Regression of Metastatic Merkel Cell Carcinoma Following Transfer of Polyomavirus-Specific T Cells and Therapies Capable of Reinducing HLA Class-I

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Abstract

Merkel cell carcinoma (MCC) is an aggressive skin cancer that typically requires the persistent expression of Merkel cell polyomavirus (MCPyV) oncoproteins that can serve as ideal immunotherapeutic targets. Several immune evasion mechanisms are active in MCC, including downregulation of HLA class-I expression on tumor cells and dysfunctional endogenous MCPyV-specific CD8 T-cell responses. To overcome these obstacles, we combined local and systemic immune therapies in a 67-year-old man, who developed metastatic MCPyV-expressing MCC. Intralesional IFN-β-1b or targeted single-dose radiation was administered as a preconditioning strategy to reverse the downregulation of HLA-I expression noted in his tumors and to facilitate the subsequent recognition of tumor cells by T cells. This was followed by the adoptive transfer of ex vivo expanded polyclonal, polyomavirus-specific T cells as a source of reactive antitumor immunity. The combined regimen was well tolerated and led to persistent upregulation of HLA-I expression in the tumor and a durable complete response in two of three metastatic lesions. Relative to historical controls, the patient experienced a prolonged period without development of additional distant metastases (535 days compared with historic median of 200 days; 95% confidence interval, 154–260 days). The transferred CD8+ T cells preferentially accumulated in the tumor tissue, remained detectable and functional for more than 200 days, persisted with an effector phenotype, and exhibited evidence of recent in vivo activation and proliferation. The combination of local and systemic immune stimulatory therapies was well tolerated and may be a promising approach to overcome immune evasion in virus-driven cancers. Cancer Immunol Res; 2(1): 27–36. ©2013 AACR.

Introduction

Merkel cell carcinoma (MCC) represents a highly aggressive neuroendocrine skin malignancy with a high disease-propensity for recurrence after initial treatment. The cause-specific mortality rate ranges from 23% to 80% at 5 years, thus making it three times as lethal as melanoma (1). The Merkel cell polyomavirus (MCPyV) is clonally integrated into at least 80% of MCC tumors and produces the viral T-antigen (T-Ag) oncoproteins that are persistently expressed by MCC and are necessary for the survival and proliferation of tumor cells (2–5). Compared with mammalian tumor-associated antigens (TAA) that all have some degree of expression within normal tissue, MCPyV T-Ag expression is restricted to MCC, is a foreign antigen not subject to T-cell self-tolerance mechanisms, and is thus an optimal target for immunotherapy. Because no viral particles are formed in the tumor cells, antiviral agents are inefficient (6).

As adoptive transfer has demonstrated clinical benefit for both viral and endogenous tumor antigens (7–9), we sought to apply the use of antigen-specific T cells to target the MCPyV large T-Ag (LT-Ag) oncoprotein. The HLA-A’2402-restricted MCPyV LT-Ag92-101-specific T cells (hereafter referred to as MCPyV-specific cells) were identified in a patient with metastatic MCC (10). These MCPyV-specific T cells, when isolated from tumors or peripheral blood mononuclear cells (PBMC) of patients with MCC, are largely associated mortality, early metastatic disease, and a high propensity for recurrence after initial treatment. The cause-specific mortality rate ranges from 23% to 80% at 5 years, thus making it three times as lethal as melanoma (1).
Here, we investigated whether adoptive transfer of polyclonal MCPyV-specific T cells might augment the probability of including and expanding cells that had an increased potential for proliferation, function, and persistence after transfer as evidenced in murine and non-human primate models (12, 13).

Similar to the observations in other virus-associated cancers, HLA class-I (HLA-I) downregulation is an immune escape mechanism present in the majority of MCC tumors (14–16). Single-dose low-dose radiation has been shown to upregulate cell surface HLA-I expression (17). Data from murine models suggest that single-dose radiation is more effective than fractionated radiation in promoting tumor immunity, because the latter may suppress the function of lymphocytes that are recruited to the tumor (18). Furthermore, IFNs direct the upregulation of HLA-I (19), and intralesional administration of IFN-β has been observed to promote immune responses in MCC (14, 20).

Here, we investigated whether adoptive transfer of polyclonal MCPyV-specific CD8⁺ T-cells following HLA-I upregulation strategies (intramuscular IFN-β or borel, single-fraction radiotherapy) could safely establish persistent anti-MCC responses, migrate to tumor tissue, and induce regression of MCPyV-positive, HLA-I-deficient MCC metastases.

Case report

A 67-year-old man with chronic renal failure secondary to a nephrectomy for renal cell carcinoma (RCC) at the age of 50 presented with a 1.6-cm (in largest dimension) MCC lesion on his left upper thigh and a negative sentinel node biopsy (American Joint Committee on Cancer stage IA). He underwent a wide local excision followed by 50 Gy of fractionated local radiation to the primary site. Eight months later while still asymptomatic, a surveillance whole body positron emission tomography (PET) scan detected a 2.9 × 1.8 cm lesion adjacent to the pancreatic head. Merkel polyomavirus-specific CD8 T cells were identified in the patient’s tumor-infiltrating lymphocytes (TIL) and peripheral blood using a MCPyV-tetramer (ref. 10; Supplementary Fig. S1). The patient underwent leukapheresis to allow MCPyV-specific T cells to be harvested. The patient was enrolled in a single-patient clinical trial of autologous T-cell therapy for MCC [Fred Hutchinson Cancer Research Center (FHCRC, Seattle, WA) protocol #2558]. Both the primary tumor and pretreatment metastases were confirmed to be classical MCC by dot-like cytokeratin-20 staining. Both lesions expressed the MCPyV T-Ag, whereas HLA-I expression was absent or sparse. Two additional metastases in the pancreatic head and neck appeared 137 days before the initiation of therapy.

The patient underwent three rounds of treatment (Fig. 1). Intramuscular IFN-β (3 × 10⁶ IU) was first administered by endoscopic ultrasound-guided fine-needle injection to the largest of the three detectable pancreatic metastases (designated as metastasis A). After 24 hours, the patient received an infusion of 10⁸/m² MCPyV-tetramer-specific polyclonal CD8⁺ T cells followed by low-dose s.c. interleukin (IL)-2 (2.5 × 10⁷ IU/m²) twice daily for 14 days as a means to increase T-cell persistence (21). Thirty-seven days later, a single 8-Gy fraction of radiation was delivered to all three remaining lesions (designated as metastases A, B, and C) followed by a second infusion of 10¹⁰/m² MCPyV-specific CD8⁺ T-cells and low-dose s.c. IL-2. On day 148 after the first infusion, metastasis B was injected with IFN-β-1b followed by a third cell infusion and low-dose s.c. IL-2 × 14 days. The patient experienced low-grade fevers and a transient, less than 72-hour grade 1–2 lymphopenia after each infusion, consistent with the expected immediate toxicities associated with T-cell infusions (22, 23). No changes in end organ function, inflammation- or autoimmune-related complications were observed. As detailed in the Results section, follow-up scans showed no evidence of new distant disease until 535 days after the original pancreatic metastasis was detected. At that time, the patient developed new brain lesions that he declined to have worked up or treated. The patient died 1 month later without additional therapeutic interventions.

Materials and Methods

Clinical protocol and patient characteristics

All clinical investigations were conducted according to the Declaration of Helsinki principles. The single-patient protocol #2558 (described above) was approved by the FHCRC Institutional Review Board and the U.S. Food and Drug Administration. The patient provided written informed consent.
Isolation and expansion of MCC-specific CTLs

PBMCs were collected by leukapheresis and all ensuing ex vivo manipulations involving processing of products destined for infusion were performed in the cGMP Cell Processing Facility of the FHCRC based on a protocol established for in vitro enrichment of low frequency antigen-specific cytotoxic T lymphocytes (CTL; refs. 24, 25). To facilitate the isolation of antigen-specific CTL, PBMCs were depleted of CD25+ T cells to eliminate regulatory T cells (Tregs; ref. 26; Miltenyi Biotec Inc.), and stimulated twice for 7 to 10 days with HLA-A2/4042-restricted MCPyV LT-Ag92-101 peptide (CPC Scientif... cellular populations from our historical controls. Cases that would not have been eligible for the trial were eliminated, including those with profound immune suppression and those who only had a single subsequent skin metastasis (these are often independent of local or regional disease (stage I, II, or III) and later developed distant metastatic disease, for which they then received treatment. The median time to second metastasis was estimated along with a point-wise estimate of the 95% confidence interval for the median time. We only included a highly analogous patient population from our historical controls. Cases that would not have been eligible for the trial were eliminated, including those with profound immune suppression and those who only had a single subsequent skin metastasis (these are often independent primary lesions or represent a very benign subset of patients). Statistical tests were performed with the GraphPad prism software version 3.0, with the R-package for statistical analysis (http://www.r-project.org), or SAS version 9.2.

Results

HLA-1 upregulation followed by adoptive transfer of MCPyV-specific T cells induces regression of Merkel cell carcinoma

Pre- and posttreatment tumor tissue immunohistochemistry was used to evaluate viral oncoprotein expression, HLA-I expression, and CD8 lymphocyte infiltration (Fig. 2A). As... the template, this method was used to capture the frequency of individual TCRs in biologic samples with accurate reproducibility and a sensitivity of 1/100,000 TCR-containing lymphocytes (31, 32). DNA from the blood and tumor samples was prepared using the DNeasy Blood & Tissue Kit (Qiagen). The sources of available cells for DNA extraction include the tetramer-sorted infusion product, cryopreserved PBMC derived using Ficoll isolation, phytohemagglutinin and IL-2–expanded TIL isolated from the primary tumor, and freshly isolated cells from the pancreatic metastatic tumor biopsy following immune therapy treatment. The results of individual TCR clonotypes are expressed as a percentage of all productive, in-frame TCRs sequenced.

Immunohistochemistry

Anti-CD8 clone 4B11 (Novocastra), anti-MCPyV T-Ag (5), and anti-HLA-I clone EMR8-5 (MBL International) were used at 1:200, 1:4,000, and 1:200 dilutions, respectively, after heat-induced epitope retrieval. Specimens were assessed for MCPyV T-Ag and HLA-I expression and scored using the Allred method (33). An Allred score of 0 to 1.5 was considered negative (0), 2 to 5 weak (+), 6 to 7.5 moderate (++), and 8 strong (+++). Peritumoral and intratumoral CD8 infiltrates were scored separately as previously described (14). The numbers of CD8+ T cells were assessed and expressed on a 0-to-5 scale with a mean of 0, 90, 306, 508, 675, and 732+ CD8s/mm², respectively. Intratumoral CD8 lymphocytes were surrounded by tumor cells only with no visualized contact with stroma.

Statistical analysis

The observed time between the first metastasis and the second metastasis for 49 patients was available and these data were used as historic controls. All of these patients presented with local or regional disease (stage I, II, or III) and later developed distant metastatic disease, for which they then received treatment. The time between metastases was treated as a time-to-event endpoint, and from these controls, the median time to second metastasis was estimated along with a point-wise estimate of the 95% confidence interval for the median time. We only included a highly analogous patient population from our historical controls. Cases that would not have been eligible for the trial were eliminated, including those with profound immune suppression and those who only had a single subsequent skin metastasis (these are often independent primary lesions or represent a very benign subset of patients). Statistical tests were performed with the GraphPad prism software version 3.0, with the R-package for statistical analysis (http://www.r-project.org), or SAS version 9.2.

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Figure 2. Response of individual MCC metastases after combined immune therapy. A, columns from left to right, hematoxylin and eosin (H&E) stains, immunohistochemistry for MCPyV T-Ag, HLA-I, and CD8 (red arrows, red asterisk indicates cell in the inset) of the primary tumor, pretreatment (metastasis A, biopsied immediately before start of immune therapy) and posttreatment (metastasis B, biopsied 148 days after start of immune therapy) tumors. Scale bar, 50 μm. B, MRI imaging (except the first datapoint, day 146, which was obtained by PET/CT) of individual metastases that included a peri-pancreatic lesion adjacent to the anterior duodenum (metastasis A, red), a pancreatic neck lesion (metastasis B, green), and a pancreatic head lesion (metastasis C, blue). The longest diameter in cm (y-axis) of each metastasis is graphed over time (x-axis). Representative images of pre- and posttreatment PET/CT scans are shown above the graph. Gray bars on x-axis indicate the timing of each treatment as detailed in Fig. 1. The corresponding RECIST 1.1 criteria are indicated below the graph. C, Kaplan–Meier curve of the probability of developing a second distant metastasis after the first detected metastasis (plotted at day zero) in 49 patients with MCC who developed distant disease. The 95% confidence interval is indicated in red.
anticipated, the primary tumor and pretreatment metastasis A
biopsies were positive for the MCPyV T-Ag oncoprotein, but
had weak or no HLA-I expression. In contrast, HLA-I was
strongly upregulated on the biopsy of the posttreatment
metastasis (metastasis B) taken 146 days after the first treat-
ment cycle, and as expected, MCPyV T-Ag expression was
persistently maintained. Scattered CD8⁺ T-cell infiltrates were
detected in the primary tumor, as well as in the pre- and
posttreatment biopsies (Fig. 2A).

Tumor burden and clinical efficacy were monitored using
MRI and PET/CT scans (Fig. 2B). Immediately before the first
treatment, the patient’s initial pancreatic metastasis had
enlarged, and two adjacent pancreatic tumors were newly
detected compared with the scan performed 146 days earlier.
Restaging 30 days after the first treatment showed that the
largest lesion, which had undergone injection with IFN-β-Ib,
had decreased in size, but the other two lesions had remained
stable or increased in size. The best overall response by
Response Evaluation Criteria in Solid Tumors (RECIST) was
a partial response observed following the second treatment
consisting of localized, single-dose 8-Gy radiation before T-cell
infusion and low-dose s.c. IL-2. After the third treatment, two
of three lesions continued to regress and one lesion remained
refractory to treatment.

To determine systemic effects of adoptively transferred T
cells, the time for new metastasis to develop after treatment
was assessed in comparison with 49 historical matched
controls undergoing standard, typically cytotoxic chemo-
therapy (Fig. 2C). The patient showed no additional systemic
metastasis until 535 days after the first treatment in the first
metastasis, which greatly exceeds the estimated median
time of 200 days (95% confidence interval, 154–260 days)
for development of new distant metastatic disease in histo-
ic controls. The scan performed 535 days after the initial
metastasis revealed new brain lesions that the patient
declined work-up. These likely represented MCC metastases;
however, as the patient also had a history of remote RCC and
no biopsy was performed, metastasis from an RCC origin
could not be excluded. Overall, these data suggest the
treatment-induced regression in two of three metastases
and may have contributed to a delay in the development of
new distant disease.

Enhanced ability to respond to antigen after transfer of
virus-specific CD8⁺ T cells

The frequency and persistence of MCPyV-specific CD8 T
cells in peripheral blood were assessed by their ability to bind
the MCPyV-specific tetramer (Fig. 3A, solid circles). The fre-
quency of these cells peaked 4 to 7 days after infusions (up to
8.8% of total CD8⁺ T cells) and remained detectable at the last
assessment (219 days after the first treatment) at frequencies of
approximately 1%, which corresponded to a more than 3-fold
increase compared with preinfusion levels (0.26%).

We next assessed whether the virus-specific T cells detected
after treatment were functional as assessed by their ability to
secrete IFN-γ in response to cognate peptide. Before the
treatment cycles, cells that secreted IFN-γ after exposure to
the MCPyV peptide could not be detected within PBMC,
suggesting that the MCPyV-specific cells binding to tetramer
in the pretreatment PBMC were nonfunctional. However, the
frequency of CD8 T cells that secreted IFN-γ in response to
MCPyV peptide increased after each infusion (5.1, 4.5, and 3%
after treatments 1, 2, and 3, respectively) and remained detect-
able at frequencies of 1% or more of total CD8⁺ T cells up to 219
days after the first infusion (Fig. 3A, open circles). Furthermore,
the frequency of MCPyV-reactive peptide-responsive CD8⁺ T
cells correlated closely with the frequency of MCPyV tetramer-
positive cells, suggesting that most of the persisting virus-
specific cells remained functional in vivo.

Because an increased frequency of virus-specific T cells over
time may reflect antigen-specific cell division, we investigated
whether the persisting MCPyV-specific T cells expressed Ki-67,
a marker of proliferation (34). Before infusion, endogenous
(presisting) MCPyV-specific T cells did not express Ki-67,
indicating minimal/absent proliferation. Cells from the infu-
sion product were overwhelmingly positive for Ki-67 (92% at
day 14 after the last stimulation cycle in vitro), consistent with
recent activation. Most transferred cells maintained Ki-67
expression early after transfer (>80% on day 1 after infusion)
and, as expected, Ki-67 expression decreased after each infu-
sion but remained well above baseline levels (Fig. 3B, solid
diamonds) 219 days after the first infusion. Ki-67 expression
on MCPyV-specific CD8 T cells was overall significantly higher
\(P \leq 0.005\) than Ki-67 expression of the host tetramer-negative
CD8 T cells (Fig. 3B, open diamonds), suggesting that the
infused cells continued to divide in the presence of persistent
antigen. The frequency of CD8 T cells that secreted IFN-
γ increased after each infusion (5.1, 4.5, and 3%
after treatments 1, 2, and 3, respectively) and remained detect-
able at frequencies of 1% or more of total CD8⁺ T cells up to 219
days after the first infusion (Fig. 3A, open circles). Furthermore,
the frequency of MCPyV-reactive peptide-responsive CD8⁺ T
cells correlated closely with the frequency of MCPyV tetramer-
positive cells, suggesting that most of the persisting virus-
specific cells remained functional in vivo.

To validate the functional status and determine the differ-
etiation profile of pre- and posttreatment virus-specific CD8
T cells, we evaluated the phenotype of the persisting trans-
ferred cells. Consistent with the absence of function and
proliferation, both PD-1 (a marker of activation/exhaustion)
and TIM-3 (a marker of exhaustion) were expressed on
MCPyV-specific cells (90% and 78%, respectively) found in
PBMC before the treatment cycles, suggesting endogenous
CD8 responses were dysfunctional and exhausted (Fig. 3C and
D). As expected, and following ex vivo expansion, the infusion
product also expressed PD-1 (34%) and TIM-3 (93%). However,
one infused into the patient, the transferred cells maintained
PD-1 expression but lost Tim-3 expression such that cells
found after transfer had a nonexhausted phenotype (Fig. 3C),
consistent with their maintained function. Furthermore,
7 days after transfer, 53% of MCPyV-specific cells transiently
expressed CD137 (4-1BB), a marker associated with very recent
activation (Fig. 3E).

On the basis of the absence of expression of the costimu-
latory molecule CD28, the IL-7 receptor CD127 as well as the
lymph node homing molecules, CD62L and CCR7 (data not
shown), the endogenous MCPyV-specific T cells found before
infusions exhibited a differentiated effector memory pheno-
type (Fig. 3F and G). Consistent with the use of IL-21 in the
ex vivo cultures (24–26), a subset of the infusion product gener-
at for this patient expressed CD28 (78%) and CD127 (19%).
After infusion into the patient, the transferred cells maintained
CD28 expression (Fig. 3F) and further upregulated CD127 (Fig.
3G). CD62L and CCR7 were not detected (data not shown).
These data suggest that the transferred cells persisted with an effector memory phenotype (13), but in a less differentiated state compared with the endogenous response, with a maintained capacity to signal through CD28 and CD127. Tregs can be sensitive to exogenous IL-2, and could have a negative impact on the persistence and function of transferred cytotoxic lymphocytes. Therefore, we assessed Treg numbers after infusions based on the expression of surrogate markers CD4 and CD25 and the absence of CD127 (24). Treg frequencies increased after each infusion from baseline levels but returned to near baseline levels within 28 days of the infusion (Supplementary Fig. S3). In TIL, unfortunately, we were severely limited by the quantity of tissue available for analysis from the patient's metastases and therefore Treg analysis could not be performed.

Overall, adoptive transfer of polyclonal, MCPyV-specific T cells was associated with several key characteristics suggestive of an enhanced ability to respond to antigen: (i) markedly increased frequency of MCPyV-specific cells that persisted in vivo, (ii) increased fraction of virus-reactive T cells, (iii) ability to proliferate (Ki-67), (iv) expression of activation markers (CD137, PD-1), and (v) facilitated costimulation and survival (CD28 and CD127; refs. 35, 36).

Transferred cells preferentially localize to metastatic MCC tissue

To capture the number and characteristics of the individual transferred MCPyV-specific CD8⁺ T-cell clones, we analyzed tetramer-sorted infusion products, TIL, and PBMC for individual TCR-β CDR3 using high-throughput TCR DNA sequencing (32). The infusion product (which was sorted a second time to yield >99% pure CD8⁺ tetramer⁺ cells for TCR-β CDR3 analysis) was polyclonal and consisted of 502 individual virus-specific clonotypes. Three clonotypes were predominant and represented 99% of MCPyV-specific TCR reads (all three clones shared V-β 7-9 and differed by their J-β subunits as depicted in Fig. 4A and B). We then used this method to determine all TCR-β CDR3 present in unsorted TIL and PBMC obtained before and after infusions and investigated whether the transferred virus-specific clonotypes were present within these samples, as well as their frequency. Before treatment, these J-β variant clones were present among primary tumor TIL (0.03% J-β1-5, 0.06% J-β1-1, 2.8% J-β2-3; Fig. 4C, left), and PBMC (0.06% J-β1-5, 0.15% J-β1-1, 0.01% J-β2-3; Fig. 4D, left), suggesting that these preexisting clonotypes had been expanded in the PBMC-derived infusion product. Analysis of the posttreatment
metastasis 146 days after the first infusion demonstrated that clone Jb1-1 now represented 4.7% of all sequences, and the clones Jb1-5 and Jb2-3 were absent (Fig. 4C, right). We then investigated whether additional clonotypes that were present in the posttreatment metastasis were also present within the low-prevalence MCPyV-specific clonotypes (<1%) in the infusion product. This analysis identified an additional MCPyV-specific clonotype (highlighted in red), with one nucleic-acid difference compared with the Jb1-1 clonotype highlighted in blue that was present at 0.6% of all TCR reads in the posttreatment metastatic biopsy and absent in the pretreatment TIL. However, because the frequency in the infusion product was so low (2.6 × 10^{-4}%), we cannot formally exclude that this clone constituted a contaminant.

Overall, MCPyV-specific CD8\(^+\) T-cell clonotypes present in the infused product increased from 2.9% in the primary tumor to 5.3% in the postinfusion metastasis (Fig. 4C). Two clones accounted for the increase in MCPyV-specific cells present in the biopsy metastasis following the treatments compared with the primary lesion. No increase in frequency of these three clones was observed in the peripheral blood before and after treatments at comparable time points (Fig. 4D, right). Using this method, the specificity of the majority of the clonotypes isolated from PBMC and TIL could not be determined and may have included (i) T cells specific for other MCPyV epitopes; (ii) T cells recognizing nonviral TAAs such as survivin (37); HIP1 oncoprotein that interacts with c-KIT (38), CD56, or other UV-induced mutational signatures; or (iii) bystander or resident T cells. In summary, as the infusion product contained clones that aggregated at a higher frequency in the metastatic lesion compared with the peripheral blood, this suggests there was preferential localization of infused virus-specific T cells to the metastatic tissue.

**Discussion**

The purpose of this study was to investigate the efficacy and safety of HLA-I upregulating agents in combination with...
polyomavirus-specific adoptive T-cell therapy in the setting of metastatic MCC. Even though preexisting MCPyV-specific CD8+ T cells were present at low frequencies in the host before infusions (10), these were unable to prevent the rapid progression of metastatic disease. We addressed two key factors that may have contributed to the inefficiency of the endogenous virus-specific T-cell responses: To reverse the observed down-regulated HLA-I expression on tumor cells necessary for the display of the MCPyV viral peptides, and render the cells accessible to specific T-cell lysis, either intraleesional IFN-β-1b and tumor-targeted, single-dose ionizing radiation (8 Gy) was administered 1 to 2 days before each T-cell infusion. To address the absence of function of the endogenous CD8 responses, polyclonal MCPyV-specific CD8 T cells were generated ex vivo from PBMC, expanded, and reinfused. This resulted in the increased frequency of detectable and functional CD8 T cells for more than 3 months in vivo and preferential localization of the infused cells in tumor tissue as evidenced by TCR clonotype analysis. The combination of HLA-I upregulation followed by the infusion of MCPyV-specific CD8 T cells was well tolerated and safe and mediated tumor regression in two of three detectable metastases consistent with T-cell-mediated lysis. The absence of new metastatic disease in this patient for a prolonged period of time compared with historic controls also suggests that the treatment may have delayed or prevented the progression of distant metastatic disease. Although the patient declined definitive work-up, it seems he developed metastatic MCC disease in the brain, a site with limited lymphocyte accessibility (39).

Although a clear increase in antitumor reactivity was achieved with this treatment, immune escape was not entirely halted. Although regressing lesions in the pancreas could not be biopsied, the escaping pancreatic lesion was amenable to a limited fine-needle aspirate. Our data indicate that HLA-I and MCPyV T-Ag expression were maintained on the escaping lesion, and thus their respective downregulation was eliminated as having played a role in immune evasion in the pancreas. Other mechanisms may have contributed to immune escape in some lesions in the patient. Infiltration of tumor tissue by CD8+ T cells has been shown to have a positive effect on survival in numerous cancer settings. TIL density and distribution were shown to independently predict sentinel lymph node status and survival in patients with melanoma (40, 41). In MCC, a clear correlation between CD8+ T-cell infiltration and favorable clinical outcome has been established, consistent with a role for the recognition of tumor cells by host T cells (14). In this patient, CD8+ T-cell infiltration in the primary tumor and pre- and posttreatment metastatic lesions remained scarce, suggesting that other factors may have prevented CD8+ infiltration and effective tumor lysis. A recent report suggests that vascular E-selectin within MCC tumors is critical in mediating intratumoral CD8+ T-cell infiltration (42); however, the intratumoral vascular architecture could not be assessed in this case. Furthermore, PD-1 expression has been shown to be higher in the tumor-infiltrating CD8+ T cells and is associated with disease progression (43–45). Although transferred cells were PD-1+ and functional in the peripheral blood, the function of the CD8+ T cells infiltrating the tumor could not be assessed. Ultimately, more effective results might also be achieved by combining adoptive transfer with immunomodulatory strategies such as blockade of the PD-1/PD-L1 axis or anti-CTLA-4 blockade (46–48).

Similar to other virus-driven cancers, MCC is an attractive target for immunotherapy because characterized, non-self, non-cross-reactive antigens are constitutively expressed and necessary for tumor survival (3). The results of this single-patient study suggest that immune-mediated antitumor activity can be established with a very limited side effect profile and offers a strategy to rapidly elucidate the requirements for MCC tumor control directly in humans by bypassing the necessity of murine models (49, 50). A multiparticipant clinical trial is currently under way (NCT01758458) to further test this combinatorial immunotherapy approach using additional viral CD8 T-cell epitopes in a broader cohort of patients with MCC.

Disclosure of Potential Conflicts of Interest
C. Desmarais has ownership interest (including patents) in Adaptive Biotechnologies. No potential conflicts of interest were disclosed by the other authors.

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