is consistent with a true rate that does not exceed 40%.

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, 9/15-16. 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).

The initial three part1 patients will be treated and followed for toxicity. All three of these patients will receive their first cycle of treatment (T cell infusions) before additional patients will be enrolled. If zero or one of the first three part I patients experiences treatment-related and grade ≥3 toxicity, the remaining (up to) 13 pts will be treated on the part II portion of the trial. If instead two or more of the first three patients experience 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, consideration given to changing the cell dose or construct pending DSMB review. If any of the first three 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. As detailed in **Section 11.7**, if at any point any patient experiences grade 3 or higher treatment-related toxicity, that particular patient will no longer receive study related active therapies, except as specified in **Section 12.6.1**. They will continue to be followed for response and outcome.

The above toxicity considerations above will be examined among all patients, regardless of their PD1-contraindication status and whether they get PD-1/PD-L1 inhibition therapy on the trial. The delay of PD-1/PD-L1 inhibition until 14 days after T cell infusion will help differentiate toxicity related specifically to PD-1 axis inhibition. However, if any patient who does not receive a checkpoint inhibitor develops a toxicity, enrollment to such patients will be suspended pending review by the DSMB and the PI, at which point a decision will be made to continue the trial as is, modify the trial, and halt enrollment of patients for whom a checkpoint inhibitor is contraindicated.

13.3 Primary Endpoint: Efficacy

13.3.1 Definition of response

Irradiated and non-irradiated lesions will be separately tracked but response determined in totality. As indicated, patients who will be evaluated for this endpoint will have at least one trackable lesion by response evaluation criteria in solid tumors (RECIST) 1.1. Response will be a defined as best overall response by RECIST 1.1 of complete or partial response for patients with at least one measurable lesion.

13.3.2 Definition of success and power of success

Responses to alternative therapies in this population of patients with advanced MCC resistant to PD1-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%, 16 patients yield 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

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study. If power is instead assessed among the anticipated 12 patients who will receive a checkpoint inhibitor, the statistical power is reduced to 78%.

13.4 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.

14. DATA AND SAFETY MONITORING PLAN

14.1 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.

14.2 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 the 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.

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A DSMB will be in place and will meet after three patients have received treatment (or sooner if requested) to review the data as it relates to AEs and determine whether trial may proceed to part 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 CTCAE 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.

15. 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 Hutch and the University of Washington Medical Center.

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.

16. 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

Assessments	Screening Evaluation (Fred Hutch/local)	Pre-treatment Evaluation[a]	Leukapheresis	Interferon Gamma	Pre-Treatment Biopsy	T-cell Infusion #1 (Day 0)	Fred Hutch Post T-cell Infusion #1 Evaluations						Fred Hutch/Local Avelumab infusion days[i]	(Fred Hutch/local- may combine w/ Avelumab eval bloodwork)		12-Week Restaging[c]	Pre-treatment Evaluation[a]	Leukapheresis (if indicated)	Interferon Gamma	Pre-treatment Biopsy	T-Cell Infusion #2 (Day 0)[d]	Fred Hutch Post T-cell Infusion #2 Evaluations						(Fred Hutch/local- may combine w/ Avelumab eval bloodwork)		Fred Hutch/Local Avelumab infusion days[i]	12-Week Restaging[c]	Short-term Follow-Up[c]
							Day +1	Day +3	Day +7	Day +10	Day +14 (Avelumab - dose 1)	Day +21	D+28, D+42, D+56, D+70, D+84	Day +28	Day +56							Day +1	Day +3	Day +7 (+ Avelumab)	Day +10	Day +14	Day +21 (+ Avelumab)	Day +28	Day +56	Approximately every 2 weeks		Month 6, 9, and 12 post T-cell Infusion 1#1
I/E criteria		X															X														
Informed consent/HIPAA	X	X																													
Medical history – Complete[b]		X											Per institutional or provider routine for Avelumab[i]																		Per institutional or provider routine for Avelumab[i]		
Medical history - Focused		X				X	X		X		X	X		X	X	X					X	X		X		X	X	X	X	X	X
Physical exam with Vital signs (including O_2 sat)		X				X	X		X		X	X		X	X	X					X	X		X		X	X	X	X	X	X
Height/weight		X																													
Karnofsky performance satus		X				X										X					X									X	X
Pregnancy test		X																													
12-lead ECG		X																													
MUGA/ECHO		X																													
Evaluation of Therapeutic Potential (may be performed at any time prior to treatment)																															
Determination of tumor viral status[e]	X																														
Fred Hutch pathology	X																														

Assessments	Screening Evaluation (Fred Hutch/local)	Pre-treatment Evaluation[a]	Leukapheresis	Interferon Gamma	Pre-Treatment Biopsy	T-cell Infusion #1 (Day 0)	Fred Hutch Post T-cell Infusion #1 Evaluations						Fred Hutch/Local Avelumab infusion days[i]	(Fred Hutch/local- may combine w/ Avelumab eval bloodwork)		12-Week Restaging[c]	Pre-treatment Evaluation[a]	Leukapheresis (if indicated)	Interferon Gamma	Pre-treatment Biopsy	T-Cell Infusion #2 (Day 0)[d]	Fred Hutch Post T-cell Infusion #2 Evaluations						(Fred Hutch/local- may combine w/ Avelumab eval bloodwork)		Fred Hutch/Local Avelumab infusion days[i]	12-Week Restaging[c]	Short-term Follow-Up[c]
							Day +1	Day +3	Day +7	Day +10	Day +14 (Avelumab - dose 1)	Day +21	D+28, D+42, D+56, D+70, D+84	Day +28	Day +56								Day +1	Day +3	Day +7 (+ Avelumab)	Day +10	Day +14	Day +21 (+ Avelumab)	Day +28	Day +56	Approximately every 2 weeks		Month 6, 9, and 12 post T-cell Infusion 1#1
review of any tumor lesion																																
HLA typing	X																															
Imaging and Tumor Measurements[f]																																
CT C/A/P or PET-CT		X												X[j]		X	X											X[j]			X	X
CT affected area (if not covered w/ above)		X														X	X														X	X
MRI Brain		X														X	X														X	X
Measurement and photo of palpable tumors		X									X					X	X														X	X
Interventions																																
Clinical Biopsy (to pathology)[g]		X														X	X														X	
Research Biopsy (may be combined w/ clinical; to Chapuis Lab D3-235)[g]		X			X[k]						X					X	X			X[k]										X	

Assessments	Screening Evaluation (Fred Hutch/local)	Pre-treatment Evaluation [a]	Leukapheresis	Interferon Gamma	Pre-Treatment Biopsy	T-cell Infusion #1 (Day 0)	Fred Hutch Post T-cell Infusion #1 Evaluations: Day +1	Day +3	Day +7	Day +10	Day +14 (Avelumab - dose 1)	Day +21	Fred Hutch/Local Avelumab infusion days[i]	(Fred Hutch/local-may combine w/ Avelumab eval bloodwork): Day +28	Day +56	12-Week Restaging [c]	Pre-treatment Evaluation [a]	Leukapheresis (if indicated)	Interferon Gamma	Pre-treatment Biopsy	T-Cell Infusion #2 (Day 0)[d]	Fred Hutch Post T-cell Infusion #2 Evaluations: Day +1	Day +3	Day +7 (+ Avelumab)	Day +10	Day +14	Day +21 (+ Avelumab)	(Fred Hutch/local-may combine w/ Avelumab eval bloodwork): Day +28	Day +56	Fred Hutch/Local Avelumab infusion days[i]	12-Week Restaging[c]	Short-term Follow-Up[c]
							Day +1	Day +3	Day +7	Day +10	Day +14 (Avelumab - dose 1)	Day +21	D+28, D+42, D+56, D+70, D+84	Day +28	Day +56	12-Week Restaging [c]						Day +1	Day +3	Day +7 (+ Avelumab)	Day +10	Day +14	Day +21 (+ Avelumab)	Day +28	Day +56	Approximately every 2 weeks	12-Week Restaging[c]	Month 6, 9, and 12 post T-cell Infusion 1#1
Leukapheresis			X															X														
Interferon Gamma[h]				X															X													
T-cell Infusion						X															X											
Avelumab[i]											X		X											X			X			X		
Clinical Blood Work / Safety Laboratory Evaluations																																
CBC w/ diff, platelet count		X				X	X	X	X	X	X	X		X	X	X	X				X	X	X	X	X	X	X	X		X	X	X
Comprehensive Metabolic Panel		X				X	X	X	X	X	X	X		X	X	X	X				X	X	X	X	X	X	X	X	X		X	X	X
LDH		X				X	X	X	X	X	X	X		X		X	X				X	X	X	X	X	X	X	X			X	X

Assessments	Screening Evaluation (Fred Hutch/local)	Pre-treatment Evaluation[a]	Leukapheresis	Interferon Gamma	Pre-Treatment Biopsy	T-cell Infusion #1 (Day 0)	Fred Hutch Post T-cell Infusion #1 Evaluations: Day +1	Day +3	Day +7	Day +10	Day +14 (Avelumab - dose 1)	Day +21	Fred Hutch/Local Avelumab infusion days[i]	(Fred Hutch/local-may combine w/ Avelumab eval bloodwork): Day +28	Day +56	12-Week Restaging[c]	Pre-treatment Evaluation[a]	Leukapheresis (if indicated)	Interferon Gamma	Pre-treatment Biopsy	T-Cell Infusion #2 (Day 0)[d]	Fred Hutch Post T-cell Infusion #2 Evaluations: Day +1	Day +3	Day +7 (+ Avelumab)	Day +10	Day +14	Day +21 (+ Avelumab)	(Fred Hutch/local-may combine w/ Avelumab eval bloodwork): Day +28	Day +56	Fred Hutch/Local Avelumab infusion days[i]	12-Week Restaging[c]	Short-term Follow-Up[c]
													D+28, D+42, D+56, D+70, D+84																		Approximately every 2 weeks		Month 6, 9, and 12 post T-cell Infusion 1#1
Amylase											X		Per institutional or provider routine for Avelumab[i]											X			X						
Lipase											X													X			X						
Uric acid		X				X	X	X	X	X	X	X		X		X	X				X	X	X	X	X	X	X	X			X	X
Serum ferritin		X														X	X														X	
CRP		X														X	X														X	
IL-6		X														X	X														X	
Cortisol		X												X	X	X	X											X	X		X	X
TSH		X												X	X	X	X											X	X		X	X
Free T4		X												X	X	X	X											X	X		X	X
DIC Panel w/o platelets		X														X	X														X	
Fred Hutch (PSBC) donor battery panel		X															X															
MCPyV Serology		X														X	X														X	X
Research Blood Work (all to Research Cell Bank)																																	
60 mL lavender top		X							X		X					X								X		X						X
20 mL serum sep		X									X					X										X						X
40 mL lavender top						X	X	X						X	X						X	X	X					X	X			

Assessments	Screening Evaluation (Fred Hutch/local)	Pre-treatment Evaluation[a]	Leukapheresis	Interferon Gamma	Pre-Treatment Biopsy	T-cell Infusion #1 (Day 0)	Fred Hutch Post T-cell Infusion #1 Evaluations: Day +1	Day +3	Day +7	Day +10	Day +14 (Avelumab - dose 1)	Day +21	Fred Hutch/Local Avelumab infusion days[i]	(Fred Hutch/local- may combine w/ Avelumab eval bloodwork)		12-Week Restaging[c]	Pre-treatment Evaluation[a]	Leukapheresis (if indicated)	Interferon Gamma	Pre-treatment Biopsy	T-Cell Infusion #2 (Day 0)[d]	Fred Hutch Post T-cell Infusion #2 Evaluations: Day +1	Day +3	Day +7 (+ Avelumab)	Day +10	Day +14	Day +21 (+ Avelumab)	(Fred Hutch/local- may combine w/ Avelumab eval bloodwork)		Fred Hutch/Local Avelumab infusion days[i]	12-Week Restaging[c]	Short-term Follow-Up[c]
							Day +1	Day +3	Day +7	Day +10	Day +14 (Avelumab - dose 1)	Day +21	D+28, D+42, D+56, D+70, D+84	Day +28	Day +56							Day +1	Day +3	Day +7 (+ Avelumab)	Day +10	Day +14	Day +21 (+ Avelumab)	Day +28	Day +56	Approximately every 2 weeks		Month 6, 9, and 12 post T-cell Infusion 1#1
10 mL serum sep						X	X	X	X			X		X	X						X	X	X	X			X	X	X			
20 mL lavender top												X															X					

NOTE: ***The proposed days of all treatments and assessments are approximate and may vary due to scheduling, clinical, or other factors***

[a] Tests obtained as part of routine clinical care may be substituted, *and may be omitted for Cycle 2 evaluation as long as within protocol-specified window*

[b] Medical history should initially include a full history of all findings at diagnosis, time of enrollment, prior history, and response to therapy, as well as relevant other medical issues, family and social history, medications, allergies. Subsequent medical histories may be focused as appropriate

[c] 12-week evaluation strongly preferred to be at Fred Hutch and 6, 9, and 12-month evaluations preferred at Fred Hutch. However, if patient cannot travel, these evaluations may be performed locally

[d] Please see section 6.4 for details on determination of one versus two cycles if T cell therapy, and guidance for timing of second T-cell administration

[e] Please see inclusion criteria for further details on determination of tumor virus status

[f] Diagnostic imaging evaluations preferred to be at Fred Hutch but may be performed elsewhere if travel or insurance issues preclude Fred Hutch scans. Baseline imaging must be obtained within 45 days prior to start of first planned FH-MCVA2TCR infusion.

[g] Biopsies may be omitted or retimed at discretion of PI based on clinical appropriateness

[h] Administered 3 times weekly through d28

[i] Please see section 6.5 for additional details and suggested guidance on standard of care avelumab or pembrolizumab. If patients receive pembrolizumab, certain evaluations may be rescheduled to match the pembrolizumab schedule (e.g., the D28 evaluations may be performed at D35, and the D84 evaluations may be performed at D77).

[j] CT chest/abdomen/pelvis (CAP) + involved areas w/ IV contrast mandatory, PET CT optional per clinical status

[k] Biopsy after 2-4 doses of IFN-G, day of or day before cell infusion

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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, RCL baseline	Lavender top	60 mL	RCB
Blood	At Enrollment	Cytokine levels and MCPyV antibody levels (research)	Serum separator	20 ml	RCB
Tumor	At Enrollment	Single cell RNA sequencing	RPMI	4 cores or equiv	ICTPL
Tumor	At Enrollment	T cell localization and tumor microenvironment characteristics	Formalin	2 core or equiv; in addition to clinical core	Chapuis D3-235
Tumor	At Enrollment	Archive	RPMI	Any addtl tissue	Chapuis D3-235
Tumor	Pre-Treatment	Single cell RNA sequencing	RPMI	4 cores or equiv	ICTPL
Tumor	Pre-Treatment	T cell localization and tumor microenvironment characteristics	Formalin	2 core or equiv; in addition to clinical core	Chapuis D3-235
Tumor	Pre-Treatment	Archive	RPMI	Any addtl tissue	Chapuis D3-235
Blood	Day 0	Cytokine levels (research)	Serum separator	10 ml	RCB
Blood	Day 0	T cell baseline persistance, function	Lavender top	40 ml	RCB

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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
Blood	Day 3	T cell persistence and functional studies	Lavender top	40 ml	RCB
Blood	Day 7	Cytokine levels (research)	Serum separator	10 ml	RCB
Blood	Day 7	T cell persistence and functional studies	Lavender top	60 ml	RCB
Blood	Day 14	Cytokine levels (research)	Serum separator	20 ml	RCB
Blood	Day 14	T cell persistence and functional studies, epitope spreading, scRNAseq, clonality	Lavender top	60 ml	RCB
Blood	Day 21	Cytokine levels (research)	Serum separator	10 ml	RCB
Blood	Day 21	T cell persistence	Lavender top	20 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, 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

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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	Annual years 2-15	serologies	Serum separator	10 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	4 cores or equiv	Chapuis D3-235
Tumor	Day + 14	T cell localization and tumor microenvironment characteristics	Formalin	2 cores or equiv	Chapuis D3-235
Tumor	Day +14	Archive	RPMI	Any addtl tissue	Chapuis D3-235
Tumor	Day + 84	Single cell RNA sequencing	RPMI	4 cores or equiv	Chapuis D3-235
Tumor	Day + 84	T cell localization and tumor microenvironment characteristics	Formalin	2 cores or equiv; in addition to clinical core(s)	Chapuis D3-235
Tumor	Day + 84	Archive	RPMI	Any addtl tissue	Chapuis D3-235
Tumor	Clinical event	Single cell RNA sequencing	RPMI	4 cores or equiv	Chapuis D3-235

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Tumor	Clinical event	T cell localization and tumor microenvironment characteristics	Formalin	2 cores or equiv; in addition to clinical core	Chapuis D3-235

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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

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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

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APPENDIX E: CRS and Neurotoxicity Grading Criteria

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

CRS grading will be as per ASTCT CRS Consensus Grading. Table reprinted for reference from the guideline of Lee et al, 2019 ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells, Biol Blood Marrow Transplant 25(2019) 625-638

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)
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 nonconvulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (>5 min); or Repetitive clinical or electrical seizures without return to baseline in between
Motor findings‡	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 (eg, no sedating medication).
‡ 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 v5.0.

Neurotoxicity/ICANS grading will be as per ASTCT CRS Consensus Grading. Table reprinted for reference from the guideline of Lee et al, 2019 ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells, Biol Blood Marrow Transplant 25(2019) 625-638

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APPENDIX F: Recommended Management of CRS

Grade (Lee et al 2019)	Suggested Treatment
1	- Manage symptomatically with acetaminophen and antiemetics prn - Fluids - Administer antibiotics per institutional guidelines, evaluate fever - If not on Keppra, initiate Keppra 500mg PO BID
2	- Manage symptomatically with fluids and oxygen - If not responsive to oxygen and fluids, Tocilizumab 8mg/kg IV, max 800 mg in a single dose, may repeat q8h if needed, to max of 3 doses in 24 hours - Consider dexamethasone 10 mg IV q12H x 2 doses - If not on Keppra, initiate Keppra 500mg PO BID - Administer antibiotics per institutional guidelines, evaluate fever - Continue symptomatic support
3	- Transfer to ICU if vasopressors are required. Other clinical settings may also require ICU transfer - Tocilizumab 8 mg/kg IV, may repeat q8h if needed, to max of 3 doses in 24 hours - Dexamethasone 10 mg IV q6-12H - Administer antibiotics per institutional guidelines, evaluate fever - If not on Keppra, initiate Keppra 500mg PO BID
4	- Transfer to ICU - Tocilizumab 8 mg/kg IV, may repeat q8h if needed, to max of 3 doses in 24 hours - Methylprednisolone 1000 IV qd x 3 days - If not on Keppra, initiate Keppra 500mg PO BID - Administer antibiotics per institutional guidelines, evaluate fever - Contact study PI and immunotherapy team for additional anti-cytokine therapy recommendations

Suggested guidelines for CRS management based on Lee et al 2019 CRS grade. Clinical flexibility is allowed by treating Immunotherapy attending physician based on his/her clinical judgement and experience. Recommendations for CRS management are evolving rapidly; guidelines may be substituted with Fred Hutch/UW institutional best practices and/or national companion guidelines to Lee et al guidelines if newer information available.

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APPENDIX G - Long Term Follow-Up

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

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

Years 1 - 15:

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

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

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

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

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