FRED HUTCHINSON CANCER RESEARCH CENTER (FHCRC)
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE (UW)

PROTOCOL 9245

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1. PROTOCOL: Study to Evaluate Cellular Adoptive Immunotherapy Using Polyclonal Autologous CD8$^+$ Antigen-Specific T Cells for Metastatic Merkel Cell Carcinoma in Combination with MHC Class I Up-regulation and the Anti-PD-L1 Antibody Avelumab.

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2. INTRODUCTION

Merkel cell carcinoma (MCC) represents a highly aggressive neuroendocrine skin malignancy with a high disease-associated mortality and a high propensity for recurrence after initial treatment. The cause-specific mortality ranges from 23% to 80% at five years thus making it three times as lethal as melanoma, and the increase in annual incidence now makes MCC the second most common cause of non-melanoma skin cancer-associated death.[1] Strong epidemiologic data have linked MCC to patients with cellular immune deficiencies.[2,3] In 2008, this association was further strengthened when a newly discovered viral agent related to SV40, the Merkel cell polyomavirus (MCPyV), was found to be clonally integrated into approximately 80% of MCC tumors.[4] The virus produces T Antigen (TAg) oncoproteins. TAg oncoproteins are persistently expressed by MCC and are necessary for the survival and proliferation of tumor cells and thus represent an optimal target for immunotherapy.

Original research in Dr. P. Nghiem's lab has led to the initial identification of an HLA-A*2402-restricted MCPyV TAg epitope recognized by autologous $CD8^+$ T cells in a patient with metastatic MCC followed by epitopes recognized by HLA-B*0702, HLA-B*3502, HLA-A*0201, HLA-A*0301, HLA-A*1101, and HLA-A*2301. As adoptively transferred antigen-specific T cells have demonstrated a significant clinical benefit in a subset of patients with metastatic melanoma targeting endogenous non-viral epitopes, we initially applied the use of antigen-specific T cells to target the TAg oncoproteins in patients with MCPyV-positive MCC tumors. Patients in Group 1 received targeted radiation therapy or intra-lesional injection of Interferon-beta 1B (IFNβ-1B) as a means to up-regulate previously down-regulated MHC expression by tumor tissue prior to adoptive transfer of antigen-specific $CD8^+$ T cells, and followed by low-dose Interleukin-2 (IL-2) to improve T-cell survival. However, the transferred cells were shown to express the negative regulator antigen PD-1 *in vivo*; and although the treatment was well tolerated and safe, this may have accounted for the lack of tumor control in Group 1 / Protocol #2586.

Therefore, we propose to assess whether the anti-tumor efficacy of adoptive transfer of MCPyV-specific T cells could be enhanced with concurrent PD-1/PD-L1 axis blockade with the anti-PD-L1 antibody Avelumab. This protocol proposes to treat 20 patients with metastatic MCC using a combined approach: Group 1 (10 pts) with MHC class I up-regulation and Avelumab every 2 weeks for 6 months, and Group 2 (10 pts) with MHC class I up-regulation, Avelumab and adoptive transfer of MCPyV-specific T cells. The safety and efficacy of this approach will be examined along with the *in vivo* persistence and function of the transferred cells.

3. BACKGROUND

3.A. MCC: An Often Lethal, Virus-driven Cancer

Despite its non-specific appearance as an asymptomatic red or purple rapidly growing nodule, MCC can often behave aggressively, spreading to local, nodal, and distant sites (**Figure 1**).

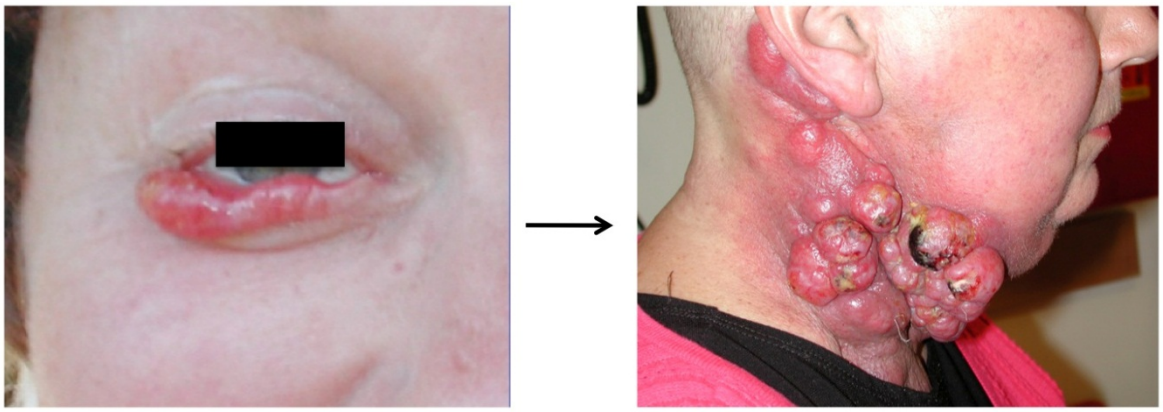


Figure 1. Clinical appearance and progression of MCC
Extensive nodal disease developed in this woman, months after a primary lesion on the eyelid. Within 1 year, she developed metastatic disease that became refractory to treatment.

Even MCC tumors smaller than 1 cm at presentation carry a significant incidence of nodal and distant metastasis; likely accounting for the largely unsuccessful attempts at long-term control with surgery alone.[5] Although radiation therapy can effectively reduce the size of MCC tumors, this treatment modality does not offer long-term disease control.[6] Previous studies with concomitant or adjuvant chemotherapy with carboplatin or etoposide have also not shown increases in overall survival.[7]

There is currently no Food and Drug Administration (FDA) approved treatment for non-resectable, recurrent, advanced, or metastatic MCC. The National Comprehensive Cancer Network (NCCN) guidelines recommend that patients with metastatic disease consult with the multidisciplinary tumor board and consider radiation, surgery, chemotherapy, or a combination thereof.[8] The specific treatment regimen for patients with distant metastasis must be individually tailored. Most NCCN institutions use only chemotherapy with or without surgery and/or radiation therapy for Stage IV, distant metastatic disease (M1). The most common regimen used for regional disease is cisplatin or carboplatin with or without etoposide.

The NCCN panel recommends cisplatin or carboplatin with or without etoposide as the choice of treatment.[9] Topotecan has also been used in some instances (for example, older patients). Cyclophosphamide in combination with doxorubicin and vincristine used to be a commonly administered regimen, but it is associated with significant toxicity.[7,10,11] Overall, although MCC is often initially chemosensitive, tumor responses are rarely

durable, with the 2-year survival rate for patients with stage IV disease at approximately 26%.[12] Furthermore, MCC reported incidence has quadrupled in the past 20 years to approximately 1,600 cases per year in the United States (US).[13] MCC risk factors include UV exposure, age over 50, and long-term T-cell immune suppression.

After recognition that MCC incidence was increased 11 fold among patients with AIDS,[2] newly developed molecular and bioinformatics methods led to the discovery of a novel polyomavirus, the MCPyV, expressed in approximately 80% of instances of MCC. MCPyV DNA shows random clonal integration into the cellular genome of MCC and is only expressed in tumor cells, as predicted for a direct viral carcinogen.[4,14,15] MCPyV encodes a multiply spliced TAg protein complex that targets several suppressor proteins, such as RB1, similarly to the SV40 and murine polyomavirus family.[15] Knockdown studies have shown that sustained TAg expression is required for the survival of virus-positive Merkel cell lines.[4,16,17]

Skin carriage of the wild-type (WT) virus has been found to be common in adults.[18] However, the WT virus must undergo at least two mutations to promote tumorigenesis. The first mutation is non-homologous recombination in the host cell genome[4], and all MCPyV genomes that have been obtained from MCC tumors have inactivating secondary mutations, such as inactivation or excision of its Helicase domain that is required to unwind the viral origin in order to allow viral DNA replication.[19] If not for this second deletion, viral replication would proceed uncontrollably in the tumor cell from the integration site, deleting the developing tumor cells via genomic fidelity sensors and apoptosis. Thus integrated, MCPyV has no mechanism to excise its genome, is no longer transmissible (analogous to HPV in most cervical carcinomas), and has no DNA-replication capacity, thus rendering the virus resistant to putative antiviral agents.[16,20]

3.B. Spontaneous Immune Responses to MCPyV TAg

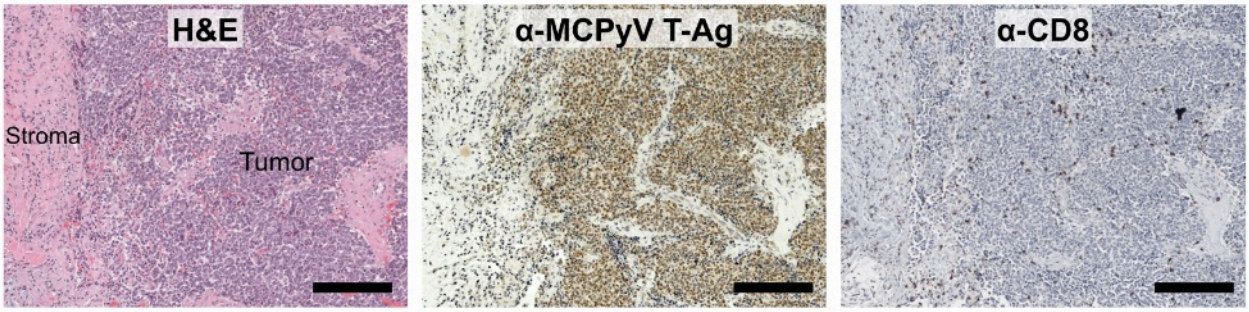
As previously mentioned, epidemiologic data suggest a strong link of MCC to the immune system. Individuals with T-cell dysfunction (transplant recipients, HIV, or chronic leukemia patients) are at 5- to 50-fold increased risk of developing MCC.[2,3] MCC tumors have been demonstrated to spontaneously regress following improvement in immune function suggesting an immune-mediated cancer control.[21,22] Furthermore, intratumoral infiltration of CD8 lymphocytes has been documented as an independent predictor of improved survival for MCC patients, suggesting that improving T-cell function may be effective in treating MCC.[23] As the TAg oncoproteins are persistently expressed in MCC tumors and are required for tumor growth, they may serve as ideal targets for immunotherapy.

Similar to the relationship between EBV and Burkitt's lymphoma, exposure to MCPyV is common in the general population (53%) as indicated by antibodies to virus-specific capsid protein VP1.[24,25] In contrast, antibodies to the MCPyV TAg are rare in the general population (less than 1%) but are commonly present (approximately 50%) at high titers in patients with active MCC.[26,27] Moreover, antibodies to TAg oncoproteins dynamically

reflect disease burden and have already been used to identify MCC recurrences prior to clinical detection.[27,28] Responses directed to MCPyV TAg, unlike antibodies directed to the capsid, are non-neutralizing and proportionate to tumor burden. Thus, the effectiveness of the treatment can be monitored by antibody titers in serial blood draws.

3.C. Identification and Characterization of MCPyV-specific CD8$^+$ T Cells from a MCC Patient's Tumor and Peripheral Blood

To determine whether the integrated MCPyV TAg peptides presented by MHC could be recognized by virus-specific T cells in MCC, primary tumor from a patient (w447) with MCC was stained with anti-MCPyV TAg antibodies (CM2B4 antibody, Santa-Cruz Biotechnology[17]) by immunohistochemistry to confirm persistent T-Ag expression and assess CD8$^+$ T-cell infiltration of the tumor tissue (**Figure 2**).



Note: Scale bar = 200 μm.

Figure 2. Persistence of MCPyV TAg expression in MCC
Histologic examination of serial sections from w447 MCC tumor. *Left:* Hematoxylin and eosin stain. *Center:* MCPyV TAg protein is expressed in the tumor. *Right:* CD8 lymphocytes are detected in the tumor.

A synthetic-peptide library spanning the MCPyV TAg was generated, and a total of 93 peptides -- 13 amino acids in length and overlapping by 9 amino acids -- were then tested against tumor-infiltrating lymphocytes (TIL). Results demonstrated TIL secreted Interferon-gamma (INFγ) when exposed to the specific 10-mer peptide EWWRSGGFS (LT-92-101) **(Figure 3).** Sequencing of DNA amplified from this patient's tumor confirmed the predicted MCPyV large-TAg amino-acid sequence (amino acids 92-101, data not shown**).**

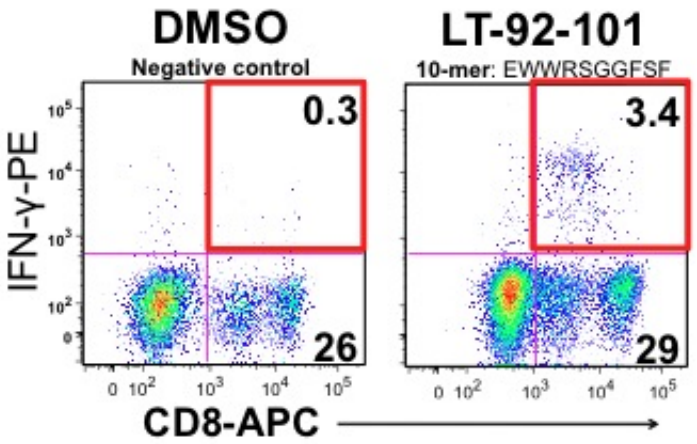


Figure 3. Reactivity to peptide

IFNγ production by TIL exposed to MCPyV TAg peptide LT-92-101 (left panel) but not to DMSO (right panel) or any other of the 93 screened peptides.

The patient's HLA type was determined and the peptide was further identified as restricted to the HLA-A*2402 allele, because only HLA-A*2402 and TAg co-transfected Cos7 cells elicited IFNγ secretion in the TIL **(Figure 4A)** and target-cell lysis **(Figure 4B)**. Specificity and HLA-restriction were confirmed with the synthesis of an MHC class I and peptide tetramer. T cells from both the tumor and peripheral blood successfully bound to the specific tetramer **(Figure 5)**. These antigen-specific T cells were expanded to sufficient numbers to be adoptively transferred back into the patient (described in **Section 3.H - Previous Human Experience**).[29]

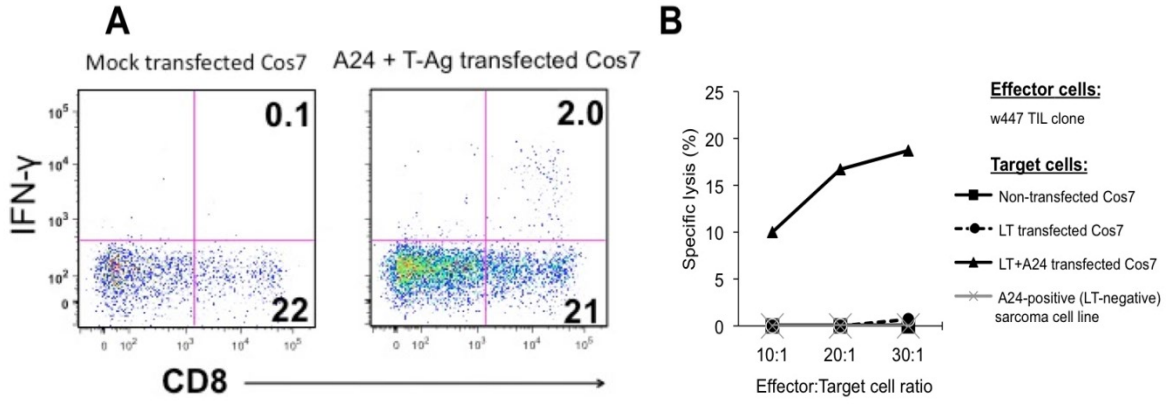


Figure 4. Reactivity to HLA-A*2402 and T-Ag transfected Cos7 cell lines

A) TIL isolated from this patient induce IFNγ after exposure to Cos7 cells that were transfected with HLA class-I A*2402 and T-Ag (right panel) but not with mock transfected Cos7 cells (left). B) w447 TIL clone was used as the effector cell against the specified target cells at the indicated Effector:Target cell ratios. Fractional cytotoxicity of Cos7 cells is presented as the percentage of all Cos7 cells, and does not take into account the decreased number of cells expected to be successfully double-transfected (50% or less).

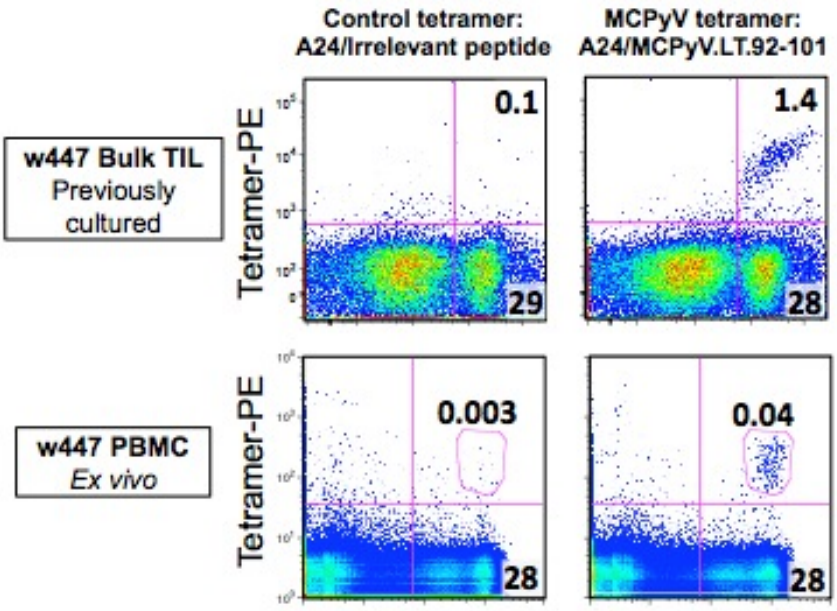


Figure 5. Binding to tetramer

TIL and T cells isolated from peripheral blood (PBMC) stain specifically with HLA-A*2402/LT.92-101 tetramer, but not with negative control tetramer.

3.D. Identification of Reactive MCPyV TAg-specific HLA-restricted Peptides

In collaboration with Sine Hadrup (Herlev Hospital, Denmark), a total of 14 $CD8^+$-restricted peptide-MHC pairs were identified within the persistently expressed LT/ST region of MCPyV (**Table 1**). All peptide-MHC pairs bind tetramers and were isolated from patients with a diagnosis of MCC, whereas no reactivity to these peptides was identified in 30 normal donors with identical HLA restrictions. Furthermore, the same epitopes were present in multiple MCC patients suggesting that these epitopes are uniquely recognized in patients with MCC (unpublished observations).

To assess the fate of the transferred cells *in vivo*, all patients eligible for this study must have an available peptide-MHC pair that can be folded into a tetramer for which MCPyV TAg-specific cells can be generated. Additionally, peptide-MHC restricted $CD8^+$ T cells must also show reactivity (lysis or INFγ secretion) in response to cell lines expressing MCPyV TAg with the corresponding HLA to verify cellular processing of the correct peptide.

Table 1. MCPyV peptide / HLA pairs for which functional tetramers exist

HLA Restriction	HLA Frequency Observed in MCC Patients (n=97)	HLA Frequency Expected (US Caucasians[a])	Viral T-Ag Domain LT: Large T ST: Small T CT: Common T	Peptide(s) from MCPyV
HLA-A*0201	55%	50%	LT LT LT ST ST	SMFDEVDEAPI KLLEIAPNC VIMMELNTL QLRDSKCACI KTLEETDYCLL
HLA-B*0702	16%	25%	LT LT LT	APNCYGNIPL APNCYGNIPLM APIYGTTKF
HLA-A*0301	25%	23%	LT	ASFTSTPPK
HLA-A*2402	14%	15%	LT	EWWRSGGFSF[b]
HLA-A*1101	15%	12%	LT	AAFKRSCLK
HLA-B*3502	3%	2%	CT ST	DKGGNPVIM FPWEEYGTL
HLA-A*2301	3%	1.3%	LT	EWWRSGGFSF

[a]US Caucasian HLA frequencies were obtained from allelefrequency.net
[b]Previously characterized and infused (see **Section 3.H - Previous Human Experience**)

3.E. Adoptive T-Cell Therapy

3.E.1 Rationale as a treatment modality

Adoptive therapy involves the transfer of *ex vivo* expanded effector cells as a means of augmenting the anti-tumor immune response. In contrast to tumor vaccination strategies, adoptive T-cell therapy can allow for far greater control over the magnitude and avidity of the targeted response by appropriate manipulation and selection *in vitro* of the T cells used for therapy.[30,31] Tumor-reactive effector cells of a desired specificity can be selected based on expression of their T-cell receptor and expanded to very high numbers. Requisite numbers of effectors can be routinely achieved and produce frequencies of antigen-specific T cells in the peripheral blood that can be many times higher than those produced using current immunization regimens alone.

To date, the majority of clinical trials in adoptive therapy have been performed in patients with metastatic melanoma for which T-cell-defined tumor antigens were first identified. Such studies designed to address the role of effector cells or conditioning regimens in optimizing the anti-tumor efficacy of adoptive cellular therapy in melanoma may be equally relevant for the use of adoptive therapy for the treatment of MCC.

3.E.2 Adoptive therapy of melanoma using antigen-specific CD8+ T-Cell Clones

Historically, the median length of survival for patients with metastatic melanoma refractory to standard therapy has been four months. Among an initial cohort of 10 patients receiving $CD8^+$ T cells targeting the tumor-associated antigens, MART1 and MelanA, and gp100 followed by low-dose IL-2 for 14 days at doses up to 3.3×10^9 cells/m^2, no serious toxicity was observed. The adoptively transferred T cells persisted *in vivo* in response to low-dose IL-2, preferentially localized to tumor sites, and mediated an antigen-specific immune response characterized by the elimination of antigen-positive tumor cells and regression of individual metastases. Eight patients experienced minor, mixed, or stable responses for periods of 2 to 21 months (average of 11 months).[32]

An updated analysis of 18 patients with refractory progressive disease receiving adoptively transferred $CD8^+$ T cells targeting, in addition to Mart1 and gp100, tyrosinase, and NYESO-1 at doses up to 10^{10} cells/m^2, demonstrated no serious toxicities and mixed responses or stable disease in 10 of 18 patients, a partial response among four patients, two of these patients with near complete responses (CR) – (no activity by PET imaging) for periods of 3 to more than 33 months. Significant tumor regression and clinical responses were reported in 50% of patients receiving infusions of *in vitro* expanded TIL and high-dose IL-2 following non-myeloablative conditioning with cyclophosphamide (CY) (60 mg/kg for 2 days) and fludarabine (25 mg/m^2 for 5 days).[33]

In two of 13 patients receiving heterogeneous populations of up to 10^{10} cells/m^2 of $CD4^+$ and/or $CD8^+$ T cells of various antigen-specificities (MART-1, gp100, tyrosinase, NYESO-1), over 90% of the reconstituted T-cell repertoire was comprised in the infused melanoma-specific T cells. T-cell receptor (TCR) analysis of circulating T cells in these patients suggested that a transferred clonal T-cell population had expanded, infiltrated tumor sites, and induced major clinical responses. However, this approach was accompanied by serious, potentially life-threatening toxicities as a consequence of profound immunosuppression and high-dose IL-2 therapy.[34,35] Thus, the prolonged immune suppression resulting from the combination of high-dose cyclophosphamide and fludarabine may significantly contribute to serious toxicities.

3.E.3 Adoptive therapy targeting viral antigens

Since 1995, more than 250 patients with EBV-related diseases received donor or autologous-derived EBV-specific cells for both prophylaxis and treatment of post-transplant lymphoproliferative diseases (PTLD) arising in stem-cell or solid-organ transplant recipients.[36] The safety of this approach was repetitively demonstrated, as no infusion-related serious adverse effects were recorded and the incidence of GVHD in allotransplant recipients was not increased after transfer.[37] Furthermore, virological as well as immunological responses were noted in almost every patient after CTL infusion.[38,39] Adoptive transfer of CTL specific for CMV after allogeneic HCT were also demonstrated to be safe in order to reconstitute deficient viral immunity, and a subset of the transferred T cells persisted long-term *in vivo*.[40] Adoptive transfer of HIV-specific CTL

targeting various HIV proteins (gag, pol, and nef), as well as CMV-specific CTL targeting pp65, were infused into HIV^+ patients and demonstrated transient efficacy in patients with high viral burdens and persistence in patients with undetectable viremias undergoing highly-active antiretroviral therapy.[41,42]

Thus, virus-specific T cells that are derived and expanded from patients with established immunity to the pathogen can be safely transferred into autologous recipients without inducing significant side-effects and are capable of persisting, mediating effector functions, and controlling infection. The cell doses used in these settings ranged from 2×10^8 to approximately 3.3×10^9 cells/m^2.[36] However, because CTL-mediated lysis of solid tumors likely requires increased frequencies of adoptively transferred cells to infiltrate tumor tissue, this protocol proposes to infuse cell doses of MCPyV-specific CTL that have shown safety and efficacy in solid tumors (10^{10} cells/m^2) as well as in one patient with metastatic MCC (see **Section 3.H – Previous Human Experience**).

3.F. Induction of MHC Expression by Tumor Tissue

Similar to other virus-associated cancers such as Kaposi sarcoma or cervical cancer that have been shown to directly down-regulate MHC I on tumor cell's surface as a mechanism of tumor immune evasion,[43,44] MHC class I expression on MCC is down-regulated in more than 50% of cases,[45] thus making tumor cells less susceptible to antigen-specific T-cell lysis. To reverse down-regulation, radiation will be administered to the tumor or an intralesional stereotactic injection of IFNβ-1B will be administered within 24 to 72 hours prior to the CTL infusion to increase MHC expression. The method used will be dependent on the localization of metastasis at the time of treatment. Of note, MHC class I expression was decreased compared to epidermis on this patient's primary tumor as shown in **Figure 6**.

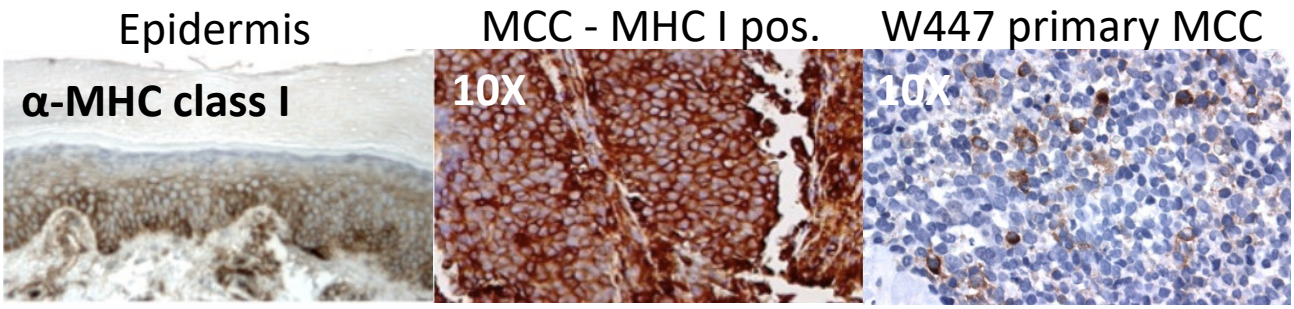


Figure 6. MHC class I downregulation in MCC
MHC class I expression in epidermis (left), MCC with maintained MHC class I expression (middle), and primary tumor from patient W447 (right). MHC class I antibody: EMR8-5.

3.F.1. <u>Localized radiation</u>

Intracellular peptide products are increased following irradiation due to increased degradation of proteins modified directly by radiation or indirectly by the oxidative stress exerted by free-radical formation after water radiolysis on amino acids.[46] Under normal

circumstances, TAP is not fully active in the cell and the peptide pool forms the limiting factor in the antigen presentation pathway.[47] However, under stressful conditions such as irradiation, MHC class I expression is up-regulated presumably as a result of the increased degradation of intracellular peptides. The effects of radiation on MHC-I up-regulation likely play an important role in MCC. In MCC cell lines (such as the virus-positive, MHC-I-negative MKL-1 line), a single dose of gamma radiation (4-8 Gy) induces up-regulation of MHC-I that persists for at least 7 days (**Figure 7**).

In mouse tumor models, irradiation of mouse colon adenocarcinoma MC38 cells with non-ablative doses of 8 or 10 Gy is not sufficient to inhibit *in vivo* tumor growth but induces MHC class I expression for up to 11 days. Furthermore, experiments in which both tumor and adoptively transferred tumor-specific T cells were infused *in vivo* in mice, only the mice which received the irradiated tumor were able to significantly reduce tumor growth.[48] In a murine B16 melanoma tumor model, the administration of a single dose of radiation to established tumors affected tumor regression in a $CD8^+$ T-cell-dependent manner; whereas sequential fractionated doses of radiation induced significantly fewer antigen-specific tumor responses, strongly suggesting that sequential doses likely contributed to ablate the induced T-cell responses.[49,50]

Although MCC is an exquisitely radiosensitive tumor,[51] the early micro-dissemination of the disease renders this modality short-lived. In a recent study of 43 MCC patients treated by radiation alone, the radiation in-field control rates were 75%, but the overall recurrences were 60%.[52] We hypothesize that a combined approach involving low-dose radiation therapy followed 24 to 72 hours later by infusion of antigen-specific T cells has an increased potential to control disease at the irradiated tumor site as well as traffic to micro-metastatic sites. Similar to what was observed in mouse models and human MCC cell lines, radiation (such as a single dose of 8 Gy) is expected to up-regulate MHC sufficiently to allow tumor recognition and lysis by transferred CTL, Although based on our clinical experience the irradiation will also likely cause stabilization and/or regression of the treated lesion in the majority of cases,[53] the primary goal of the radiation therapy is MHC upregulation/enhanced antigen presentation and 'priming' of a systemic immune response.

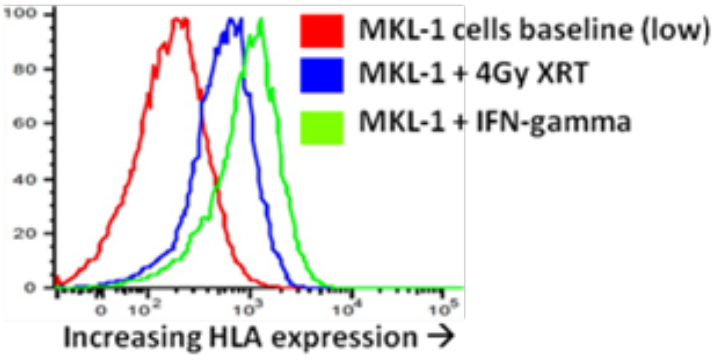


Figure 7. MHC class I up-regulation

Up-regulation of MHC class I by gamma radiation and IFNγ administration in a MCPyV$^+$ MCC cell line, MKL-1. MHC-I up-regulation persists up to 7 days with a single-dose radiation administration.

3.F.2. Intralesional IFNβ-1B injections

This method will be used preferentially over radiation, if the tumor is accessible to stereotactic injections as, compared to radiation, a single injection of IFNβ-1B may less confound the effects of MCC T-cell-mediated lysis and tumor regression. Interferons mediate antiviral immune responses by up-regulation of MHC class I.[54] Multiple interferons are approved for use in the US for varied indications such as chronic HCV, melanoma, and multiple sclerosis.[55-57] Intralesional IFNβ-1B injections have previously been reported as primary therapy for MCC.[58,59] Of nine patients treated at this institution with multiple IFNβ-1B injections at cutaneous MCC sites, in whom this was used as a unique treatment modality, tumor regression was observed in eight cases (see **Appendix C).**[45] In patients in whom MCC tissue could be biopsied before and after treatment, MHC class I was significantly up-regulated (**Figure 8**). Expression of the MCPyV TAg by MCC was not modified, and intralesional CD8$^+$ T-cell infiltrates were increased after treatment. In contrast, systemic interferon has not been reported to be effective in inducing MCC regression and may be due to inadequate delivery into the tumor micro-environment.[16,60,61]

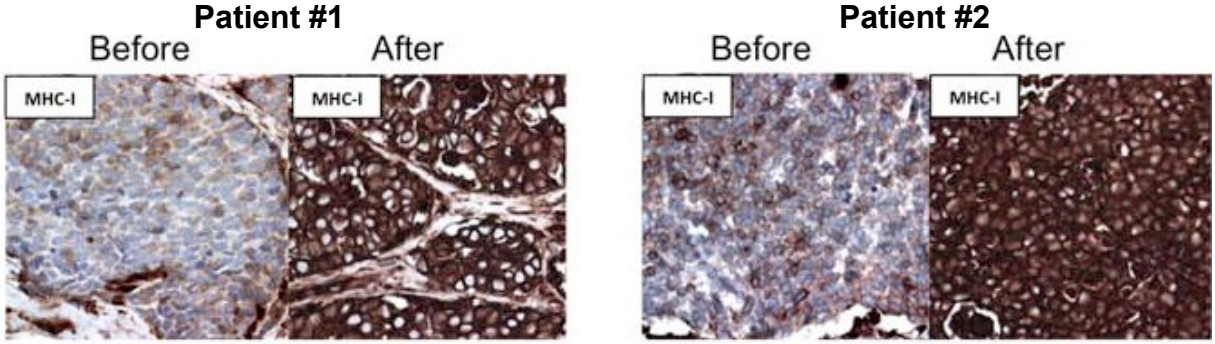


Figure 8. IFNβ-1B intralesional injections to induce MHC class I
MHC class I expression in MCC tissue biopsies before (left) and after (right) IFNβ-1B intralesional injections for two individual patients.

3.G. Previous Human Experience: Adoptive Therapy of MCC Targeting MCPyV TAg

MCPyV TAg represents an exceptionally attractive model of viral tumor antigen for evaluating the safety and efficacy of antigen-specific immunotherapy in MCC. MCPyV TAg is expressed in approximately 80% of MCC and, compared to mammalian tumor associated antigens that all have some degree of expression within normal tissue, MCPyV TAg expression is restricted to MCC. As described in **Section 3.C**, antigen-specific CD8$^+$ T cells targeting the HLA-A*2402-restricted MCPyV TAg epitope EWWRSGGFSF could be generated and infused in patient w447 (Protocol FHCRC #2558) and the results are now published.[29] As soon as a first metastasis was detected

(**Figure 9A**), the patient underwent a leukapheresis to collect sufficient PBMC to derive and expand TAg-specific cells in the FHCRC cGMP facility.

At the time of follow-up scans 140 days later, the patient's initial metastasis had expanded and two additional metastases were detectable. To up-regulate MCH class I (see **Section 3.F**), the patient underwent intralesional IFNβ-IB injections to the largest metastasis before receiving a first infusion of 10^{10} MVPyV TAg-specific $CD8^+$ T cells followed by low-dose subcutaneous (SC or s.c.) IL-2 (2.5 x 10^5 IU/m^2 BID x 14 days) (**Figure 9B**). Restaging 28 days later showed a response in the IFNβ-IB infiltrated lesion, consistent with T-cell-mediated lysis following MHC class I up-regulation, but growth in the non-infiltrated lesions. The patient then went on to receive 8 Gy radiation to all three remaining lesions before a second infusion of 10^{10} MVPyV TAg-specific $CD8^+$ T cells.

Although two of three detected lesions disappeared 140 days after the first infusion, the pancreatic neck lesion first decreased in size and then re-increased, suggesting the possibility of tumor antigen escape. However, the patient did not develop additional metastasis elsewhere 274 days after starting treatment, which suggests a long-term control of the transferred T cells on MCC. Overall, the infusions were well tolerated: apart from the expected immediate toxicities associated with T-cell infusion (transient, <72 hours Grade 2 Cytokine Release Syndrome [CRS] and Grade 2 lymphopenia), the patient did not develop changes in end organ function or inflammation-related complications such as an autoimmune process.

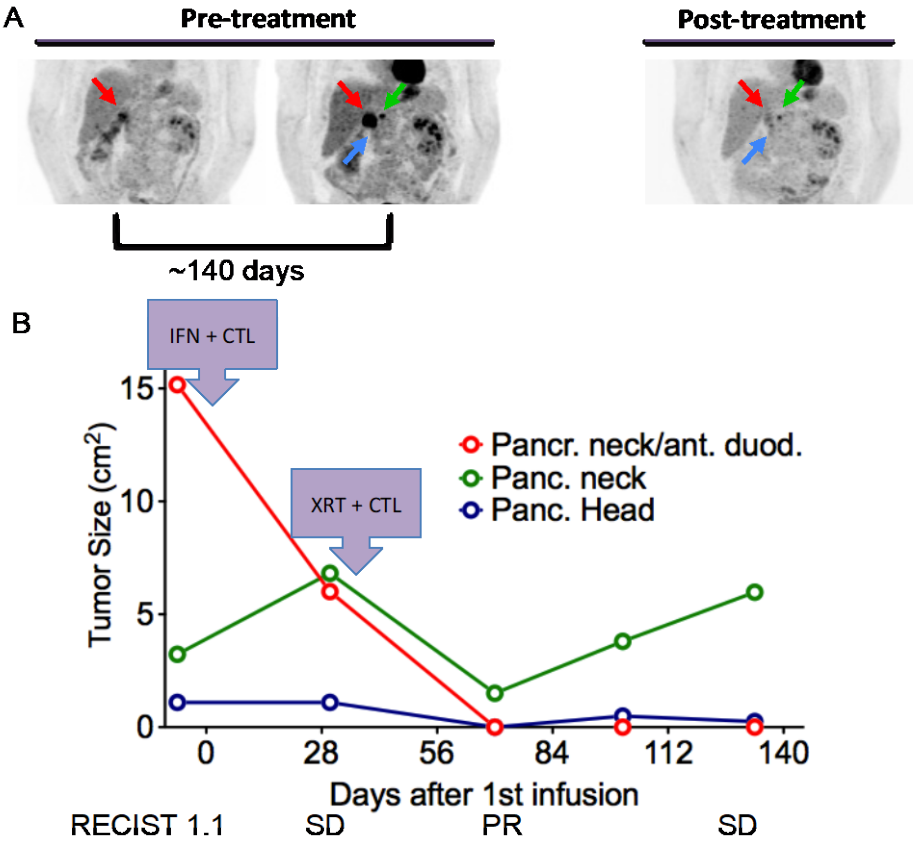


Figure 9. w447 response to MHC class I up-regulation and MCPyV-TAg-specific CD8⁺ T-cell infusions

A) pre- (left) and post (right)-treatment PET scans. B) Evolution of the individual metastasis size in cm^2 (y axis) over time (x axis) and corresponding RECIST 1.1 criteria. SD: stable disease, PR: partial remission.

Assessment of the frequency of infused CD8⁺ T cells by their ability to bind tetramers *in vivo* demonstrated the cells peaked from 1 to 3 days after infusions (~8%) before persisting at least 140 days at frequencies of 1% to 1.5% or a 10-fold increase compared to pre-infusion levels (1.3%) (**Figure 10**).

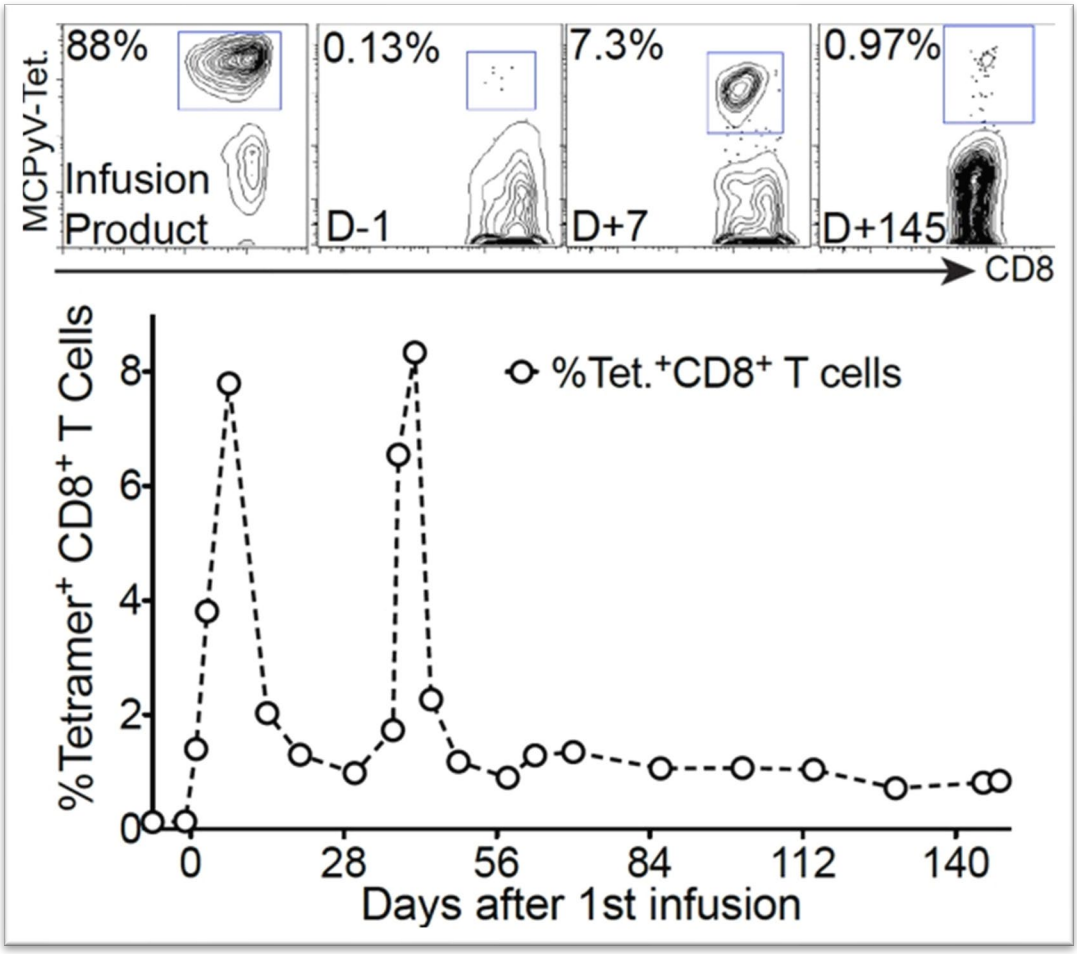


Figure 10. Persistence of MCPyV TAg-specific CD8⁺ T cells

Top panels show flow plots of the infusion product and selected timepoints, and bottom panel shows the frequency of tetramer⁺ MCPyV-specific CD8⁺ T cells (x axis) over time (y axis).

Prior to infusions, no MCPyV TAg-reactive cells could be detected within PBMC suggesting the detectable tetramer⁺ cells were non-functional. However, after infusions,

the frequency of MCPyV TAg-reactive cells measured by their ability to secrete IFNγ in response to cognate peptide increased with each infusion and were detectable at frequencies of 1% to 2% at least 140 days after the first infusion. These values correlated with the frequency of tetramer$^+$ cells (**Figure 11**).[29]

Overall, these results suggest the combination of MHC class I up-regulation with infusion of 10^{10} polyclonal *ex vivo*-expanded MCPyV TAg-specific cells was safe in this patient, established detectable cellular reactivity to MCPyV TAg for at least 140 days, and mediated tumor regression in 2 out of 3 detectable lesions without the occurrence of new distant metastasis for at least 274 days.

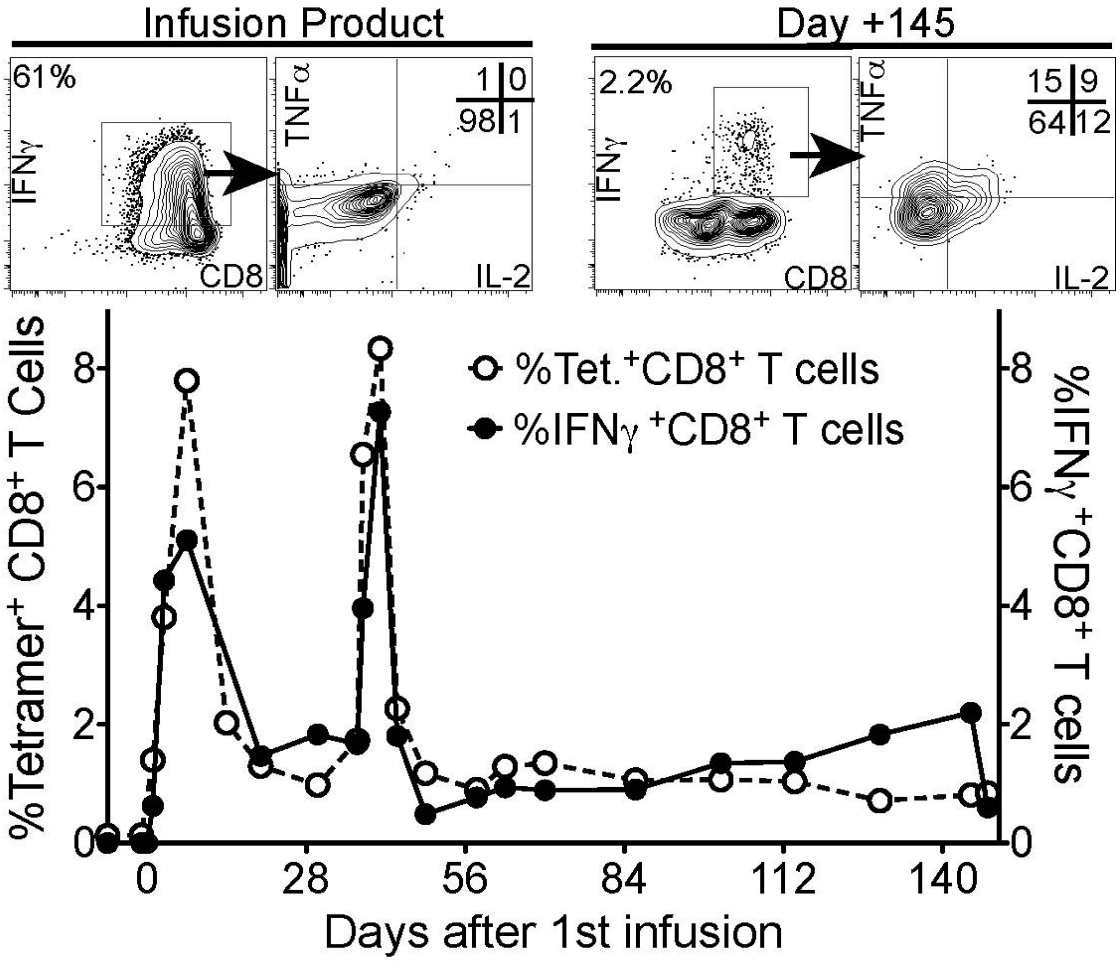


Figure 11. Establishment of MCPyV TAg-reactive cells *in vivo* after CD8$^+$ T-cell transfer

Frequencies of IFNγ$^+$CD8$^+$ T cells (right y axis) correlate with frequencies of tetramer$^+$ CD8$^+$ T cells (left y axis) over time after transfer.

3.H. Previous Clinical Experience: Results of Protocols FHCRC #2558 and #2586

To date, 4 additional patients have been treated in Group 1 of protocol 2586 bringing the total number of patients who have received infusions of 10^{10} cells/m^2 MCPyV-specific T cells to 5, for a total of 15 infusions, each followed by low-dose s.c. IL-2 for 14 days (**Table 2**).

Table 2. Patient Characteristics

Pt No	Previous treatments	Disease Burden (cm^2)	MHC class I up-regulation	Targeted epitope	Infusions received
Pt-1 #2558	Surgery, 50Gy radiation	Intermediate (20.03)	IFNβ -1B and 8Gy radiation	HLA A*2402-EWW	3
Pt-1 #2586	Surgery, IL-12 electroporation, radiation, chemotherapy: cisplatin and etoposide x 5.	High (51)	IFNβ -1B	HLA A*A0201 - KLL and HLA A*2402-EWW	2
Pt-2 #2586	Surgery, radiation x 2.	High (54)	8Gy radiation	HLA A*A0201 – KLL	4
Pt-3 #2586	Surgery x 3, radiation x 4.	Low (1.25)	IFNβ -1B	HLA A*2402-EWW	4
Pt-4 #2586	Surgery x 4, radiation x 6, chemotherapy x 2, intratumoral IFNβ-1B	High (56)	8Gy radiation	HLA B*3502	2

AEs were evaluated starting from the time of the first infusion to 30 days after the patients had taken the last dose of s.c. IL-2. AEs that were deemed possibly, probably or likely related were collected and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (NCI CTCAE v4.0) (**Table 3**).

Table 3. Adverse events

Categories	NCI CTCAE v4.0[a]	Grade 1	Grade 2	Grade 3	Grade 4
Cytokine Release Syndrome	Fever	6	2		
	Chills	6			
	LBB[b]		1		
Hematological Abnormalities	White blood cell count decreased	4			
	Lymphopenia		3	4	1
	Thrombocytopenia	1	2		
IFNβ-1B-related	Pain at injection site	1			

	Wound infection		1		
IL-2 related	Injection site reaction	2			

[a] National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.
[b] Left bundle branch block (new) in a patient with pre-existing coronary artery disease.

Expected transient symptoms were observed due to a cytokine release syndrome (CRS) associated with activation of large numbers of antigen-specific CTL transferred into patients with targets expressing the antigen and/or low-dose s.c. IL-2. Specifically, all patients experienced fevers (≥ 38.3°C) with or without chills after at least 1 infusion. Blood cultures were all negative for bacterial or fungal growth. All symptoms were managed on the general hospital ward and resolved within 24 hours. One patient with pre-existing coronary artery disease experienced a transient new left bundle branch block while he was febrile. The symptoms resolved and he went on to receive a coronary artery stent 10 days later. The hematological abnormality most commonly encountered was lymphopenia, which is a predictable, transient side effect of T-cell infusions presumably reflecting redistribution of peripheral lymphocytes.[62,63] The temporary drop in total lymphocyte counts returned to pre-infusion levels within 7 to 11 days in all patients. After the treatment, one patient experienced a temporary drop in platelet counts which was most likely associated with radiation administration to the pelvis. Of the patients who received IFNβ-1B injections, one experienced pain after the injection and another developed a localized wound infection which resolved with antibiotic treatment. One patient experienced injection-site reactions with low-dose s.c. IL-2 administration.

3.I. Rationale for Combining MHC Class I Upregulation, MCPyV-Specific T-Cell Infusions and PD1 - PD-L1 Axis Blockade

Of the patients that could be analyzed beyond 16 weeks (**Table 4**), one patient showed rapidly progressive disease (PD) at 8 weeks and was deceased by 16 weeks. Although the remaining 3 patients had either a partial response (PR) or stable disease (SD) at 8 weeks, only 1 with SD continued on to have a PR at 16 weeks. The other 2 patients had progressed at 16 weeks from their maximal response at 8 weeks. Of the 3 patients who demonstrated progression after infusions, 2 had detectable transferred cells at the time of progression suggesting that the presence of transferred cells was not sufficient to suppress tumor growth.

Table 4. Clinical outcomes

Patient-Number Protocol #	Clinical Response by mWHO criteria[a] (weeks after 1st infusion)		T-cell persistence?
	8 weeks	16 weeks	
Pt-1 #2558	PR (-79%)[b]	Progression of 1 remaining lesion (-38%)	Yes
Pt-1 #2586	PD (+100%)	Deceased	No

Pt-2 #2586	SD (-10%)	Mixed response (-34%): progression of non-irradiated lesions	Yes
Pt-3 #2586	SD (-13%)	PR (-58%)	Yes

[a]mWHO criteria=modified World Health Organization criteria based on the products of diameters of tumor lesions [64]; [b] Note: numbers in parenthesis refer to an increase [positive number] or decrease [negative number] in tumor burden as a percentage of baseline values at the indicated timepoint. Definitions: Pt=patient, PR=partial response, PD=progressive disease, SD=stable disease.

Analysis of the phenotype of the transferred cells revealed that most products expressed programmed death 1 (PD-1) before infusions (**Figure 12, top row**) and that products that expressed PD-1 continued to express PD-1 when they could be detected *in vivo* (Pt-1 #2558, Pt-1 #2586) (**Figure 12, bottom row**). Pt-3 #2586 received a product with low PD-1 expression which remained detectable and continued to express low levels of PD-1 *in vivo.* This observation coincided with the best clinical outcome as it occurred in the only patient whose MCC remained in a PR for 11 months.

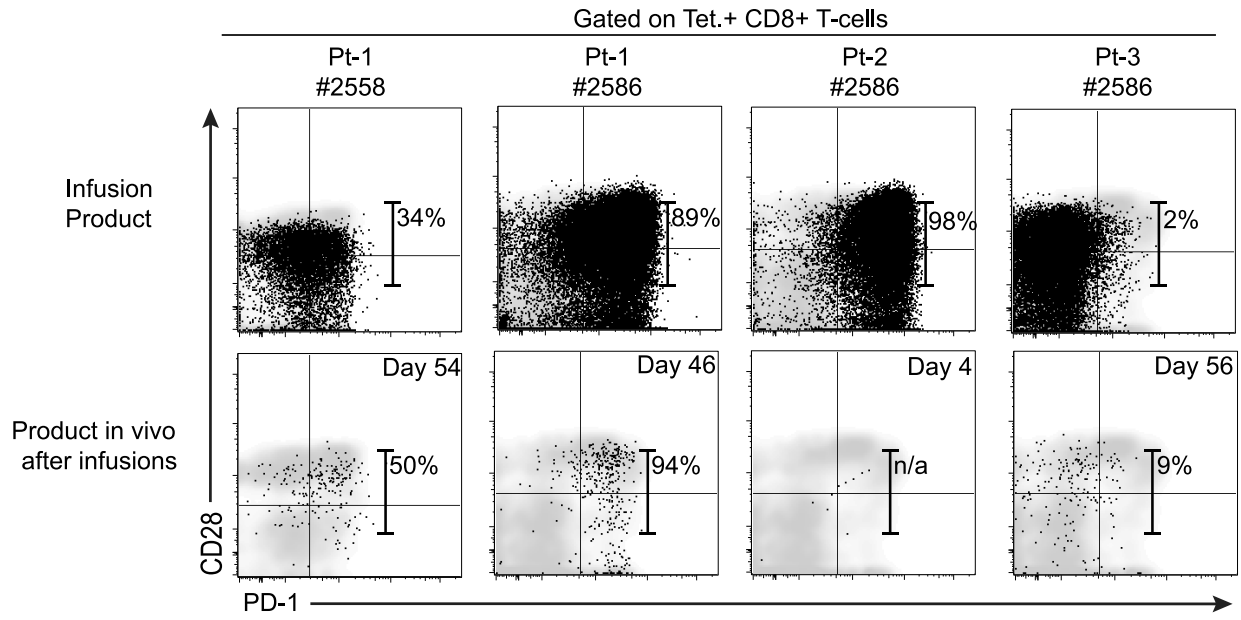


Figure 12. Phenotype of infused products before and after infusions
Vertical bars indicate cumulative percentages of ecentss in the upper and lower left quadrants and represent percentages PD1-expressing cells.

Because the ligand for PD-1 (PD-L1), is expressed on 50% of MCC, on 55% of MCC tumor infiltrating lymphocytes, and can be induced by local inflammation, this may provide a strong inhibitory signal by the tumor microenvironment, shielding the tumor from the transferred MCPyV-specific T cells upon PD-1/PD-L1 ligation.[65,66] Thus, blocking this interaction could reverse the inhibitory signal and allow enhanced responses by the transferred T cells that could in turn more efficiently eliminate the MCC.

3.I.1. Programmed death receptor and ligands

The PD-1 receptor as well as PD-L1 and PD-1 ligand 2 (PD-L2) play integral roles in immune regulation. Expressed on activated T cells, PD-1 is activated by PD-L1 and PD-L2 expressed by stromal cells, tumor cells, or both, initiating T-cell death and localized immune suppression,[67-70] potentially providing an immune-tolerant environment for tumor development and growth. Conversely, inhibition of this interaction can enhance local T-cell responses and mediate antitumor activity in nonclinical animal models.[67,71]

In the clinical setting, treatment with antibodies that block the PD-1 – anti-PD-L1 interaction have been reported to produce objective response rates of 7% to 38% in patients with advanced or metastatic solid tumors, with tolerable safety profiles.[72-74] Notably, responses appeared prolonged, with durations of 1 year or more for the majority of patients.

Of importance to the current trial: previous studies have demonstrated PD-L1 tumor-cell expression in 49% of MCC patient samples (49 patients sampled) and expression in tumor-infiltrating lymphocytes in 55% of patient samples. Of note, of the PD-L1 positive samples, 28 of 29 samples showed co-localization with immune infiltrates.[66] These findings, coupled with the sited response rates and long response durations in other solid tumors make PD-L1 an attractive target in patients with MCC.

3.I.2. The anti-PD-L1 therapeutic antibody Avelumab

The Investigational Medicinal Product (IMP) for the present trial is Avelumab, a fully human monoclonal antibody of the immunoglobulin (Ig) G1 isotype. This anti-PD-L1 therapeutic antibody concept is intended to be developed in oncological settings by Merck KGaA, Darmstadt, Germany, and by its subsidiary, EMD Serono Inc, Rockland, MA, USA.

Avelumab 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.[75] For complete details of the *in vitro* and nonclinical studies, please refer to the Investigator's Brochure (See **Appendix C**). Avelumab is currently in clinical development with 2 ongoing Phase 1 studies in subjects with solid tumors:

Trial EMR100070-001 is "a Phase 1, open-label, multiple-ascending dose trial to investigate the safety, tolerability, pharmacokinetics, biological, and clinical activity of Avelumab in subjects with metastatic or locally advanced solid tumors."
Trial EMR100070-002 is "a Phase 1 trial to investigate the tolerability, safety, pharmacokinetics, biological, and clinical activity of Avelumab in Japanese subjects with

metastatic or locally advanced solid tumors, with expansion part in Asian subjects with gastric cancer."

3.I.3. Preliminary results of trial EMR10070-001

This trial consists of two parts, a dose-escalation phase and an expansion phase, which is performed in selected tumor indications.
A maximum of 42 subjects with advanced malignancies with no established therapy available were planned to be enrolled in the dose-escalation phase of this trial. Avelumab was administered intravenously at the assigned dose level as a 1-hour intravenous (IV) infusion once every 2 weeks. Dose escalation (3 + 3 design) was performed at the following dose levels:

- Dose level 1: 1.0 mg/kg (Cohort 1)
- Dose level 2: 3.0 mg/kg (Cohort 2)
- Dose level 3: 10.0 mg/kg (Cohort 3)
- Dose level 4: 20 mg/kg (Cohort 4)

As of 29 August 2013, a total of 13 subjects were enrolled in the dose-escalation phase of the trial, with 4, 3, and 6 subjects being treated with Avelumab of 1, 3, and 10 mg/kg, respectively. All subjects completed the dose-limiting toxicity (DLT) evaluation period and received all planned treatment administrations except for 1 subject in the 1 mg/kg cohort (Subject 101-0002 who was replaced). Overall, the data reported under this section are based on a total of 51 administrations of Avelumab: 14 administrations of 1 mg/kg, 20 administrations of 3 mg/kg, and 17 administrations of 10 mg/kg.

Based on data of 13 subjects treated at 1, 3, or 10 mg/kg, the decision to use a dose of 10 mg/kg for further evaluation in the expansion cohorts was made.

No subject experienced a DLT. The maximum administered dose level of 10 mg/kg at this time was thus considered a safe and well-tolerated dose.

Subsequently, the recruitment of Cohort 4 started (3 + 3). In addition, subjects are currently being enrolled at 3 mg/kg and 10 mg/kg for the purpose of generating additional PK and PharmDyn data.

A total of 7 expansion cohorts are currently being enrolled in the US, EU, and Asia. Three cohorts (non-small-cell lung cancer, gastric cancer, metastatic breast cancer) are each planned to enroll 150 subjects to generate data that will support the development of a companion diagnosis based on immune-staining of PD-L1 that is specific to the membrane expression of the molecule. The other 4 cohorts (metastatic colorectal cancer, castrate-resistant prostate cancer, advanced melanoma, and ovarian cancer) are each planned to enroll 20 subjects.

3.I.3.A. Safety results of trial EMR10070-001

All adverse events (AE)
All 13 subjects (100%) at all dose levels experienced at least 1 treatment-emergent AE (TEAE). The TEAEs at the Preferred Term level reported for the greatest proportion of subjects (observed in ≥ 2 subjects) included lymphopenia (9 subjects; 69.2%), anemia (8 subjects; 61.5%), fatigue (7 subjects; 58.8%), pyrexia and white blood cell count decrease (each in 6 subjects; 46.1%), hyperglycemia (5 subjects; 38.5%), hypoalbuminemia and aspartate aminotransferase (AST) increase (each in 4 subjects; 30.8%), chills and influenza-like illness (each in 3 subjects; 23.1%), and thrombocytopenia, blood alkaline phosphatase increase, blood creatinine increase, neutrophil count decrease, hypocalcemia, vomiting, and rash (each in 2 subjects; 15.4%). There were no relevant differences with regard to the frequency of the AEs across different dose levels.

AEs of Grade ≥ 3
Five subjects (38.5%), 4 receiving the 1 mg/kg dose and 1 receiving the 10 mg/kg dose, experienced at least 1 TEAE with a Grade ≥ 3. Of the Grade ≥ 3 TEAEs, anemia was reported in 2 subjects (15.4%) and all others, which included the Preferred Terms of peritonitis bacterial, staphylococcal infection, pneumonia, lymphopenia, hyperglycemia, hypoalbuminemia, hyponatremia, AST increase, blood alkaline phosphatase increase, dyspnea, ascites, pain, and gastric outlet (duodenal) obstruction, occurred in 1 subject each. No dose dependency for the occurrence of Grade ≥ 3 TEAEs was observed. In addition, Subject 101-0002 experienced a Grade 5 event of hypoxia, caused by a bacterial pneumonia, which led to the death of this subject. A drug-induced pneumonitis caused by the drug was excluded by the Investigator.

Treatment-related TEAEs and treatment-related Grade ≥ 3 AEs
Eleven subjects (84.6%) experienced TEAEs assessed as related to Avelumab by the Investigator, including 3 of 4 subjects treated with the 1 mg/kg dose, 2 of 3 subjects treated with the 3 mg/kg dose, and all 6 subjects treated with the 10 mg/kg dose.

The TEAEs reported for the greatest proportion of subjects and deemed to be related to Avelumab by the Investigator were fatigue and lymphopenia (each in 5 subjects; 38.5%), pyrexia (4 subjects; 30.8%), influenza-like illness and chills (each in 3 subjects; 23.1%), and AST increase (2 subjects; 15.4%). All other treatment-related TEAEs were reported in 1 subject each.

One subject (101-0004) treated with the 1 mg/kg dose experienced Grade ≥ 3 blood alkaline phosphatase increase and AST increase, both considered by the Investigator to be related to the trial treatment.

Subsequent to the cutoff date of 29 August 2013, 2 Grade 4 infusion reactions have been reported (see **Section 3.I.3.B**)

Serious adverse events (SAE)

As of 29 August 2013, 2 of 13 subjects (15.4%) experienced treatment-emergent SAEs (Subject 101-0003: gastric outlet obstruction, abdominal ascites, and bacterial peritonitis; and Subject 101-0002: hypoxia, shortness of breath, and lung infection bilateral pneumonia). Both subjects were treated with 1 mg/kg of Avelumab. None of the SAEs in either subject were considered related to trial treatment as assessed by the Investigator.

As of 29 August 2013, 1 death as a result of a TEAE has been reported:

- Subject 101-0002 (1 mg/kg of Avelumab): The subject was reported to have died of bilateral pneumonia, with the SAE of hypoxia being considered as the fatal event. The SAEs leading to death, and thus the event of the death, were not considered related to trial treatment by the Investigator. In particular, the diagnosis of drug-induced pneumonitis was excluded by the Investigator.

TEAEs leading to permanent discontinuation of Avelumab treatment
As of 29 August 2013, 3 subjects (23.1%), all receiving 1 mg/kg of Avelumab, discontinued trial treatment permanently for TEAEs. These included the 2 subjects (101-0003 and 101-0002) who experienced SAEs as described above and 1 additional subject (101-0004) who discontinued trial treatment for Grade 3 AST increased.

Laboratory abnormalities
Abnormal laboratory findings were reported as AEs if they were associated with clinical signs or symptoms, or led to treatment discontinuation, or were considered otherwise medically important by the Investigator.

Hematology parameters were stable, except for transient decreases in white blood cell (WBC) count during trial treatment. No clinically significant hematology abnormalities were evident and no dose-dependent changes in hematology parameters were observed.

Clinically significant increases in AST and alanine aminotransferase (ALT) leading to treatment discontinuations as well as increases in alkaline phosphatase were observed in 1 subject treated with 1 mg/kg of Avelumab, which were observed following 5 doses of trial drug infusion and were transient, with the ALT and AST abnormalities returning to normal within a week. An elevated gamma-glutamyl transferase (GGT) was observed in 1, 2, and 1 subjects treated with 1, 3, and 10mg/kg of Avelumab respectively. In all these subjects, decreases in GGT were seen over time. Total bilirubin remained unchanged during the treatment.

No clinically relevant changes in amylase, lipase, glucose, albumin, total protein, creatine kinase, lactate dehydrogenase (LDH), creatinine, urea, uric acid, electrolytes, cholesterol, and triglycerides were observed. Overall, no evidence of a dose dependency of Avelumab was observed for changes in blood chemistry variables during the trial.
No clinically relevant changes in blood coagulation parameters, such as international normalization ratio, and activated partial thromboplastin time were identified in all subjects.

Vital signs, body weight, and electrocardiogram
During trial treatment, most variations in vital sign parameters, including heart rate, systolic and diastolic blood pressures, respiratory rate, and body temperature as well as body weight were small and the values remained stable and within the normal range. No obvious differences were observed among different dose cohorts. Overall, no clinically significant changes in blood pressure or heart rate were observed during trial treatment. The electrocardiograms (ECGs) in 5 of 13 subjects were normal at baseline. During trial treatment, none of the 5 subjects had abnormal ECGs assessed by the Investigator as clinically significant. Eight subjects had abnormal ECG findings at baseline. All these abnormal ECG findings were discussed at the Safety Monitoring Committee meetings and had no impact on the continuation of trial treatment.

Summary of safety
Overall, Avelumab was well tolerated up to 10 mg/kg, no DLT has been reported, and no safety concerns have been identified. The majority of the observed AEs were consistent with the subjects' underlying cancer disease.

3.I.3.B. Infusion-Related Reactions and Hypersensitivity

As of 23 January 2014, 7 subjects with infusion-related hypersensitivity have been reported in 119 subjects treated with Avelumab in the 2 Phase 1 trials, including 3 SAEs (2 infusion-related reactions, 1 anaphylactic reaction). Of the 3 SAEs, 2 were assessed by the Investigator as Grade 4 infusion-related reactions; however, review of the cases revealed that the described reactions fulfilled the criteria for Grade 2 infusion-related reactions. Follow-up with the Investigator is ongoing. The remaining 4 subjects were reported as having non-serious infusion-related reactions, including 3 subjects with Grade 2 and 1 subject with Grade 3 infusion-related reactions.

3.I.3.C. Pharmacokinetic and Pharmacodynamic Results

At the analysis cut-off date of 29 August 2013, 13 evaluable subjects were included for PK analysis. The PK profile of Avelumab at the highest dose level of 10 mg/kg (compared with the 1 and 3 mg/kg doses) is characterized by the following parameters:

- Average maximum concentration observed postdose (C_{max}) was 230.0 µg/mL, with individual values ranging from 182.6 to 299.4 µg/mL compared with 18.41 µg/mL for the 1 mg/kg dose (14.49 to 22.64 µg/mL) and 86.54 µg/mL (76.89 to 101.1 µg/mL) for the 3 mg/kg dose.
- Median time to reach C_{max} (t_{max}) was approximately 1 hour after the start of infusion for all doses.
- At Day 15 before the second dose administration, an average minimum postdose (trough) concentration (C_{min}) of 17.14 µg/mL was observed for the subjects who received a dose of 10 mg/kg with individual values ranging from 11.72 to 26.87 µg/mL compared with 0.23 µg/mL for the 3 subjects treated at a 1 mg/kg dose

(0 to 0.68 µg/mL) and 5.66 µg/mL (4.56 to 6.99 µg/mL) for the 3 subjects treated at a 3 mg/kg dose.

- The average area under the concentration-time curve (AUC_{tau}) for the first infusion was 23485 µg/mL·h (16439 to 29316 µg/mL·h) compared with 1286 µg/mL·h for the 1 mg/kg dose (854 to 1669 µg/mL·h) and 8067 µg/mL·h (7516 to 8485 µg/mL·h) for the 3 mg/kg dose.
- Interindividual variability in exposure parameters (C_{max} and AUC_{tau}) was low. In the 10 mg/kg cohort, the coefficient of variation for the geometric mean was 19.1% for C_{max} and 21.9% for AUC_{tau}.
- No significant accumulation of drug concentrations was recorded in all dose levels. After the first infusion, trough levels did not fall beyond the lower limit of detection, but were very low (C_{min}). Based on the limited number of PK data available after the second infusion, it was concluded that these residual trough values did not increase up to the fourth infusion. The peak values also remained at a similar level over time.
- Half-life ($t_{1/2}$) was 92.8 hours (77.2 to 117.4 hours) compared with 63.4 hours for the 1 mg/kg dose (45.9 to 85.6 hours) and 91.3 hours (88.5 to 95.1 hours) for the 3 mg/kg dose.

From the PK perspective, no unexpected observations or findings that would raise safety concerns could be identified. The shift to a longer $t_{1/2}$ from 1 to 3 mg/kg doses is consistent with a progressing target occupancy, which indicated that the fast target-mediated clearance reached to a saturated stage, while a slow unspecific elimination became prominent. Similar half-lives for the 3 and 10 mg/kg doses indicated that the target-mediated clearance was nearly saturated by the 3 mg/kg dose. The PK parameters determined, support a DLT evaluation period of 21 days, which covered approximately 5 half-lives.

These theoretical considerations, extrapolated from the PK behavior were supported by an initial set of target occupancy (TO) data. The TO, that is, the binding to the PD-L1 molecules, was analyzed on circulating $CD3^+$ lymphocytes. The investigation was performed by flow cytometry on blood samples collected on Day 1 before the start of the infusion, at 4 and 48 hours (Day 3) after the start of infusion, and before the start of each infusion on Days 15, 29, 43, and 85.

At the Avelumab doses of 3 and 10 mg/kg, the TO prior to the second infusion on Day 15 was greater than 90% for all subjects, while the Avelumab trough serum levels ranged from 4.56 to 6.99 µg/mL and from 19.42 to 26.87 µg/mL, respectively, at this time point. At the Avelumab dose of 1 mg/kg, 2 of 3 subjects displayed a TO of less than 90% at trough serum concentrations on Day 15, while the Avelumab serum levels were below the lower limit of quantification of 0.2 µg/mL in these 2 subjects.
Before the initiation of the Phase 1 trial, a target trough concentration of 58.5 µg/mL was calculated (Avelumab Investigator Brochure Version 1.0) on the basis of TO data for Avelumab in mouse blood, fitted by a receptor binding model, and considering that 95% receptor occupancy on the peripheral blood mononuclear cell (PBMC) correlated with

tumor growth inhibition in a murine disease model. Subsequently, additional target occupancy data for primate blood cells showed that PD-L1 density was much lower on primate $CD3^+$ lymphocytes than mouse PBMCs.
Target occupancy was investigated on PBMC by flow cytometry-based assay, previously validated using PBMC from healthy volunteers. Validation data showed that near complete TO was achieved at anti-PD-L1 concentrations greater than 0.2 µg/mL. These new data explain why a near-complete TO on peripheral $CD3^+$ was achieved at lower Avelumab serum levels than predicted.

Though a TO of > 90% was observed for trough concentrations at the 3 mg/kg dose, the 10 mg/kg dose was selected as the clinical dose for the expansion cohorts because

- it was considered conservative to compensate for possible differences in receptor density between $CD3^+$ cells present in peripheral blood and tumor cells or immune-infiltrating cells that are present in the tumor micro-environment;
- to compensate for differences in Avelumab serum concentration and tissue (tumor) concentrations;
- there was no difference in terms of safety for any of the 3 doses, which was consistent with the findings reported for another anti-PD-L1 monoclonal antibody (0.3 to 10 mg/kg), in which the spectrum, frequency, and severity of treatment-related TEAEs were similar among the dose levels, with the exception of infusion reactions (29);
- the finding that the $t_{1/2}$ for the 3 and 10 mg/kg doses were nearly identical is consistent with the view that the target-mediated clearance was saturated and that higher doses were not expected to result in a higher percentage of TO.

3.J. Rationale for Assessing T-Cell Effectiveness by 'Time to New Metastasis'

It cannot be excluded that up-regulation of MHC class I with either radiation therapy or intralesional IFNβ-1B injections may have an independent effect on the targeted metastasis, which could then be measurable by Response Evaluation Criteria In Solid Tumors (RECIST) criteria. Furthermore, as transferred T cells circulate systemically, their effect may be better assessed by the prevention of the establishment of micrometastasis and the occurrence of new detectable metastatic lesions. To capture the systemic effects of adoptively transferred T cells, the time for new metastasis to develop after treatment will be assessed, and this parameter will be compared to historical matched controls undergoing standard therapy by radiation or chemotherapy.

4. OBJECTIVES

4.A. Primary Objectives

1. Assess and compare the safety and potential toxicities associated with treating patients with metastatic MCC with either MHC up-regulation and PD1-axis blockade (Group 1), or MHC up-regulation, PD1-axis blockade and adoptive transfer of MCPyV TAg-specific polyclonal autologous $CD8^+$ T cells (Group 2).

2. Assess and compare the antitumor efficacy associated with treating patients with metastatic MCC with either MHC up-regulation and PD1-axis blockade (Group 1), or MHC up-regulation, PD1-axis blockade and adoptive transfer of MCPyV TAg-specific polyclonal autologous $CD8^+$ T cells (Group 2).

4.B. Secondary Objectives

1. Examine the *in vivo* persistence and, where evaluable, migration to tumor sites of adoptively transferred polyclonal $CD8^+$ T cells targeting the MCPyV TAg (Group 2).

2. Examine the *in vivo* functional capacity of adoptively transferred polyclonal $CD8^+$ T cells targeting the MCPyV Tag (Group 2).

3. Examine and compare evidence of epitope spreading with either MHC up-regulation and adoptive transfer of MHC up-regulation and PD1-axis blockade (Group 1), or MHC up-regulation, PD1-axis blockade and adoptive transfer of MCPyV TAg-specific polyclonal autologous $CD8^+$ T cells (Group 2).

5. STUDY ENDPOINTS

5.A. Primary Endpoints

1. Evidence and nature of toxicity related to the treatment.

2. Evidence of response based on “median time to new metastasis” (See **Section 12.D.1**).

5.B. Secondary Endpoints

1. Persistence of transferred T cells in blood and in tumor (Group 2).

2. Functional capacity of transferred T cells (Group 2) (Please see **Section 12.E.3**).

3. Evidence of epitope spreading (See **Section 12.F**).

4. Disease response by RECIST criteria, and MCC-specific survival (See **Section 12.D.2**).

6. STUDY DESIGN

The proposed study is a Phase I/II trial aimed at treating 20 individuals with metastatic MCC.

Group 1: Due to the availability of the anti-PD-L1 antibody, ten patients with metastatic MCC who do not have an HLA type for which T cells can be generated (for example, who DO NOT express HLA A*0201, HLA A*2402 or HLA B*3502) or for whom T cells cannot be generated for technical issues, will be treated on Group 1 (**Figure** 13). They will be planned to receive the anti-PD-L1 antibody as an infusion every 2 weeks for 12 months and an MHC class I up-regulation intervention (either localized radiation or an intra-tumor injection of 3.3 x 10^6 IU of IFNβ-1B) between 7 and 10 days after the preceding infusion of anti-PD-L1 (or 7 to 4 days before the next anti-PD-L1). Patients may receive preferably one, but up to 3 infusions of anti-PD-L1 before the first MHC class I up-regulation intervention. Depending on the continued presence of detectable metastatic disease and if they remain clinically eligible, patients could undergo MHC class I up-regulation during the time anti-PD-L1 infusions were ongoing starting within four weeks of the preceding restaging scans.

Group 2: Ten patients with metastatic MCC who have an HLA type for which T cells can be generated (for example, who express HLA A*0201, HLA A*2402 and/or HLA B*3502) will be treated on Group 2. They will be planned to receive an infusion of anti-PD-L1 antibody every 2 weeks for 12 months, an MHC class I up-regulation intervention (either localized radiation or an intra-tumor injection of 3.3 x 10^6 IU of IFNβ-1B) between 7 and 10 days after the first infusion of anti-PD-L1 and 2 to 5 days prior to receiving a first of 2 infusions (approximately 28 days apart) of no greater than 10^{10} cells/m^2 of polyclonal autologous MCPyV TAg-specific $CD8^+$ T cells. For example, a patient with a surface area of 2 m^2 would receive no greater than 2 x 10^{10} total polyclonal autologous MCPyV TAg-specific $CD8^+$ T cells. Restaging studies were performed 8 weeks after the first interventions to induce local MHC Class I upregulation. Similar for patients in Group 1, they may receive preferably one, but up to 3 infusions of anti-PD-L1 before the first MHC class I up-regulation intervention. A second attempt will be made to generate MCPyV TAg-specific $CD8^+$ T cells if the initial cell production was unsuccessful and the final cell product will be infused as soon as it becomes available.

If patient has disease progression while on Group 1 alone, treatment may be stopped/interrupted to allow for disease control with other anti-cancer therapies. If T cells are generated and made available in the interim, the patient may resume therapy on Group 2 as long as eligibility criteria are met.

For safety considerations, low-dose SC IL-2 will not be administered after the T-cell infusions due to concurrent anti-PD-L1 treatment. Restaging studies will be performed 8 weeks after interventions to induce local MHC Class I upregulation by tumor tissue. Depending on the continued presence of detectable metastatic disease and if the patient

remains clinically eligible, patients could undergo the same treatment (MHC class I up-regulation, two CTL infusions approximately 28 days apart) during the time anti-PD-L1 infusions were ongoing starting within four weeks of the preceding restaging scans.

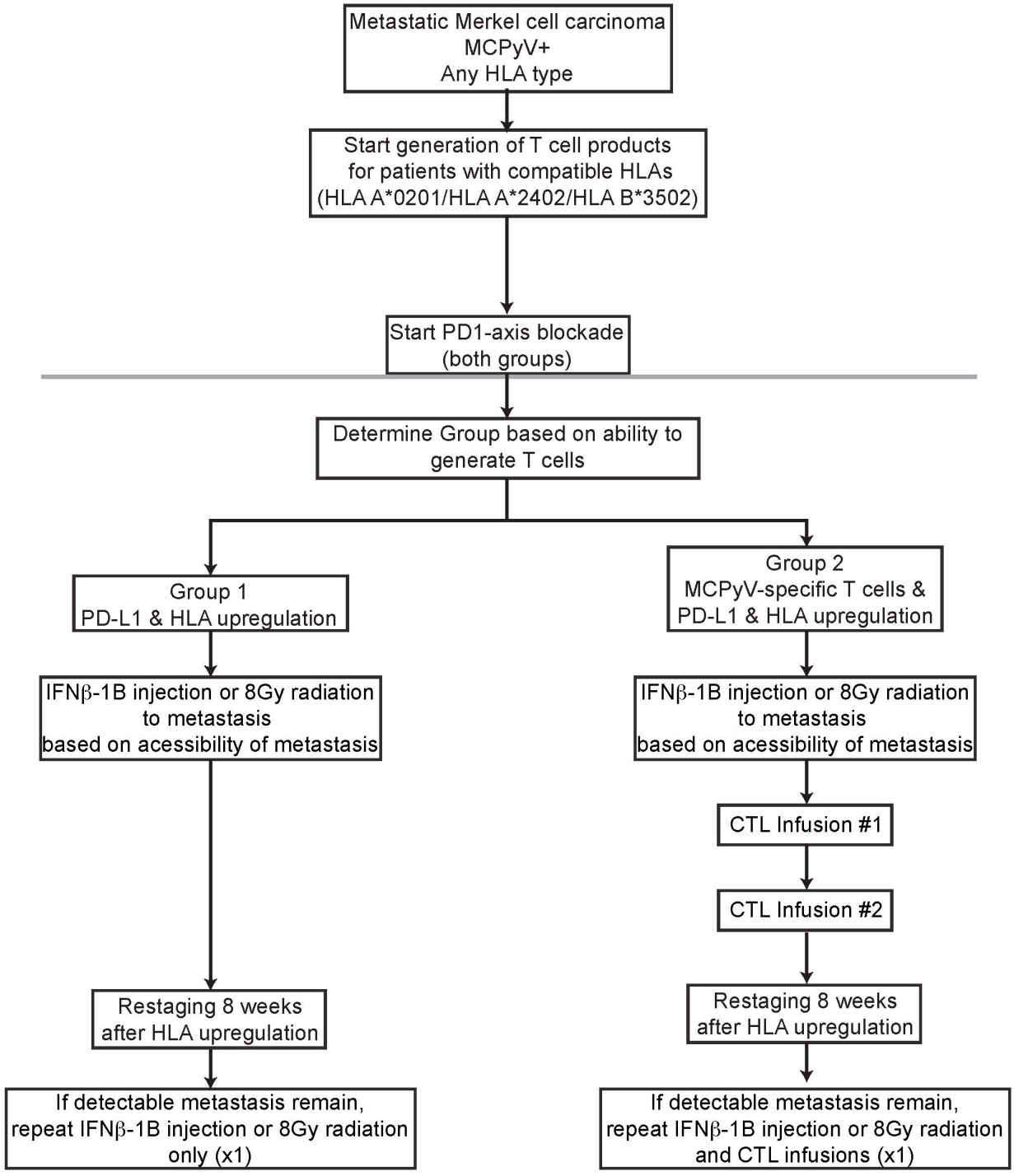


Figure 13. Study decision tree (Groups 1 and 2)

7. ELIGIBILITY FOR TREATMENT

7.A. Inclusion Criteria

1. Signed written informed consent.

2. Confirmation of MCC by internal pathology review of initial or subsequent biopsy or other pathologic material.

3. If an accessible lesion is present, a biopsy will be performed within 6 weeks of the start of study intervention. The results of the biopsy must be obtained prior to initiation of study intervention.

4. Evidence of MCPyV TAg tumor expression by immunohistochemistry on any prior or current tumor specimen or viral oncoprotein antibody confirmation within 6 weeks of the start of study intervention.

5. ECOG PS ≤ 2 at trial entry.

6. 18 years of age or older.

7. Patients must have at least one bi-dimensionally measurable lesion by palpation, clinical exam, or radiographic imaging within 6 weeks of the start of study intervention (X-ray, CT scan, PET scan, MRI, or Ultrasound).

8. For patients designated to be treated on Group 2: cardiac ejection fraction ≥35%. For patients with significant risk factors for coronary artery disease (Framingham risk score > 15%), a cardiac stress test is recommended.

9. At least 3 weeks must have passed since any of the following: systemic corticosteroids, immunotherapy (for example, T-cell infusions, immunomodulatory agents, interleukins, MCC vaccines, intravenous immunoglobulin, expanded polyclonal TIL or LAK therapy), pentoxifylline, other small molecule or chemotherapy cancer treatment, other investigational agents **or other systemic agents that target Merkel cell carcinoma.**

7.B. Exclusion Criteria

1. Known active infections or oral temperature >38.2 C fewer than 72 hours prior to receiving study treatment or systemic infection requiring chronic maintenance or suppressive therapy.

2. CBC profile prior to trial entry:

WBC < 2000/mcl
Hb < 8 g/dL
ANC < 1000/mcl
Platelets < 50,000/mcl

3. New York Heart Association functional class III-IV heart failure, symptomatic pericardial effusion, stable or unstable angina, symptoms of coronary artery disease, congestive heart failure, clinically significant hypotension, or history of an ejection fraction of ≤ 30 % (echocardiogram or MUGA).

4. Clinically significant pulmonary dysfunction, as determined by medical history and physical exam. Patients so identified will undergo pulmonary functions testing and those with FEV1 < 2.0 L or DLco (corr for Hgb) < 50% will be excluded.

5. Creatinine clearance < 30 ml/min which cannot be attributed to MCC metastasis.

6. Total bilirubin > 1.5 x ULN, AST/ALT > 2.5 x ULN. For patients with liver metastases, AST/ALT > 5x ULN

7. Active autoimmune disease (e.g. systemic lupus erythematosus, vasculitis, infiltrating lung disease, inflammatory bowel disease) whose possible progression during treatment would be considered unacceptable by the investigators.

8. Symptomatic and untreated central nervous system (CNS) metastasis. However, patients with 1 to 2 asymptomatic, less than 1 cm brain/CNS metastases without significant edema may be considered for treatment. If sub-centimeter CNS lesions are noted at study entry, then repeat imaging will be performed, if more than 4 weeks have elapsed from the last scan.

9. Any condition or organ toxicity that is deemed by the PI or the attending physician to place the patient at unacceptable risk for treatment on the protocol.

10. Pregnant women, nursing mothers, men or women of reproductive ability who are unwilling to use effective contraception or abstinence. Women of childbearing potential must have a negative pregnancy test within 2 - 6 weeks prior to treatment.

11. Clinically significant and ongoing immune suppression including, but not limited to, systemic immunosuppressive agents such as cyclosporine or corticosteroids, CLL, uncontrolled HIV infection, or solid organ transplantation.

12. Patients may not be on any other treatments for their cancer aside from those included in the protocol. Patients may not undergo another form of treatment concurrently with this study.

13. Known severe hypersensitivity reactions to monoclonal antibodies (Grade ≥ 3 NCI-CTCAE v 4.0), any history of anaphylaxis, or uncontrolled asthma.

14. Vaccination with live inactivated viral strains for the prevention of infectious diseases within 4 weeks of the start of the study treatment. Inactivated influenza vaccines are permitted while on trial.

15. Known alcohol or drug abuse.

16. Legal incapacity or limited legal capacity.

8. CONSENTING

Prior to consenting to this study, patients will have been HLA typed. If they express an HLA type for which T cells can be generated (HLA A*0201, HLA A*2402 or HLA B*3502), they will have undergone a leukapheresis under protocols **2365** (see **Section 10.A.1**)**.** Thus: at the time of the consent, they will already be designated for Group 1 or 2. An enrollment conference will be held with the patient. The PI or a delegated representative will discuss this study and alternative treatments available for metastatic MCC. All known risks and potential hazards of treatment with IFNβ-IB, radiation and PD-L1 and MCPyV-specific $CD8^+$ T polyclonal cells (Group 2) will be discussed. Informed consent will be obtained from the patient using forms approved by the Institutional Review Board (IRB) of the FHCRC. The patient will also be informed that they may withdraw from the study at any time and for any reason without jeopardizing their future treatment. Patients who are designated for treatment on Group 2 will also be informed that they will receive alternative treatment on Group 1 in the unlikely case that MCPyV-specific T-cells cannot be generated for them. In accordance with federal regulations (21 CFR 312), the patient must sign the IRB-approved consent form in the presence of a witness.

9. PROTOCOL REGISTRATION

Patients will be assigned to the protocol by the Clinical Coordinator who will register the patient with the Registration Office, (206) 667-4728, between 8:30 am and 4:00 pm, Monday through Friday. After hours, the Registration Office can be reached by paging (206) 995-7437.

10. STUDY AGENTS

10.A. MCPyV-specific $CD8^+$ Cells

10.A.1. <u>Procedure to obtain PBMC for generation of MCPyV-specific $CD8^+$ cells</u>

Patients will have undergone leukapheresis under FHCRC IRB-approved **Protocol 2365** to obtain PBMC for the generation of antigen-specific T cells in the lab. PBMC

may be cryopreserved under therapy conditions and used to generate T cells for this study.

10.A.2. Generation of MCPyV-specific CD8$^+$ cells

All T cells administered will be autologous antigen-specific T cells targeting MCPyV TAg and derived from the peripheral blood lymphocytes of a patient with an established diagnosis of MCC. Methods employed to generate and qualify product for infusions are outlined in **IND 14279** (Sponsor Dr. Aude Chapuis). T cells demonstrating antigen specificity are further qualified using mycoplasma, fungal, and bacterial sterility testing.

10.A.3. Handling of T-cell products before infusion

For each infusion, the cell product is formulated at the desired cell dose in a final volume of 500 mL. The final product will be prepared and labeled according to standard of operation procedure in the Cellular Processing Facility. The cell product will be transported to the infusion facility by a protocol 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 to the patient over one to two hours (as described in **Section 11.4**).

10.B. Anti PD-L1 Antibody Avelumab

10.B.1. Packaging and labeling

Avelumab is a sterile, clear, and colorless solution intended for IV administration supplied by the Sponsor in single-use glass vials at a concentration of 20 mg/mL closed with a rubber stopper and sealed with an aluminum yellow polypropylene flip off seal. Packaging and labeling will be in accordance with applicable local regulatory requirements and applicable Good Manufacturing Practice (GMP) guidelines. Avelumab will be packed in boxes containing a suitable number of vials. The information on the IMP will be in accordance with approved submission documents. Avelumab will be shipped in transport cool containers (2°C to 8°C) that are monitored with temperature control devices.

10.B.2. Storage, handling, and preparation

The contents of the Avelumab vials are sterile and nonpyrogenic, and do not contain bacteriostatic preservatives. Any spills that occur should be cleaned up using the facility's standard cleanup procedures for biologic products.

Avelumab drug product must be stored at 2°C to 8°C until use, with a temperature log maintained daily. All medication boxes supplied to each trial site must be stored carefully, safely, and separately from other drugs.

Avelumab drug product stored at room temperature (23°C to 27°C) or at elevated temperatures (38°C to 42°C) for extended periods is subject to degradation.

Avelumab must not be frozen. Rough shaking of Avelumab must be avoided.
For application in this trial, Avelumab drug product must be diluted with 0.9% saline solution (sodium chloride injection) supplied in an infusion bag. Detailed information on infusion bags and medical devices to be used for the preparation of the dilutions and subsequent administration will be provided in the MOP.

Avelumab must not be used for any purpose other than the trial. The administration of IMPs to subjects who have not been enrolled into the trial is not covered by the trial insurance.

Any unused portion of the solution should be discarded in biohazard waste disposal with final disposal by accepted local and national standards of incineration.

10.B.3. Avelumab product accountability

The Investigator is responsible for ensuring accountability for IMP, including reconciliation of drugs and maintenance of drug records.

- Upon receipt of IMP, the Investigator (or designee) will check for accurate delivery and acknowledge receipt by signing (or initialing) and dating the documentation provided by the Sponsor and returning it to the Sponsor. A copy will be retained for the Investigator File.
- The dispensing of the IMP will be carefully recorded on the appropriate drug accountability forms provided by the Sponsor and an accurate accounting will be available for verification by the Sponsor's Monitor at each monitoring visit.
- IMP accountability records will include:
  - confirmation of IMP delivery to the trial site;
  - the inventory at the site of IMP provided by the Sponsor and prepared at the site;
  - the use of each dose by each subject;
  - the return to the Sponsor or alternative disposition of unused IMP; and
  - dates, quantities, batch numbers, expiry dates and (for IMP prepared at the site) formulation, as well as the subjects' trial numbers.
- The Investigator should maintain records that adequately document
  - that the subjects were provided the doses specified by the clinical trial protocol/amendment(s); and
  - That all IMP provided by the Sponsor was fully reconciled.

Unused IMP must not be discarded or used for any purpose other than the present trial. Any IMP that has been dispensed to a subject must not be redispensed to a different subject.

The Sponsor's Monitor will periodically collect the IMP accountability forms and will check all returns (both unused and used containers) before arranging for their return to the Sponsor or authorizing their destruction by the trial site.

At the conclusion or termination of this trial, trial site personnel and the Clinical Trial Monitor (CTM) will conduct a final product supply inventory on the Investigational Drug Accountability Forms and all unused containers will be destroyed. Instructions for destruction of product will be provided to the site. The CTM will be supplied with a copy for filing of the Investigational Drug Accountability Forms. This documentation must contain a record of clinical supplies used, unused, and destroyed and shall include information on

- all administered units,
- all unused units,
- all destroyed units (during the trial),
- all destroyed units at the end of the trial,
- date of destruction(s),
- name and signature of the Investigator/pharmacist.

It must be ensured at each trial site that the IMP is not used

- after the expiry date, and
- after the retest date unless the IMP is reanalyzed and its retest date extended.

This is to be closely monitored by the trial monitor.

10.C. Radiation therapy

The goal of the radiation is not to kill tumor cells, although some lesional stabilization/shrinkage is expected in many cases, but to induce the tumor cells to up-regulate previously down-regulated MHC Class I to prime a systemic immune response mediated by the transferred CTL. A suggested regimen based on a large case series as well as preclinical data supporting MHC upregulation effect is 8 Gy dose given in single fraction,[53] however we recognize that there are clinical scenarios in which alternate dosages and/or fractionated regimens may be more appropriate, and in this scenario the treating radiation oncologist may select an alternate regimen for the patient if felt that it is in the best interest of patient safety. Given that radiation is being given for MHC Class I upregulation/antigen presentation, the GCT (gross tumor volume) and the CTV (clinical tumor volume) are the same. For the planning tumor volume (PTV) which accounts for

day-to-day set-up variations, it is recommended a 5-10mm margin be added to the GTV (subject to individualization by the treating Radiation Oncologist). CT-based 3D-conformal planning (e.g. Intensity Modulated Radiation Therapy [IMRT]) will be utilized to minimize dose to the surrounding critical structures. Image Guided Radiotherapy (IGRT) will be utilized to verify the target location at treatment with the planning CT scan. Choice of radiation modality will be at discretion of radiation oncologist. It is recommended that the field size be kept under 10 x 10 x 10 cm. Re-irradiation is allowed although it is recommended to limit re-irradiation to the spinal cord, neural structures and small bowel to no more than 2x single fraction 8Gy radiation unless no appropriate alternatives. Radiation will be completed within 2 to 5 days prior to a T-cell infusion and 7 to 4 days prior to the infusion of the next anti-PD-L1 infusion.

If MHC class I up-regulation is not clinically indicated, (for example tumor site reduction without clearance and/or no discrete tumor metastasis that can be irradiated or infiltrated with intralesional IFNβ-1B) the second set of T cell infusions can proceed without MHC class I up-regulation.

10.D. Intralesional IFNβ-1B Injection

IFNβ-1B (Betaseron, Bayer, Montville NJ; or Extavia, Novartis, East Hanover, NJ) will be used. One vial contains 0.3 mg or approximately 10 x 10^6 IU. The lyophilized contents will be suspended in the provided 1.2 ml sodium chloride 0.54%. 1/3 of the vial (0.1mg or approximately 3.3 x 10^6 UI or 0.1mg) is administered intralesional into an identified lesion after US-guided tumor core biopsy. IFNβ-1B administration will be completed 2 to 5 days prior to a T-cell infusion and 7 to 4 days prior to the infusion of the next anti-PD-L1 infusion.

10.E. Guidelines for selecting radiation therapy or intralesional IFNβ-1B Injections

The decision to proceed to radiation of the lesions or administer intralesional IFNβ-1B will be based on clinical judgment with the following guidelines: 1. If more than one tumor lesions are present at different locations and no critical organ is involved, if feasible, approximately 50% of the lesions should receive radiation or intralesional IFNβ-1B initially; 2. At a given time, one patient should receive either radiation or intralesional IFNβ-1B but not both modalities; 3. Intralesional IFNβ-1B is the preferred modality for patients who have easily palpable superficial lesions (cutaneous, subcutaneous, or lymph nodal metastasis) that can be accurately localized, stabilized by palpation, and are superficial enough to enable intratumoral injections, or patients who have lesions that are attainable through endoscopy; 4. Radiation to a specific lesion or to multiple lesions within a specific field will be administered in the case that multiple radiographically detectable lesions are identified on the pre-treatment scans, lesions are inaccessible by interventional radiology, or the patient refuses intralesional injections. 5. If multiple lesions are present within a tight field, it is recommended not to irradiate some and not others, but rather deliver radiation to the whole field.

11. PLAN OF TREATMENT

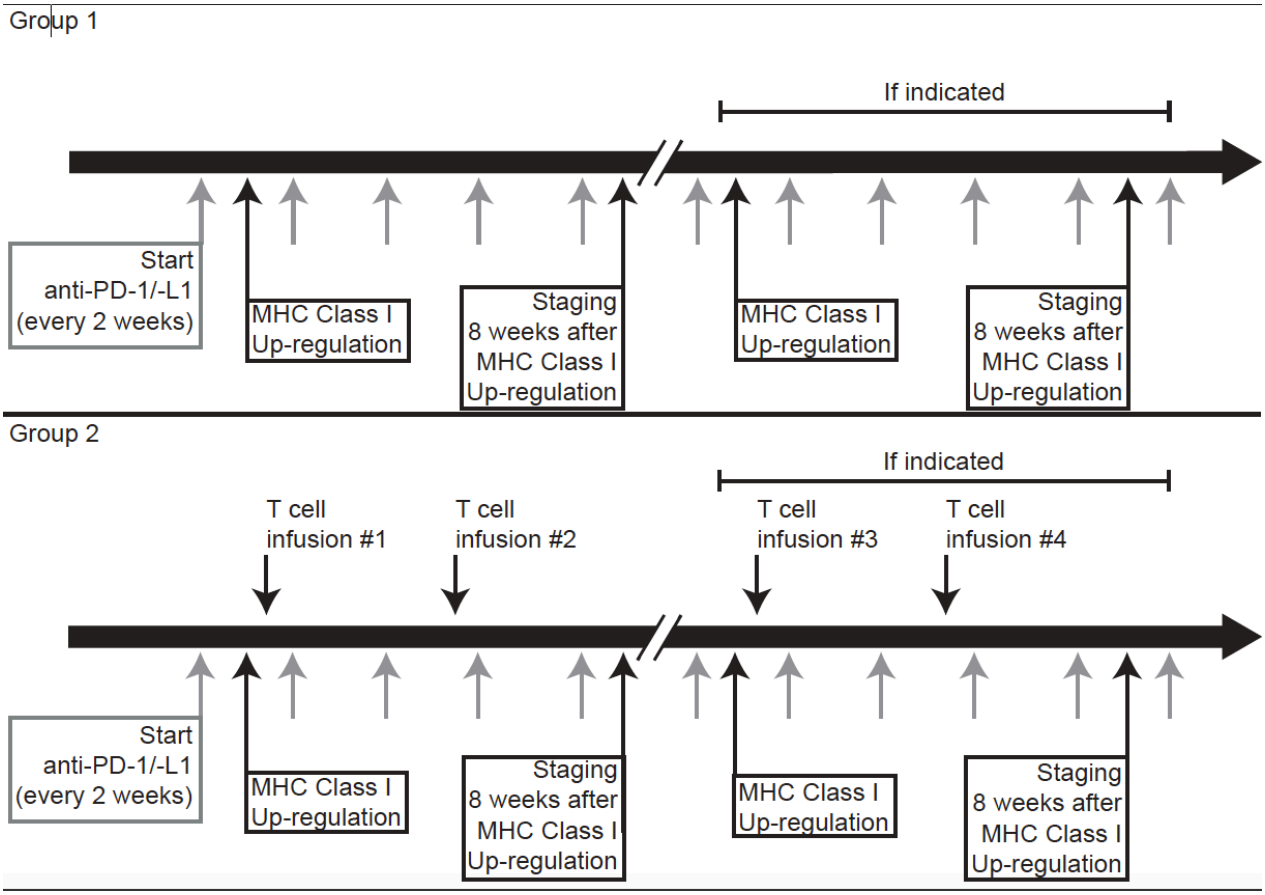


Figure 14. Treatment schema

1. **Initial Evaluation/Consent (all Groups)**
   Patients will be screened for their eligibility on the study and consented during this visit. Baseline anthropometry will be obtained.

2. **Administration of Avelumab (Groups 1 and 2)**
   Subjects will receive an IV infusion of Avelumab within 4 weeks of the baseline staging scans at a dose of 10 mg/kg over the duration of 1 hour (-10/+20 minutes) following pretreatment with H1 blockers (diphenhydramine 12.5mg to 50 mg PO or IV, or equivalent), and acetaminophen po 500 to 650 mg, once every 2 weeks (See **Appendix B)**. The dose of Avelumab will be calculated based on the weight of the subject determined at trial entry. Following Avelumab infusions, subjects will be observed for 1 hour post infusion for potential infusion-related reactions. Subjects will receive Avelumab once every 2 weeks for 12 months or until the criteria in **Section 13.G.** (Off Study Criteria) are met. Modifications of the infusion rate due to infusion-related reactions are described in **Section 13.C**.

In this trial, subjects will receive Avelumab IV infusions at the SCCA. Well trained medical staff will monitor and perform the trial drug administration.

3. **MHC class I up-regulation: Radiation or intralesional IFNβ-IB injection**
   Radiation therapy or intralesional IFNβ-1B injection will be scheduled as an outpatient treatment at the SCCA or the University of Washington Medical Center (UWMC) between 7 and 10 days after the preceding dose of Avelumab (for Groups 1 and 2) and within 2 to 5 days prior to a subsequent T-cell infusion (Group 2).

4. **T-Cell Infusions**
   T-cell infusions will be given at the Seattle Cancer Care Alliance at least 48 hours after MHC class I upregulation and preferably at least 48 hours before the next dose of Avelumab. All patients will be observed for 2 hours post infusion and will be released if no AE requiring prolonged hospitalization have occurred (see **Section 13**).
   T cells will be infused intravenously at a rate of not more than 500 cc/hour through an 18- or 20-gauge catheter inserted into a peripheral vein or through a central catheter. The infusion bag will be gently mixed periodically during the infusion. Subjects will have vital signs per **Section 12.B**.

12. EVALUATION

See **Protocol Appendix B** for a summary of patient evaluation for Groups 1 and 2. The dates listed on the study calendar are approximate as many patients reside out of the area and may not always follow the time points as dictated by the protocol. Results of tests and, or procedures conducted as standard of care may be used for research purposes. Potential patients will initially be identified and recruited through the SCCA MCC clinic. The informed consent form will be discussed and signed in the presence of a GCP-trained investigator. Patients who are found eligible to participate in this protocol will be followed closely by a multidisciplinary oncology team that includes medical oncologists, dermatologists and radiation oncologists (as appropriate).

12.A. Patient Evaluation at Each Planned Visit

Patients will have a history taken, ECOG performance status (Appendix A), a physical exam with vital signs prior to the T-cell infusion and 1, 14, and 28 days after each infusion. Thyroxine 3 (T3), free thyroxine 4 (FT4) and thyroid stimulating hormone (TSH) at baseline, at cycle 6 and then every 6 cycles. Merkel virus oncoprotein serology at baseline and at time of radiographic evaluation. A comprehensive chemistry panel, and a complete blood count at each planned visit including the pre-treatment visit, prior to Avelumab infusion, prior to IFNβ-IB injections or radiation, prior to tissue biopsy or aspiration, prior to the T-cell infusion and 1, 3, 7, 14, and 28 days after each T cell infusion (Appendix B). It is anticipated that some patients reside outside of the area and

may have their +7, +14 and +28 day clinical evaluations completed locally (visits can occur+/- 1 day, weekly visits can occur +/- 3 days before or after the planned date).

12.B. Patient Evaluation During T-Cell Infusions

Blood pressure, heart rate, temperature, respiratory rate, and O_2 saturation (by pulse oximetry) will be recorded at time 0, every 15 minutes during the 1 to 2 hour T-cell infusion, end of infusion, hourly for 2 hours following the T-cell infusion. Adverse events will be managed by standard medical practice (see also **Section 13**).

12.C. Clinical and Laboratory Evaluation for Toxicity (General Toxicity Assessment)

The period of monitoring for treatment-related toxicity will start with the first Avelumab infusion. All grade ≥ 3 CTCAE v.4.03 AEs will be recorded up to the last T cell or avelumab infusion. Beginning 4 weeks after the last study infusion, only study related toxicities will be collected per section 15. To evaluate for potential toxicities, patients will undergo a history and physical and, or laboratory evaluations (comprehensive chemistry panel and complete blood count) immediately prior to Avelumab infusion, prior to radiation therapy or intralesional IFNβ-1B injection, immediately prior to infusions, days +1, +3, +7, +14, and +28 after each T cell infusion, and then as clinically indicated after the last infusion (for example, every 3 months for the first year, every 6 months for a year and then yearly see **Appendix B**). Clinical assessments at days +7, +14 and +28 after T-cell infusions may be performed by the patient's local provider and records assessed by the investigator within 14 days for any toxicities not previously assessed.

12.D. Efficacy Assessment

12.D.1. Clinical response by 'median time to new metastasis' (primary endpoint)

All patients treated on this protocol will have developed metastatic disease. It is plausible that up-regulation of MHC class I with either radiation therapy or intralesional IFNβ-1B injection may have an independent effect on the targeted metastasis (as assessed by RECIST criteria). It is unlikely however, that localized radiation or intralesional interferon will have a significant effect on the risk of distant disease development. In contrast, T-cell infusions and/or anti-PD-L1 have the potential to generate a broader systemic effect by preventing the occurrence of new distant metastasis. To assess the potential systemic effects of adoptively transferred T cells, the time for a new metastasis to develop after treatment will be assessed, and this parameter will be compared to historical matched controls undergoing standard therapy. A preliminary assessment was carried out on 49 of the MCC patients in our cohort who originally presented with local or regional disease and later developed metastatic disease, at which time they underwent standard therapy (radiation or chemotherapy). Patients with systemic immune suppression were eliminated from the analysis as they would not be candidates for the combined MHC and T-cell therapy trial. Among these patients, the median time to developing a new metastasis was 200 days (see **Section**

18 for further statistical details). Patients will be followed and restaged at least once every 3 months and as clinically indicated after 12 months since the last infusion has elapsed until new metastasis occur (see **Appendix B**).

12.D.2 Clinical response by RECIST criteria (secondary endpoint)

Tumor lesions and tumor responses will be assessed according to the **Response Evaluation Criteria in Solid Tumors (RECIST) *v* 1.1**. Radiographic imaging and clinical assessment of residual disease will be compared with pre-infusion assessment. Responses will be reported separately for lesions which did or did not received MHC Class I upregulation interventions, if the latter are present. Overall responses will be reported as well.

Clinical response assessment of each individual lesions: Response assessment will be done through clinical (for example bi-directional measurements of palpable lesions) or radiographic (for example CT, PET, or MRI) measurement of each lesion at 8 weeks after the first intervention to induce MHC class I upregulation, 8 weeks after the second MHC class I upregulation, if the patient undergoes a second set of T cell infusions, and then at least once every 3 months (12 weeks) or as clinically indicated. Consistency of imaging modality over time is recommended for a given patient. Objective responses in lesions which received MHC Class I upregulation and lesions which did not receive MHC Class I upregulation, will be reported separately. For each individual lesion or group of lesions, a complete response (CR) will be defined as total regression of the lesions, a PR as 30% or greater decrease in the sum of the longest diameter of the lesions compared to baseline and PD as 20% increase in the sum of the longest diameter of the lesions compared to the smallest prior diameter (RECIST v1.1 criteria).

Overall Response Assessment:
A complete response (CR) will be defined as total regression of all tumors, a PR as 30% or greater decrease in the sum of the longest diameter of target lesions compared to baseline and PD as 20% increase in the sum of the longest diameter of target lesions compared to the smallest prior diameter (RECIST v1.1 criteria). This assessment will be performed 8 weeks after the first MHC class I upregulation, 8 weeks after the second MHC class I upregulation, if the patient undergoes a second set of T cell infusions, and then at least once every 3 months (12 weeks) or as clinically indicated.

12.E. Evaluation of Persistence and Function of Adoptively Transferred T Cells

12.E.1. Blood samples

Patients will have 50-60 ml of heparinized blood (green top) and 6 ml of serum (red top) tube drawn during the pre-treatment visit, prior to Avelumab infusion, prior to receiving radiation or intralesional IFNβ-1B injections (pre-treatment baseline), prior to tissue biopsy or aspiration, prior to infusion, and at selected time points following infusion (**Appendix B**, research labs). Samples will be used to evaluate the duration of *in vivo* persistence and function of infused T cells, as well as evaluation of MCPyV TAg titers.

All blood samples will be kept at room temperature and sent to room E1-305, FHCRC Research Cell Bank.

12.E.2. Tumor samples

Only patients with histologically confirmed MCC will be enrolled on the study. It is anticipated that most patients will undergo a confirmatory biopsy of metastatic lesions; although this may not be possible for patients with a high clinical likelihood of metastatic Merkel carcinoma and lesion(s) that are difficult to access. Patients undergoing intralesional IFNβ-1B injections will have an aspirate or biopsy prior to the injections. When obtained, the aspirates or biopsies will serve as a pre-treatment baseline for the presence of MCPyV TAg-specific cells. Localization of transferred MCPyV TAg-specific CTL to tumor sites after treatment will be evaluated in selected patients with surgically accessible disease (disease that is accessible by needle or core biopsy, or that can be excised from skin or superficial lymph node).
Post study treatment biopsy will be done at approximately 4 weeks after the first Avelumab infusion (group 1) and two weeks after the first T cell infusion (group 2). If accessible a 2nd post treatment biopsy may be performed if patients undergo another cycle of MHC class I up-regulation and CTL infusions. If enough material is obtained, single cell suspensions will be prepared and stained to identify antigen-specific CTL by tetramer analysis. Alternatively, if limited material is obtained, DNA will be isolated from samples and used for high throughput TCR analysis. All results will be compared to pre-treatment peripheral blood and, where possible, to pre-infusion tumor samples.

12.E.3. Assessment of the Functional Capacity of Transferred Cells

To evaluate the direct *ex vivo* function of the transferred cells, where possible, tetramer^{+} cells within collected PBMC will be evaluated for production of intracellular cytokines including IFN?, TNFα and IL-2 in response to cognate antigen using an intracellular cytokine assay (ICS).

Intranuclear Ki-67 expression will be assessed on infused tetramer^{+} T cells to evaluate the *in vivo* proliferation of the transferred cells. To evaluate the *ex vivo* proliferative capacity of infused cells compared to the infusion product, carboxyfluorescein succinimidyl ester (CFSE) dye dilution in response to peptide stimulation will be used.

The phenotype of tetramer^{+} antigen-specific cells in the peripheral blood will also be assessed using established immunophenotyping panels including assessment of CD45RO (memory T cells), CD28, CD27, CD127, CD62L and CCR7 (central memory T cells), CTLA-4 (CD152), PD-1, Tim-3, and CD57 (evidence of negative regulation or senescence).

The persistence, function, proliferative capacity and phenotype of infused T cells will be correlated to clinical responses.

12.F. Assessment of Epitope Spreading

Quantification of the overall recognition of the MCPyV T-antigen for each patient will likely be performed by testing the reactivity of whole PBMC before and at indicated timepoints after treatment to peptides 15 amino acids (aa) in length offset by 5 aa bases spanning the whole T-antigen protein to include both CD8 and CD4 responses regardless of the HLA type of the patient. The reactivity will be detected using IFNγ secretion in a human IFNγ ELISpot assay.[76] Results will be presented as the number of spot forming cells/10^5 PBMCs.

13. MANAGEMENT OF TOXICITIES AND COMPLICATIONS

13.A. Toxicity Grading

Toxicity grading will be evaluated according to the current guidelines in NCI Common Toxicity Criteria version 4.0.[77] The full text of the NCI CTCAE is available online at: http://evs.nci.nih.gov/ftp1/CTCAE/About.html.

13.B. Regimen Related Toxicity

If the patient develops excessive Toxicity attributable to one or more component of the regimen, the patient will not receive additional study treatment and a treatment of corticosteroids will be given, if clinically indicated (please refer to **Section 13.C**). Excessive toxicity will be considered when a non-pre-existing Grade 3 or higher toxicity develops after the start of treatment with the following exceptions:

13.B.1. Expected toxicities attributable to **anti-PD-L1 (Avelumab) infusions** and considered exceptions to criteria for discontinuation include:

1. Infusion-related reactions (see **Section 13.C** for management):
    i. Transient (≤ 6 hours) Grade 3 CRS, flu-like symptoms or fever, which is controlled with medical management.
    ii. Transient (≤ 24 hours) Grade 3 fatigue, local reactions, headaches, nausea, emesis that resolved to ≤ Grade 1.
    iii. Single laboratory values out of normal (excluding Grade ≥ 3 liver function tests increase) that are unlikely related to Avelumab according to the investigator, do not have a clinical correlate, and resolve to ≤ Grade 1 within 7 days with adequate medical management.
    iv. Urticaria lesions responding to antihistaminics (Grade 2)
    v. Allergic reaction (flushing or rash) responding to antihistaminic treatment (Grade 2).

2. Tumor flare phenomenon defined as local pain, irritation, or rash localized at sites of known or suspected tumor.

3. Tumor-lysis syndrome: If symptoms of tumor lysis occur after the Avelumab infusions they will be managed according to best clinical practice (see **Section 13.C.4**). Tumor lysis will not constitute a reason to discontinue the Avelumab infusions.

4. Change in ECOG PS to ≥ 3 that resolved to ≤ 2 within 14 days (infusions should not be given on the following cycle if the ECOG PS is ≥3 on the day of the Avelumab administration.

13.B.2. Expected toxicities attributable to **localized radiation** of pre-existing symptomatic metastasis and considered exceptions to criteria for discontinuation include:

i. Non-life-threatening - diarrhea (i.e. 4-6 stools over baseline stools a day)
ii. Target organ inflammatory responses resulting in Grade 3 or 4 toxicities that return to baseline within 4 weeks. For example: elevated lipase and/or amylase after radiation to a pancreatic lesion; or elevated transaminases after radiation to a liver lesion.
iii. Hyperglycemia (Grade 3) lasting <14 days.
iv. Dry skin, skin pain, and erythema lasting <14 days.
v. Single laboratory values out of normal that according to the investigator do not have a clinical correlate and resolve to ≤ Grade 1 within 7 days with adequate medical management.

13.B.3. Expected toxicities attributable to **intralesional IFNβ-1B injections** and considered exceptions to criteria for discontinuation include:

i. Non-life-threatening infections (i.e. absence of hemodynamic compromise requiring pressor support and non-opportunistic infection).
ii. CRS Grade 3 or less, including but not limited to asthenia, flu-like symptoms, myalgia, lymphopenia, and rigors.
iii. Target organ inflammatory responses resulting in Grade 3 or 4 toxicities that resolve within 4 weeks. For example: elevated lipase and/or amylase after injection to a pancreatic lesion; or elevated transaminases after injection to a liver lesion.
iv. Single laboratory values out of normal that according to the investigator do not have a clinical correlate and resolve to ≤ Grade 1 within 7 days with adequate medical management.

13.B.4. Expected toxicities attributable to **T-cell infusions** and considered exceptions to criteria for discontinuation include:

i. CRS Grade 3 or less, including but not limited to asthenia, flu-like symptoms, myalgia, lymphopenia, and rigors.
ii. Skin rash or erythroderma (Grade 3 toxicity) lasting for <7 days.

iii. Hypoxemia requiring continuous oxygen <72 hours, but not requiring mechanical ventilation or intubation.
iv. Fever that resolves within 36 hours of the T-cell infusion.
v. Lymphopenia (lymphocytes <500) that resolves to baseline levels (pre-therapy) within 4 weeks.
vi. Single laboratory values out of normal that according to the investigator do not have a clinical correlate and resolve to ≤ Grade 1 within 7 days with adequate medical management.

13.C. Management of Symptoms During Avelumab Infusions

13.C.1. Special precautions

As infusion reactions have been observed with Avelumab (see **Section 3.J.3**), 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 each Avelumab infusion to mitigate infusion-related reactions including hypersensitivity reactions. As a routine precaution, subjects enrolled in this trial must also be observed for 1 hour post infusion, in an area with resuscitation equipment and emergency agents.

Infusion of Avelumab 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 according to the NCI are as outlined in **Sections 13.C.2**, **13.C.3,** and **13.C.4** below.

13.C.2. Infusion-related reactions

A. Symptoms
- Fever
- Chills
- Rigors
- Diaphoresis
- Headache

B. Management

Table 5. Treatment modification for symptoms of infusion-related reactions caused by Avelumab

NCI-CTCAE Grade	Treatment Modification for Avelumab
Grade 1 – mild Mild transient reaction; infusion interruption not indicated; intervention not indicated.	Decrease the Avelumab infusion rate by 50% and monitor closely for any worsening. The total infusion time for Avelumab 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 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 infusion immediately and disconnect infusion tubing from the subject. Subjects must be withdrawn immediately from Avelumab treatment and must not receive any further Avelumab treatment.

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

Once the Avelumab 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 subject has a second infusion-related reaction of Grade ≥ 2 on the slower infusion rate, the infusion should be stopped and the subject should be removed from Avelumab treatment. If a subject experiences a Grade 3 or 4 infusion-related reaction at any time, the subject must discontinue Avelumab.

13.C.3. Management of severe hypersensitivity reactions and flu-like symptoms due to Avelumab infusions

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

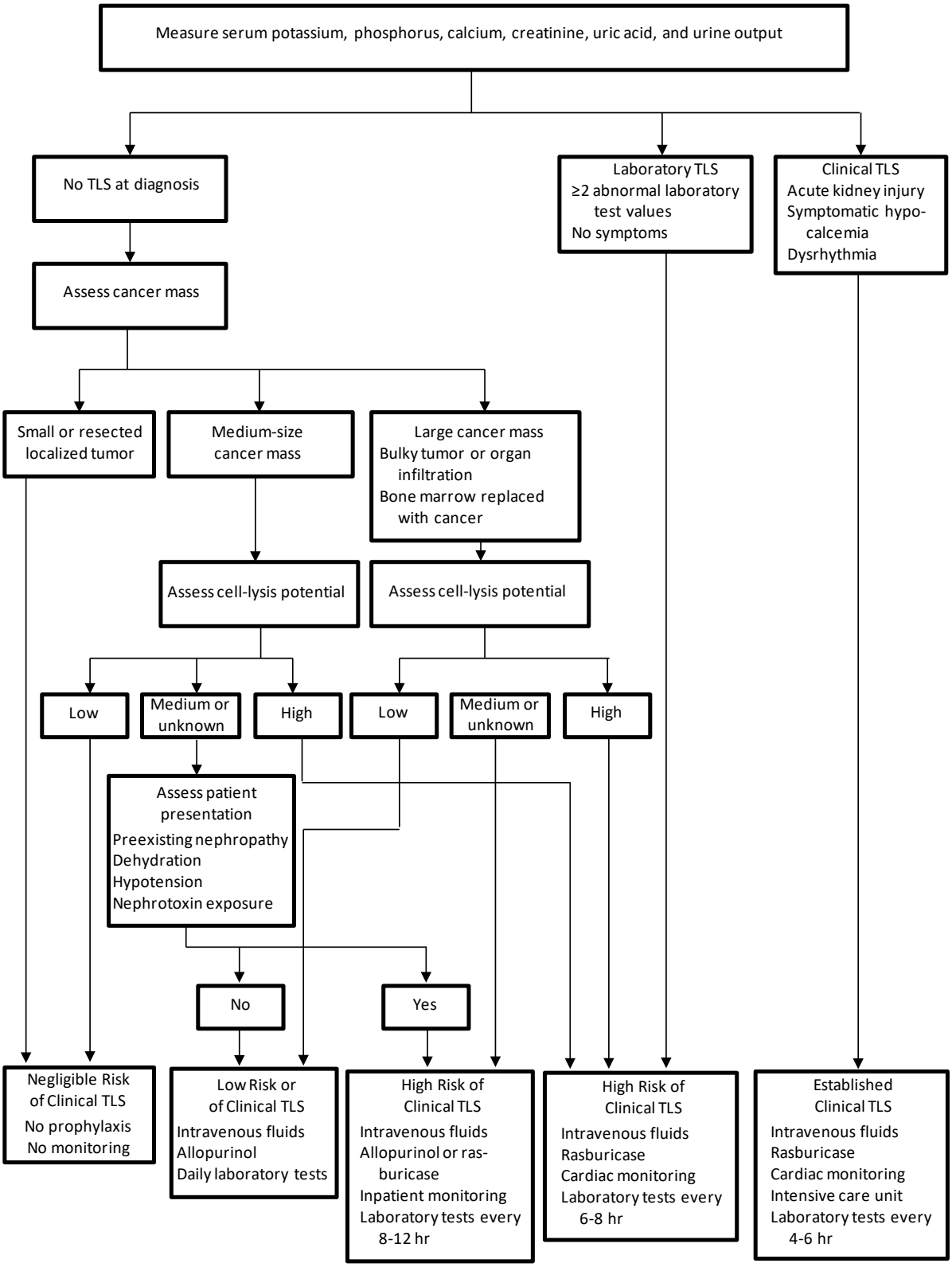
A. Symptoms
- Impaired airway
- Decreased oxygen saturation (< 92%)
- Confusion
- Lethargy
- Hypotension
- Pale / clammy skin
- Cyanosis

B. Management
1. Epinephrine injection and dexamethasone infusion
2. Patient should be placed on monitor immediately
3. Alert ICU for possible transfer if required

For prophylaxis of flu-like symptoms, 25 mg of indomethacin or comparable NSAID dose (for example, ibuprofen 600 mg, naproxen sodium 500 mg) may be administered 2 hours before and 8 hours after the start of each dose of Avelumab IV infusion. Alternative treatments for fever (for example, paracetamol) may be given to subjects at the discretion of the Investigator.

13.C.4. Tumor lysis syndrome

In addition, since Avelumab can induce antibody-dependent cell-mediated cytotoxicity (ADCC), there is a potential risk of tumor lysis syndrome. Should this occur, subjects should be treated per the local guidelines and the management algorithm below (**Figure 135**).[78]



Definitions: TLS=tumor lysis syndrome

Figure 135. Assessment and initial management of tumor lysis syndrome

13.C.5. Delayed toxicities and immune-related adverse events (irAE) secondary to

Avelumab

Patients will also be closely monitored for potential irAEs,[79,80] 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). IrAEs will be managed according to **Section 13.E** with steroids.

Suggested management for Grade 2 adverse drug reactions (ADR):

- If a Grade 2 ADR resolves to Grade ≤ 1 by the last day of the current cycle, treatment may continue.
- If a Grade 2 ADR does not resolve to Grade ≤ 1 by the last day of the current cycle, infusions should not be given on the following cycle. If at the end of the following cycle the event has not resolved to Grade 1, the subject should permanently discontinue treatment with a Avelumab ADR (except for hormone insufficiencies, that can be managed by replacement therapy; for these hormone insufficiencies, up to 2 subsequent doses may be omitted).
- Upon the second occurrence of the same Grade 2 ADR (except for hormone insufficiencies that can be managed by replacement therapy) in the same subject, treatment with Avelumab must be permanently discontinued.

13.D. Management of Symptoms During T-Cell Infusion

Immediate reaction to infusion (i.e. defined as those occurring either during the first 24 hours following T-cell infusion) may occur due to release of cytokines from T cells stimulated by the recognition of targets. Mild transient symptoms have been observed with lymphokine-activated killer cell (LAK) and TIL cell infusions, and with infusions of antigen-specific T-cell clones (**FHCRC Protocol 2140**).

13.D.1. Milder reactions (i.e. <Grade 3 CTCAE v.4 or less severe than specified below) would include symptoms such as:

- Fever, chills, fatigue.
- Dyspnea, chest tightness, or myalgia,
- Alteration in vital signs such as:
  - Lowering of blood pressure, but with systolic BP ≥ 90 mm Hg, or ≤ 20 mm Hg below baseline.
  - Tachycardia, but with HR ≤ 130 or ≤ 30 above baseline.
  - Tachypnea, but with RR ≤ 32/min or ≤ 10 above baseline.
  - Hypoxemia, but O_2 saturation ≥ 88% on room air, or ≤ 5% fall from baseline.
  - Skin changes such as erythema, urticaria, or other rash.

Management will be by **decreasing the rate of infusion and/or appropriate supportive care** such as:

- Acetaminophen or Demerol for fever and chills. (All subjects who develop fever or chills should have a blood culture drawn).
- Acetaminophen for headache.
- Diphenhydramine for nausea and vomiting.
- Fluid administration for hypotension.
- Supplemental oxygen for hypoxemia.

13.D.2. More severe reactions occurring during the 1-2 hour infusion would include symptoms such as:

- Hypotension with systolic BP <90 mm Hg and >20 mm Hg below baseline
- Tachycardia with HR >130 and >30 above baseline.
- Tachypnea with RR >32 and >10 above baseline.
- Hypoxemia with O_2 saturation of <88% and >5% fall from baseline.

Management will be by **terminating the infusion and administering supportive medical care**.

- If patient responds to supportive care by normalization of vital signs or resolution of hypoxemia and PI deems it safe to continue, the infusion will be restarted at slower rate.
- If the patient does not respond by normalization of vital signs or hypoxemia after supportive care alone, **methylprednisolone to ablate the infused T cells will be administered as per Section 13.E**.

13.D.3. Any unexpected severe toxicity (see Section 13.B) occurring in the first 24 hours (due to the T-cell infusion and not attributable to a non-infusion-related cause).

Management will be by **supportive medical care and methylprednisolone will be administered as per Section 13.E**.

13.D.4. Delayed toxicities secondary to T-cell infusions

Although unlikely, it remains possible that the infusion of MCPyV TAg-specific T cells will result in delayed toxicities related to the recognition of tumor tissue. It is anticipated that the symptoms and signs will occur within hours to four weeks after completion of the T-cell infusion.

Any toxicities requiring treatment discontinuation (see **Section 13.B) occurring within 4 weeks of study treatment** (potentially due to the T-cell infusion and not attributable to a non-infusion-related cause) **will be managed by supportive care and MP will be administered as per Section 13.E**.

13.E. Management of Severe Treatment-related Toxicities with Corticosteroids

i. The patient will receive corticosteroids if treatment-related toxicity warranting ablation of T cells is observed.
ii. The patient will be hospitalized for the first 48 hours for monitoring. The following dose schedule is recommended:

Day 1	Intravenous Methylprednisolone at 2 mg/kg
Day 2	Intravenous Methylprednisolone at 2 mg/kg
Day 3-4	Prednisone at 30 mg po b.i.d.
Day 5-6	Prednisone at 15 mg po b.i.d.
Day 7-8	Prednisone at 10 mg po b.i.d.
Day 9-10	Prednisone at 10 mg po q.d
Day11-12	Prednisone at 5 mg po q.d.

iii. The *in vivo* frequency of infused $CD8^+$ T cells will be assayed immediately prior to and between 48 and 72 hours after the start of steroid therapy.

13.F. Concomitant Therapy

13.F.1. As with all monoclonal antibody therapies, Avelumab carries a risk of allergic reaction. Avelumab will be administered at the SCCA in a setting that allows for immediate access and administration of therapy for severe allergic or hypersensitivity reactions, such as the ability to implement immediate resuscitation measures. Steroids (dexamethasone 10 mg), epinephrine (1:1000 dilution), allergy medications (antihistamines), or equivalents are available for immediate access. If hypersensitivity reaction occurs, the subject must be treated according to the best available medical practice.

13.F.2. Subjects will be pretreated with H1 blockers (diphenhydramine 25-50 mg PO or IV, or equivalent), and acetaminophen 500 to 650 mg (orally), 30 to 60 minutes prior to each Avelumab and T-cell infusion.

13.F.3. Any medications (other than those excluded by the clinical trial protocol) that are considered necessary for the subjects' welfare and will not interfere with the IMP may be given at the Investigator's discretion. For example, active infections occurring after initiating the study should be treated according to the standard of care. Other agents might include analgesics, antinausea medications, antihistamines, diuretics, anti-anxiety medications, and medication for pain management, including narcotic agents.

13.F.4. The following agents are not allowed while on study:

- Systemic corticosteroids (except as outlined for management of toxicity of Avelumab and non-transduced CTL)
- Immunotherapy (for example, interleukins, MCC vaccines, intravenous immunoglobulin, expanded polyclonal TIL or LAK therapy)
- Pentoxifylline, other small-molecule or chemotherapy cancer treatment
- Other investigational agents
- Any vaccine therapies for the prevention of infectious disease (for example, seasonal flu vaccine, human papilloma virus vaccine) except administration of the inactive influenza vaccine
- Growth factors (granulocyte colony stimulating factor or granulocyte macrophage colony stimulating factor). Exception: erythropoietin and darbepoietin alpha may be prescribed at the Investigator's discretion
- Bisphosphonate treatment is not allowed unless it has been initiated more than 14 days prior to receiving the first administration of Avelumab

13.G. Off-study Criteria

A patient's participation on the protocol will be terminated for any of the reasons listed below:

- Progressive disease or development of new metastasis requiring urgent additional drugs (for example chemotherapy)
- The participant withdraws consent
- Patient death
- Occurrence of pregnancy
- Participation in another 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 and / or avelumab infusions are not in the best interest of the patient
- Occurrence of any non-pre-existing AEs / repetitive Grade 2 AEDRs that are deemed related to the treatment as defined in **Section 13.C.2.**

- Use of a non-permitted concomitant drug such as steroids, or as defined in **Section 13.F.4** for which the predefined consequence is withdrawal from the trial
- 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

14. TARGETED/PLANNED ENROLLMENT

TARGETED/PLANNED ENROLLMENT: 20 Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	0	0	0
Not Hispanic or Latino	9	11	20
Ethnic Category: Total of All Subjects *	9	11	20
Racial Categories			
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	9	11	20
Racial Categories: Total of All Subjects *	9	11	20

* The “Ethnic Category: Total of All Subjects” must be equal to the “Racial Categories: Total of All”

All racial groups and ethnicities will be included. **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.**

<u>Sex/Gender</u>	
Males	60%
Females	40%
<u>Ethnicity</u>	
Hispanic/Latino	3.2%
Not Hispanic/Latino	96.8%
<u>Race</u>	
American Indian/Alaska Native	0.18%
Asian	1.4%
Native Hawaiian/Pacific Islander	0.98%
Black/African American	0.84%
White	96.6%

15. GUIDELINES FOR ADVERSE EVENTS REPORTING

15.A. Reporting of Adverse Events

All unexpected and serious AEs which may be due to study treatment or intervention must be reported to the FHCRC Institutional Review Office (IRO) per their current reporting requirements.

All grade ≥ 3 CTCAE v.4 AEs will be collected through 4 weeks after the last T-cell infusion and per section 12.C. Beginning 4 weeks after the last study infusion, only study related toxicities will be collected.

All SAEs that are unexpected and related to study treatment will be reported to the FDA on a MedWatch 3500 reporting form that will be submitted to the FDA within 15 calendar days. The reports will include the date and time of onset, severity and duration of the event, the relationship to study treatment, the treatment given, and the eventual outcome.

15.B. Definitions

Definitions associated with reportable events can be found on the FHCRC IRO extranet website. (Relevant FHCRC policies include, but are not limited the documents listed [**Table 6**]. Please also refer to the FHCRC IRO website.)

Table 6. FHCRC IRB policies for reportable events

IRB Policy 2.6	Adverse Events and Other Unanticipated Problems Involving Risks to Subjects or Others	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.9	Noncompliance with the Office of the Director's Human Research Protection Program Policy	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.1	Reporting Obligations for Principal Investigators	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html
IRB Policy 2.2	Continuing Review	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html
IRB Policy 1.13	Investigational New Drugs (IND), Biologics and Investigational Device Exemptions (IDE)	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html

16. DATA AND SAFETY MONITORING PLAN

16.A. Primary Monitoring

The PI of the study will have primary responsibility for ensuring that the protocol is conducted as approved by the Scientific Review Committee and IRB. The PI will ensure that the monitoring plan is followed, that all data required for oversight of monitoring are accurately reported to the Protocol Office and Data Safety Monitoring Board (DSMB), that all AEs are reported according to protocol guidelines, and that any AEs reflecting patient safety concerns are appropriately reported.

16.B. Monitoring Plan

The PI, or a co-investigator on the study designated by the PI, will personally review with the Study Nurse the clinical course of all MCPyV TAg-specific T-cell infusions. The PI or his/her designee will review with the Study Nurse the progress of each patient undergoing therapy as well as the clinical course of all patients who have completed a course of Tcell therapy.

16.C. Monitoring the Progress of the Trial and the Safety of Participants

The FHCRC PI is responsible for monitoring this clinical trial, with oversight by a DSMB, the Protocol and Data Monitoring Committee (PDMC) at the FHCRC, and the FHCRC IRB. This is a phase I/II study and the assessment of risk is considered above minimal.

A DSMB will be in place and will meet after four patients on Group 2 have received treatment (or sooner if requested) to review the data as it relates to AEs and response to treatment. 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 III or greater NIH CTC v4.0 toxicities and SAEs and determine whether the study should be prematurely discontinued due to excessive toxicity consistent with **Section 13.B**. Ad hoc meetings may be scheduled as needed.

A report from the DSMB will be submitted to the Coordinating Center IRB (FHCRC) and the PI. The DSMB will discontinue the review of outcomes when all subjects on this trial have completed all protocol-specified follow-up. The DSMB for this study will be composed of Drs. Scott Tykodi, Barry Storer, and Michi Shinohara (**Table 7**).

Table 7. DSMB Members

Name	Affiliation	Position
Dr. Scott Tykodi	FHCRC	DSMB Chair
Dr. Barry Storer	FHCRC	DSMB Biostatistician
Dr. Michi Shinohara	University of Washington	DSMB Clinical Investigator

All members have experience in the management of patients with skin cancers and in the conduct and monitoring of clinical trials. Any DSMB member who has or develops a significant conflict of interest will be required to resign from the DSMB. DSMB membership is for the duration of the clinical trial. If any members leave the DSMB during the course of the trial, the PI will promptly appoint their replacement.

A monitor will be retained to monitor study progress. The scope of monitoring will be based on the FHCRC/UW Data and Safety Monitoring Plan (DSMP): http://www.cancerconsortium.org/rto/prr/DSMPlan.pdf. Per the DSMP, an initial monitoring visit is expected after the first 4 patients have received treatment with 100% of records verification. Monitoring reports will be forwarded to the DSMB, the IND sponsor, and the PI at FHCRC.

Content of Reports for the DSMB:

- Study number and title. Brief summary of the study design
- Protocol amendments
- Status of accrual. If accrual is slower than expected, a plan for increasing enrollment
- Compliance
- Analyses of primary and secondary endpoints
- Analyses of AEs and overall safety data. A listing of SAEs by subject and by body system
- Analysis of lab values

DSMB Meeting Minutes

The CTSO will provide staff to assist with minutes for the DSMB meeting. The FHCRC IRB and Regulatory Affairs Manager will be included on the distribution list. At the time of IRB annual renewal, DSMB minutes will be required, if not already provided.

Statistical Monitoring Guidelines

The DSMB will review all Grade 3 or greater toxicities as defined by version 4.0 of NCI Common Toxicity Criteria and determine whether the study should be prematurely discontinued due to toxicity. Toxicity grading will be evaluated by the clinical investigators. Criteria for discontinuing of therapy in an individual patient are described in protocol section titled “Management of Toxicities and Complications”. Criteria for discontinuing the trial is described in section titled “Data and Safety Monitoring”.

The type and grade of toxicities noted during therapy will be summarized for each dose level. All AEs noted by the investigator will be tabulated according to the affected body system. Descriptive statistics will be used to summarize changes from baseline in clinical laboratory parameters. Tumor responses will be determined as specified above.

17. RECORDS

The Clinical Research Division at the FHCRC maintains a patient database that allows for the storage and retrieval of specific types of patient data including demographic

information, protocol registration information, and data from the treatment course. These data are collected from a wide variety of sources and conform to institutionally established guidelines for coding, collection, key entry, and verification. Each patient will be assigned a unique patient number (UPN) to assure patient confidentiality. Any publication or presentation will refer to patients by this number and not by name. Information about patients enrolled on this protocol that is forwarded to agencies such as the FHCRC IRB, EMD Serono, Inc., NIH, and FDA will refer to the patients only by their UPN.

Original inpatient and outpatient medical records will be maintained by the medical records departments at the institutions where the patients receive their care. The majority of their care related to this protocol will be received at the SCCA and UW Medical Center. The study nurse and/or data coordinator will maintain a Case Report Form (CRF) Notebook for each patient treated on this protocol. The CRF notebooks and their contents will be identified by the patient's initials and UPN only. All supporting documents used to verify the accuracy of the data in the case report forms will be kept separately. Patient research files will be kept in a locked, controlled-access building. At least monthly, the PI or a designated co-investigator will review and cross check the data entered on the case report forms with the source documents.

18. STATISTICAL CONSIDERATIONS

The **primary objective** of this trial is to assess and compare the safety and potential efficacy associated with treating patients who developed metastatic MCC by combined MHC up-regulation and anti-PD-L1 antibody treatment (Group 1); and combined MHC up-regulation, anti-PD-L1 antibody treatment, and adoptive transfer of MCPyV TAg-specific polyclonal autologous $CD8^+$ T cells (Group 2). The treatment for each group will be considered to have an acceptable safety profile, if the observed toxicity rate is consistent with a true rate that does not exceed 30%, whereas toxicity is defined in **Section 13**. The potential efficacy of treatment within each group will be assessed primarily by examining the time from first to second metastasis. Among 49 patients who would be considered eligible for this trial and who received standard treatment (localized radiation or chemotherapy) at UW, the median time to second metastasis was an estimated 200 days (**Figure 146**). This is the benchmark that we shall use in the current trial for assessment of potential efficacy.

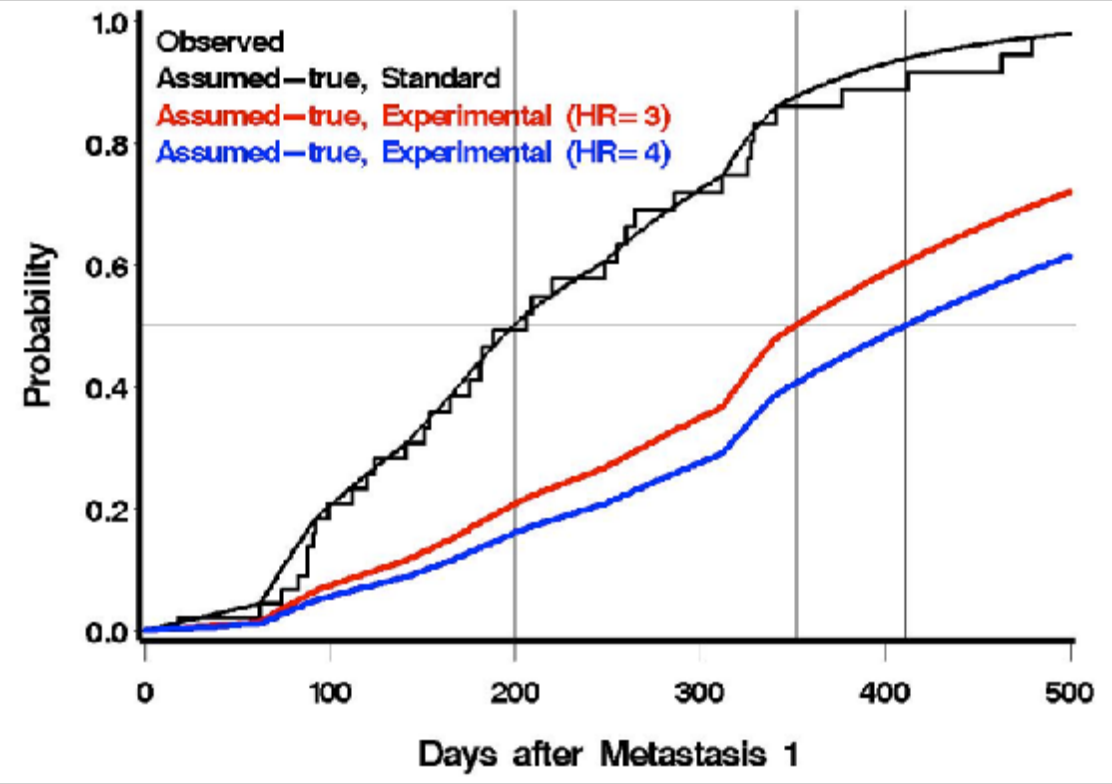


Figure 146. Observed and predicted times to second metastasis

A total of 20 patients will be treated in the trial, including 10 in each of Groups 1 and 2 as long as the level of safety is deemed to be acceptable. This number was not derived based on any formal statistical considerations; rather, it is a number that is a compromise between the number of patients that can be enrolled in a reasonable time frame and the number of patients whose treatment will provide enough information to gain a preliminary assessment of efficacy. From our database of over 850 patients, we are in active contact with ~300 living MCC patients. Approximately 120 new patients are recruited each year to our cohort. One-third of MCC patients will have a first metastasis within 3 years of diagnosis. Each year, approximately 40 patients in our cohort experience a first metastasis. Because we now have tetramers that cover >80% of MCC patients based on their HLA alleles, we anticipate that generation of functional tetramer-positive MCPyV-specific T cells from the peripheral blood will be feasible on a sufficient number of cases so that we can readily meet targeted enrollment of four cases per year.

The observed time to second metastasis for the 49 patients is summarized in **Figure 146**. Based on these data, and in collaboration with our study biostatistician (Ted Gooley, PhD), we fit a piecewise exponential model wherein we assumed a constant hazard of metastasis in each of nine different time windows as a means of specifying an assumed-true distribution for time to metastasis. At the end of each window, the assumed-true probability of metastasis is required to equal the observed value at this time. From this assumed-true distribution, the assumed-true distribution for the current

therapy was generated by assuming a hazard of failure that is proportional to that from the assumed-true distribution from the historical data. From this distribution, 5,000 simulated trials were randomly generated, and the lower limit of a point-wise confidence interval was estimated for each simulated trial. The proportion of trials where this confidence limit exceeded 200 days was taken as the estimated power to observe a statistically significantly improved median time to metastasis compared to the fixed time of 200 days. **Table 8** below summarizes the power estimated in this manner for a variety of assumed-true distributions and one-sided significance **(□)** levels with 10 patients.

Therefore, with 10 patients treated, we'll have sufficient power to observe a statistically significant improvement in median time to metastasis relative to the fixed historical time of 200 days for scenarios with assumed-true parameters as summarized above. For the analysis, we will use 'Time 0' as the first day of treatment as opposed to the time of 1st metastasis. Potential efficacy of Groups 1 and 2 will be assessed in this manner. If each group is deemed to be potentially efficacious, then we will use a "play-the-winner" approach to choose the treatment that will move forward to our next trial, wherein the group with the longest median time to metastasis will be declared the winner. If only one group is deemed to be potentially efficacious as described above, then this is the treatment that will be taken forward. If neither treatment is considered to be potentially efficacious, then neither treatment will be taken forward.

Table 8. Power estimates

Assumed-true Median	Assumed-true Hazard Ratio	Significance level of 0.10	Significance level of 0.15
352 days	3	70%	88%
411 days	4	85%	92%

Secondary efficacy endpoints include response to treatment by RECIST criteria and MCC-specific survival. Although we will report the overall response rate (See **Section 12.D.2**) using RECIST criteria to determine clinical responses in all patients, radiation therapy or intralesional IFNβ-1B injection may have an independent effect on the targeted metastasis (see **Section 12.D.1**) and may not adequately capture the effect of transferred T cells or anti-PD-L1 antibody treatment. Furthermore, it is estimated that 10 patients will not be sufficient to evaluate the effect of this treatment on MCC-specific survival.

However, in patients who had both lesions which did and did not receive MHC Class I upregulation interventions, we will report the rate of response of either group of lesions. Response assessment will be carried out per RECIST v1.1 in all lesions which received MHC Class I upregulation and all lesions which did not receive MHC Class I upregulation. Responses will be classified as CR, PR, SD or PD per RECIST definitions. The Objective response rate (ORR) and 90% confidence interval will be calculated as the proportion of evaluable lesions that achieve a CR or PR. The ORR for lesions which

did and did not receive MHC Class I upregulation will be calculated separately, and confidence intervals will account for correlation of lesions within a patient.

The study will be suspended pending a review by the DSMB if there is ever sufficient evidence to suggest that the true toxicity rate exceeds 30% in any group. In terms of toxicity of conventional therapy, fractionated ionizing radiation of 30-60Gy is typically effective in controlling the target MCC lesion, but it is commonly associated with transient grade ≤ 3 local toxicities such as mucositis and erythema, depending on the target site. However, radiation therapy cannot be used to repetitively control multiple recurrences.[81] The rate of grade ≥ 3 toxicities associated with platinum-based chemotherapy regimens for the systemic treatment of MCC is approximately 30% to 40%.[82] Thus, in order not to exceed the toxicity rate induced by standard systemic treatment, enrollment to any group will be suspended if evidence suggests that the true toxicity rate exceeds 30%. Sufficient evidence will be taken to be an observed proportion of toxicities for which the associated lower 80% confidence limit exceeds 30%. Operationally, this limit will be met if any of the following proportions is observed: 2/2-3, 3/4-5, 4/6-8, 5/9-10. Based on this rule, if the true probability of toxicity is 10%, the probability of suspending one group after 10 patients is approximately 0.03. If the true probability of toxicity is 50%, the probability of suspension is 0.76.

19. ADMINISTRATIVE CONSIDERATIONS

19.A. Institutional Review Board

In accordance with federal regulations (21 CFR 312.66), an IRB that complies with regulations in 21 CFR 56 must review and approve this protocol and the informed consent form prior to initiation of the study.

19.B. Termination of Study

The study will be stopped if any of the following events occur:

- All 20 patients have completed treatment.
- Stopping rules for toxicity have been met. Accrual will be put on hold for discussion with the DSMB regarding a possible change in study design.
- The PI reserves the right to terminate the study at any time. The FDA may also terminate the study.

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APPENDIX A

ECOG / Zubrod Performance Status

0 Fully active, able to carry on 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 house work, office work

2 Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours

3 Capable of only limited self-care, confined to bed or chair more than 50% of waking hours

4 Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair

5 Dead

APPENDIX B

Group 1 Monitoring Schedule[a]

GROUP 1		Event	History and PE	Chemistry Panel	CBC	T3 FT4 TSH	Tumor Biopsy	Blood for Research	Radiographic Evaluation	Oncoprotein Serology
Pre-treatment		Evaluation for treatment	X	X	X	X[f]	X[g]	X	X[b]	X
	Day 0	PD-L1 infusion[c]	X	X	X			X		
Week +1/+2	**Day +7 - +10**	Administer Radiation OR Intralesional IFNβ-1B	X	X	X			X		
Week +2	**Day +14**	PD-L1 infusion	X	X	X			X		
Week +4	**Day +28**	PD-L1 infusion	X	X	X		X[g]	X		
Week +6	**Day +42**	PD-L1 infusion	X	X	X			X		
Week +8	**Day +56**	PD-L1 infusion	X	X	X			X		
Week +9	**Day +63**	Staging							X (Day 56-70)	X
Week +10	**Day +70**	PD-L1 infusion	X	X	X	X[f]		X		
Week +11/+12	**Day +77-+80**	Administer Radiation OR Intralesional IFNβ-1B (if indicated)[d]	(X)	(X)	(X)			(X)		
Week +12	**Day +84**	PD-L1 infusion	X	X	X			X		
Week +14	**Day +98**	PD-L1 infusion	X	X	X			X		
Week +16	**Day +112**	PD-L1 infusion	X	X	X			X		
Week +18	**Day 126**	PD-L1 infusion	X	X	X			X		
Week +19	**Day +133**	Staging							X (Day 126-140)	X
Week +20	**Day +140**	PD-L1 infusion	X	X	X			X		
Week +22	**Day +154**	PD-L1 infusion	X	X	X	X[f]		X		
Week +24	**Day +168**	PD-L1 infusion	X	X	X			X		
Beyond +52 weeks		Study ends	X[e]	X[e]	X[e]			X[e]	X[e]	X
Beyond +24 weeks infusion Q2 weeks for up to 1 year total on treatment		PD-L1 infusion	X	X	X			X		

Test results and procedures conducted as per standard of care may be used for eligibility determination and evaluation for toxicity.
[a] The dates listed on the study calendar above are approximate as many patients are expected to reside out of state and his oncologists and laboratories may not always be able to follow the time points as dictated by the protocol.
[b] Includes brain evaluation.
[c] Patients may receive preferably one, but up to 3 infusions of anti-PD-L1 before the first MHC class I up-regulation intervention.
[d] The MHC class I upregulation can be repeated once, at any time point, if there is radiological disease present. Treatment must start within 4 weeks of the preceding radiological evaluation.
[e] At least every 3 months for one year, every 6 months for one year then yearly and as clinically indicated.
[f] at baseline, at cycle 6 and then every 6 cycles.
[g] If accessible.

Group 2 Monitoring Schedule[a]

GROUP 2		Event	History and PE	Chemistry	CBC	T3 FT4 TSH	Tumor Biopsy	Blood for Research	Radiographic Evaluation	Oncoprotein Serology
Pre-treatment	**Evaluation for treatment**	**X**	X	X	X	X[f]	X[g]	X	X[b]	X
	Day 0	**PD-L1 infusion[c]**	X	X	X			X		
Week +1/+2	**Day+7 - +10**	**Administer Radiation OR Intralesional IFNβ-1B**	X	X	X			X		
	Day +12 (T1+0)	**Infusion #1 (10^{10}cells/m2)**	X	X	X			X		
	Day +13 (T1+1)	*** Day after infusion 1**	X	X	X			X		
Week +2	**Day +14**	**PD-L1 infusion**								
Week +2	**Day +15 (T1+3)**	**Day 3 after Infusion #1**		X	X			X		
Week +3	**Day +19 (T1+7)**	*** Day 7 after Infusion #1**		X	X			X		
Week +3/+4	**Day +26 (T1+14)**	**Day 14 after Infusion #1**	X	X	X			X		
Week +4	**Day +28**	**PD-L1 infusion**	X	X	X		X[g]	X		
Week +5	**Day 40 (T1+28 / T2+0)**	**Infusion #2 (10^{10}cells/m2)**	X	X	X			X		
	Day +41 (T2+1)	*** Day after infusion 2**	X	X	X			X		
Week +6	**Day +42**	**PD-L1 infusion**								
Week +6	**Day +43 (T2+3)**	**Day 3 after Infusion #2**		X	X			X		
	Day +47 (T2+7)	*** Day 7 after Infusion #2**		X	X			X		
Week +7	**Day +54 (T2+14)**	**Day 14 after Infusion #2**	X	X	X			X		
Week +8	**Day +56**	**PD-L1 infusion**	X	X	X			X		
Week +9	**Day +63**	**Staging**	X	X	X				X (Day 56-70)	X
Week +9	**Day +68 (T2+28)**	**Day 28 after Infusion #2**	X	X	X			X		
Week +10	**Day +70**	**PD-L1 infusion**	X	X	X	X[f]		X		
Week +11/+12	**Day +77- +80**	**Administer Radiation OR Intralesional IFNβ-1B (if indicated)[d]**	(X)	(X)	(X)			(X)		
	Day +82 (T3+0)	*** Infusion #3 (10^{10} cells/m2) (if indicated)**	(X)	(X)	(X)			(X)		
	Day +83 (T3+1)	*** Day after infusion 3 (if indicated)**	(X)	(X)	(X)			(X)		
Week +11	**Day +85 (T3+3)**	**Day 3 after Infusion #3**		X	X			X		
Week +12	**Day +84**	**PD-L1 infusion**	X	X	X			X		
Week +13	**Day +89 (T3+7)**	*** Day 7 after Infusion #3 (if indicated)**		X	(X)			(X)		
Week +13	**Day +96 (T3+14)**	**Day 14 after Infusion #3**	X	X	X			X		
Week +14	**Day +98**	**PD-L1 infusion**	X	X	X			X		
Week +15	**Day +110 (T3+28 / T4+0)**	*** Infusion #4 (10^{10} cells/m2) (if indicated)**	(X)	(X)	(X)			(X)		
	Day +111 (T4+1)	*** Day after infusion 4 (if indicated)**	(X)	(X)	(X)			(X)		
Week +16	**Day +112**	**PD-L1 infusion**	X	X	X			X		
Week +16	**Day +113 (T4+3)**	**Day 3 after Infusion #4**		X	X			X		

Week +17	**Day +117 (T4+7)**	*** Day 7 after Infusion #4 (if indicated)**		X	(X)			(X)		
Week +17	**Day +124 (T4+14)**	**Day 14 after Infusion #4**	X	X	X			X		
Week +18	**Day 126**	**PD-L1 infusion**	X	X	X			X		
Week +19	**Day +133**	**Staging**							X (Day 126-140)	X
Week +19	**Day +138 (T4+28)**	**Day 28 after Infusion #4**	X	X	X					
Week +20	**Day +140**	**PD-L1 infusion**	X	X	X			X		
Week +22	**Day +154**	**PD-L1 infusion**	X	X	X	X[f]		X		
Week +24	**Day +168**	**PD-L1 infusion**	X	X	X			X		
Beyond 52 weeks		**Study ends**	X[e]	X[e]	X[e]			X[e]	X[e]	X
Beyond +24 weeks infusion Q2 weeks for up to 1 year total on treatment		PD-L1 infusion	X	X	X			X		

Test results and procedures conducted as per standard of care may be used for eligibility determination and evaluation for toxicity.

[a] The dates listed on the study calendar above are approximate as many patients are expected to reside out of state and his oncologists and laboratories may not always be able to follow the time points as dictated by the protocol.

[b] Includes brain evaluation.

[c] Patients may receive preferably one, but up to 3 infusions of anti-PD-L1 before the first MHC class I up-regulation intervention.

[d] The MHC class I upregulation and infusions can be repeated once, at any time point, if there is radiological disease present. Treatment must start within 4 weeks of the last radiological evaluation. A cumulative total of 4 infusions can be administered. Radiation therapy or intralesional IFN□-1B injection will typically be administered before the first infusion of subsequent cycles. Monitoring will end at least 20 weeks after the last infusion or with the development of new metastasis.

[e] At least every 3 months for one year, every 6 months for one year then yearly and as clinically indicated.

[f] at baseline, at cycle 6 and then every 6 cycles.

[g] If accessible.

APPENDIX C

Outcomes of patients with MCC who received IFNβ-1B injections

Patient	Injected lesion(s)	Number of injections	Response	Draining node swelling
1 *	3 in-transit metastases	27 (to each of 3 lesions) over 37 days.	Complete response	Unknown
2 (w419)	Primary tumor	14 over 14 days.	Partial response	Yes, no tumor by IHC
3 (w380)	2 recurrences	15 injections per each of 2 lesions over 15 days.	Partial response	No
4 (w438)	Primary tumor	9 over 9 days.	Complete response	Yes, no tumor by IHC
5 (w425)	Recurrence	15 over 15 days.	Progressive disease	No
6 (w390)	Recurrence	12 over 24 days. 4 week rest. 3 over 5 days.	Partial response	No
7 (w131)	Recurrence	12 over 15 days.	Partial response	No
8 (w732)	Multiple recurrences	21 over 31 days.	Complete response**	No
9 (w668)	2 nodal metastases	10 over 14 days	Partial response	N.A.

* Some details of this case were previously reported (Nakajima, et al 2009)

** Ten un-injected lesions on the same leg also had complete responses to interferon.