Cover Sheet
scRNA-seq Exploratory Awards

Principal Investigator(s): Aude Chapuis, MD

Project Title: ScRNA-seq to Identify Mechanism of Merkel Cell Carcinoma Response and Escape from T Cell Immunotherapy.

Key Personnel

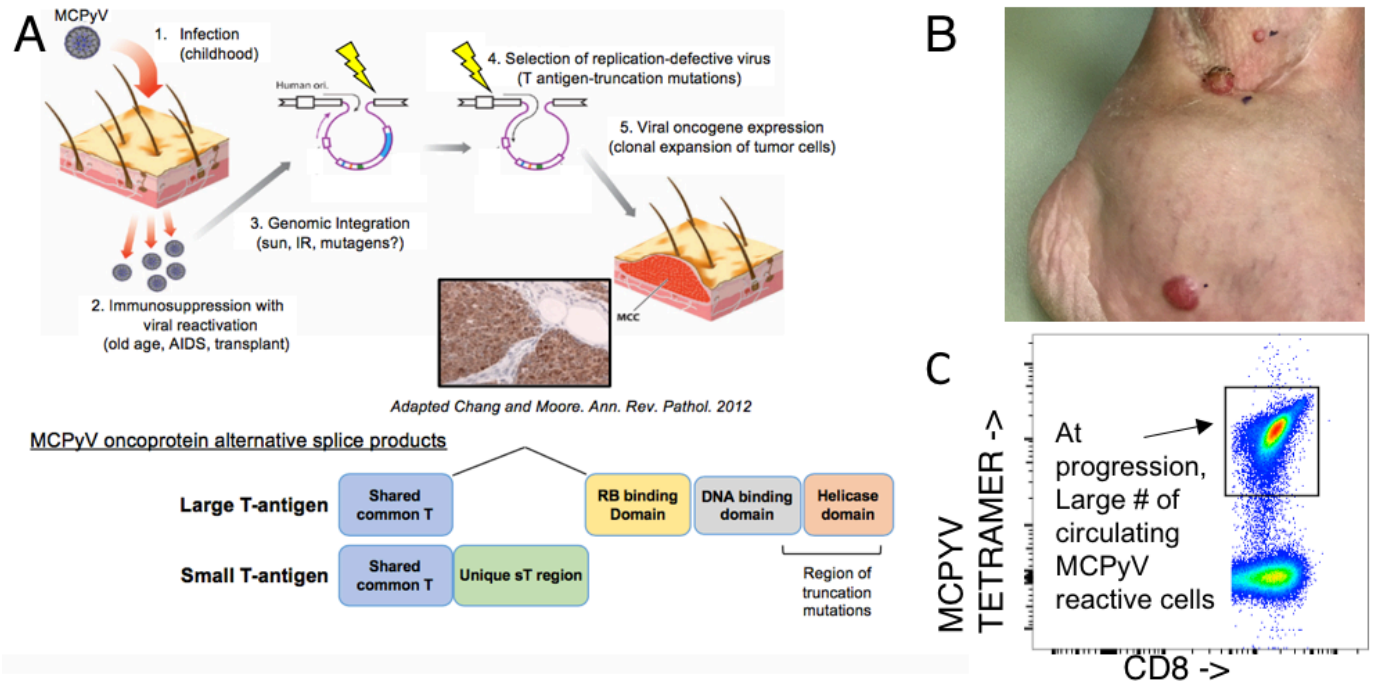
Name	Professional Title	Role on Project	Institution
Aude Chapuis, MD	Assistant Member	***PI***	FHCRC
Kelly Paulson, MD, PhD	Oncology Fellow	Post-doc	FHCRC (Chapuis Lab)
Paul Nghiem, MD, PhD	Professor, Division Chair	Collaborator	UW

These studies will be performed in the laboratory of Dr. Aude Chapuis, Assistant Member in the program in Immunology, in collaboration with Dr. Paul Nghiem (chair of UW Dermatology and expert on Merkel cell carcinoma and MCC immunity). No subcontracts to UW will be performed in the exploratory phase. The proposed studies will further benefit from the collaborative expertise of the Bielas laboratory.

Does this study involve human subjects? (Y / N) Animals? (Y/ N)
IRB/ IACUC Number: _FHCRC 1765, FHCRC 2586_____

ABSTRACT *(500/500 words)*

Merkel cell carcinoma (MCC) is a skin cancer with high disease-associated mortality. 80% of MCCs are driven by obligatory expression of the immunogenic Merkel cell polyomavirus (MCPyV) oncoproteins,[1-3] and these viral antigens represent appealing targets for cellular immunotherapy[4](**Figure1A**). PD1-axis blockade is effective in ~30-50% of MCCs[5,6], but half of patients do not respond, which likely reflects inadequate antigen-specific T cells. Our recent results suggest administering MCPyV-specific T cells alone is insufficient to control $MCPyV^+$-MCC, but combination with upfront PD1-axis blockade in PD1-naive patients produced 4/4 responses. **Understanding the mechanisms of response and resistance to virus-directed cellular therapy is critical to improve the efficacy of adoptive-transfer-based therapies for MCC and solid tumors more broadly**. One $MCPyV^+$-MCC patient mostly treated 'off trial' is particularly interesting. This patient with widely-metastatic MCC was treated first with PD-1 blockade, then MCPyV-specific T cells without response. PD1-axis blockade was resumed and again ineffective. He then received anti-CTLA and experienced immediate tumor regression, with a partial remission lasting ~1 year. Unfortunately, recently the patient again recurred implying immune escape (**Figure1B**). Remarkably, since adoptive transfer >1 year ago, 40% of the patients' peripheral T cells are MCPyV-specific and these cells infiltrate the tumors suggesting both robust persistence (**Figure1C**) and intratumoral localization; and biopsies taken after treatment show tumor specific pan class I HLA expression. No significant changes in the MCPyV specific T cell population at time of response or progression were seen in functional, clonotype, or epitope spreading analyses. Thus, the mechanism for his likely initial immune-mediated response followed by subsequent evasion remains frustratingly unclear. Candidate mechanisms for immune avoidance include loss of antigen-presentation machinery, loss of specific HLA necessary for epitope presentation (HLA-A24), T-regs or Th2 CD4s in the tumor microenvironment, expression of matrix proteins blocking immune infiltration, loss of epitope, among many others. An unbiased exploratory approach is critical to elucidate this patient's course and could identify broadly applicable mechanisms of adoptive immunotherapy success and failure, similar to a recently-reported colorectal cancer patient.[7] 10X-Genomics technology is uniquely poised to provide a detailed multidimensional view of the tumor microenvironment and cellular response. In the **exploratory phase**, to understand first the success then failure of immunotherapy in this patient directly at the tumor site, we will detail his tumor microenvironment including tumor cells, stromal elements, and innate and cellular immune subsets. This is possible as we have collected key material from time points of response and progression including **viable frozen single cell tumor digests** (contain tumor & stroma & infiltrating immune cells), TIL cultures, and PBMCs (**Figure1D**). We will complement data obtained from 10x-Genomics with T cell functional and phenotype data and FFPE/IHC. If the 10X-Genomics technology is successful, there is publication potential as a single case. If selected for the **pilot phase** we will compare the tumor microenvironment and TIL of patients with MCC in whom T cell therapies failed or succeeded. Specifically, we will expand our investigations for additional patients with MCC treated with T cells with specimens available, including responders (n=5) and non-responders (n=4) .



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Time point:	Pre-ImmunoTx	Failing T cells & α-PD-1	Responding to T cells & α-PD-1 & α-CTLA	Failing T cells & α-PD-1 & α-CTLA
Samples for 10X-Genomics	Single-cell tumor digest (viable) TIL (viable) PBMC	PBMC	PBMC	Single-cell tumor digest (viable) TIL (viable) PBMC
Complementary Samples	BX: IHC. TIL, PBMC: T cell phenotype, Adaptive	PBMC: T cell phenotype, adaptive	BX: IHC TIL: Adaptive PBMC: T cell phenotype, adaptive	BX: IHC. TIL, PBMC: T cell phenotype, Adaptive

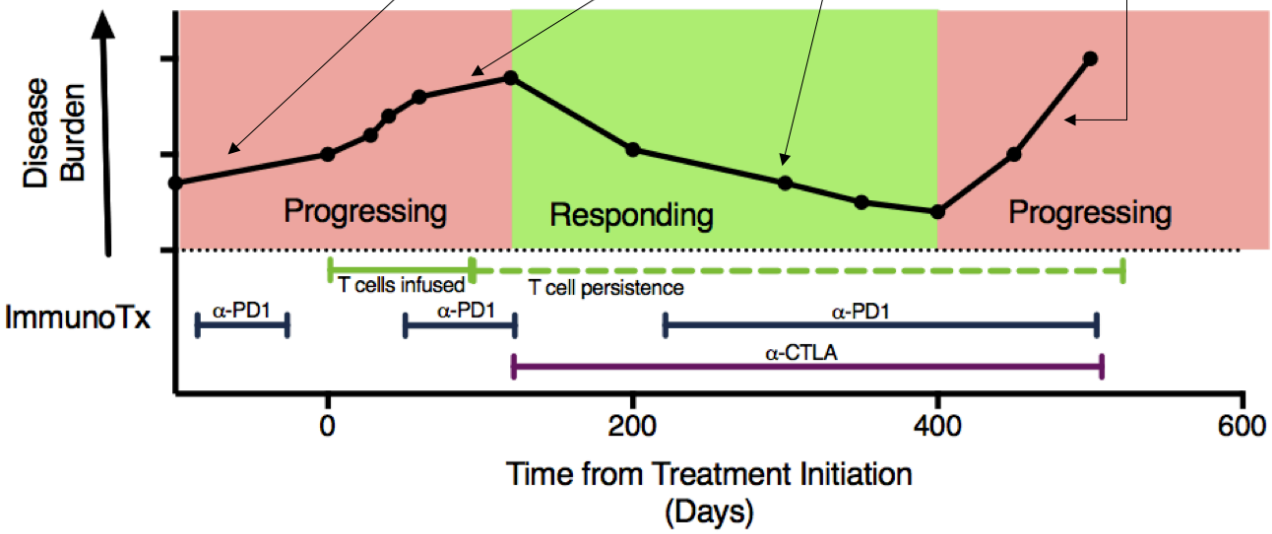

FIGURE 1: Overview of Merkel Cell Carcinoma and Proposed Studies

A) Schematic of Merkel cell polyomavirus life cycle and oncogenesis. 80% of MCCs persistently express viral oncoproteins which are targets for cellular immunotherapy.

B) Clinical photograph of patient with MCC of the left ankle who developed local, nodal, and distant (multiple visceral, orbit) metastases. Black dots mark MCC tumors. This is the patient on whom further analyses are planned for the exploratory phase.

C) Flow cytometry of peripheral blood for this patient at time of progression, demonstrating ample MCPyV-specific CD8+ lymphocytes, implying failure of immune therapy not due to lack of persistence of infused cells.

D) This patient developed progression upon treatment with anti-PD1 and T cells, followed by brisk response to anti-CTLA 4 therapy, and then again diffuse progression. Materials are available from many time points at the patient's course. Note that the tumor material is **single cell digest suspension of frozen, viable cells.** The 8 samples proposed for 10x analysis are listed.

REFERENCES

1. Paulson KG, Carter JJ, Johnson LG, et al. Antibodies to merkel cell polyomavirus T antigen oncoproteins reflect tumor burden in merkel cell carcinoma patients. *Cancer Res* 2010; **70**(21): 8388-97.
2. Iyer JG, Afanasiev OK, McClurkan C, et al. Merkel cell polyomavirus-specific CD8(+) and CD4(+) T-cell responses identified in Merkel cell carcinomas and blood. *Clin Cancer Res* 2011; **17**(21): 6671-80.
3. Miller NJ, Church CD, Dong L, et al. Tumor-Infiltrating Merkel Cell Polyomavirus-Specific T Cells Are Diverse and Associated with Improved Patient Survival. *Cancer Immunol Res* 2017; **5**(2): 137-47.
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6. Kaufman HL, Russell J, Hamid O, et al. Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial. *Lancet Oncol* 2016; **17**(10): 1374-85.
7. Tran E, Robbins PF, Lu YC, et al. T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer. *N Engl J Med* 2016; **375**(23): 2255-62.

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM 3/2017	THROUGH 3/2018

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Aude Chapuis, MD	PD/PI					0		0
SUBTOTALS →								0

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* 10x Genomics Reagants - $1500/sample x 8 samples = $12,000 Miscellaneous laboratory disposables = $500	12,500
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)* NGS sequencing through Genomics Core - $1500/sample x 8 samples = $12,000 Publication fees = $500	12,500

CONTRACTUAL COSTS	DIRECT COSTS	25,000
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		**$** 25,000
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE	0
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$** 25,000