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November 8-12
NATIONAL HARBOR
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Society for Immunotherapy of Cancer

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Single Cell RNA Sequencing Reveals Mechanisms of Merkel Cell Carcinoma Escape from Intense Pressure of T cell Immunotherapy

Kelly Paulson, MD, PhD
Dr. Aude Chapuis Laboratory
Fred Hutchinson Cancer Research Center
November 11, 2017

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Society for Immunotherapy of Cancer

Presenter Disclosure Information

Kelly Paulson, MD, PhD

The following relationships exist related to this presentation:

No Relationships to Disclose

This presentation discusses the following investigational or off-label therapies: endogenous T cell therapy (NCT01758458), pembrolizumab (off-label), ipilimumab (off-label)

Acquired Resistance has Substantial Clinical Impact

Acquired Resistance Is Challenging to Study

- Thousands of antigens/mutations, often "private epitopes"
- Multiple relevant cell populations in the microenvironment
- Multiple mechanisms of resistance

Adoptive cellular tx trials offer a window into interactions between T cells, Tumor, Microenvironment

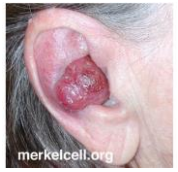
- Defined antigen/epitope & trackable T cell populations

Review: Sharma et al, Cell, 2017
Challenges of AR study: Riaz et al, Cell, 2017
Trackable T cells: Chapuis et al, Sci Immunol 2017

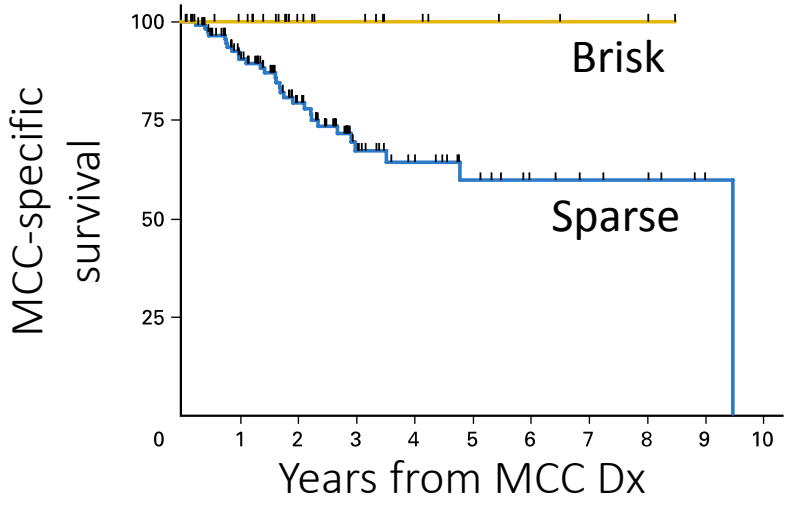
Merkel Cell Cancer: Ideal to Study Immunotherapy

Highly metastatic skin cancer

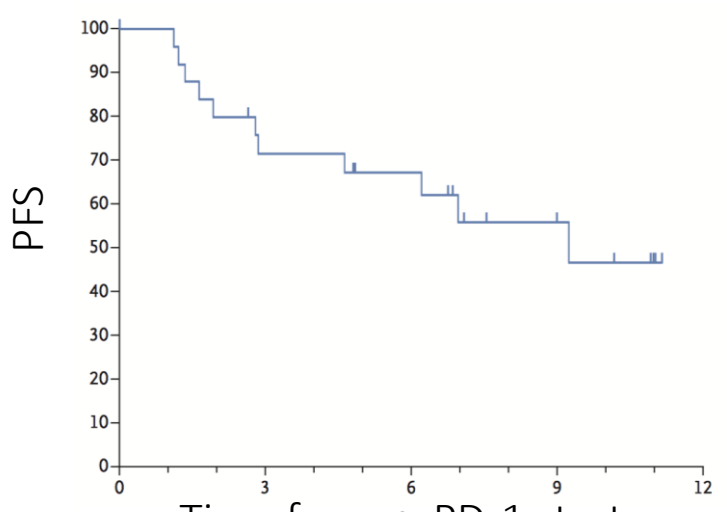
- More aggressive than melanoma
- 2500 cases/year in US
- Biopsiable skin/node mets



T cell sensitive: Intratumoral CD8+s at dx favorable



Immunotx responsive:
56% RR to pembrolizumab



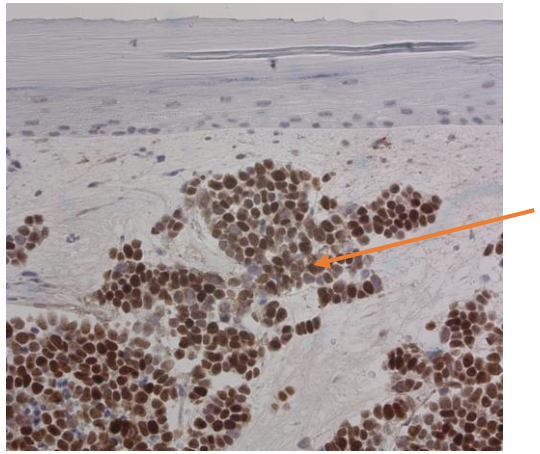

Dr. Shailender Bhatia


Dr. Mac Cheever


Dr. Paul Nghiem

Clinical Image: Nghiem P et al, merkelcell.org
Incidence: Paulson KG et al JAAD 2017
CD8 Infiltration: Paulson KG et al JCO 2011
Pembrolizumab: Nghiem P et al NEJM 2016

Merkel Cell Polyomavirus Causes 80% of MCCs



MCC tumor expressing viral oncoproteins (brown)

Discovered 2008 by U. Pitt team led by Drs. Chang and Moore

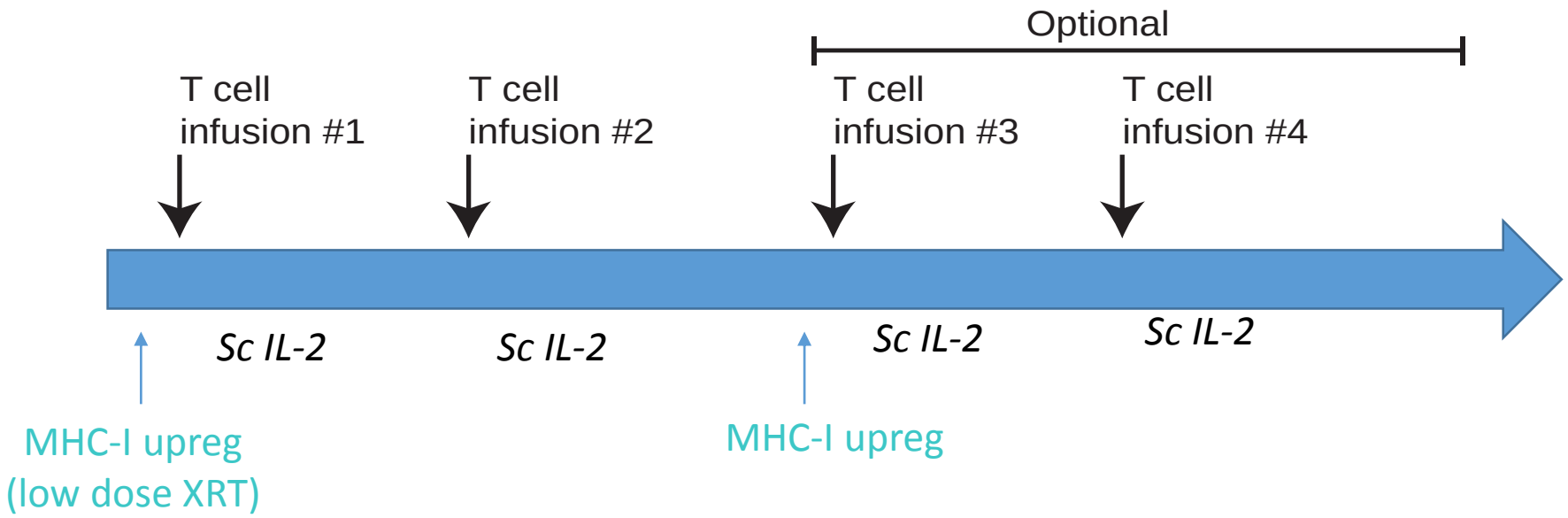
(image: post-gazette)

Feng et al, Science, 2008

- MCPyV Oncoproteins Exemplary Target Antigens:
  - Necessary for growth & Highly Expressed
  - Tumor Specific & Non-Human
  - Conserved between patients
- MCPyV+ MCCs have few non-viral Neoantigens

Autologous MCPyV-Specific T cells for MCC

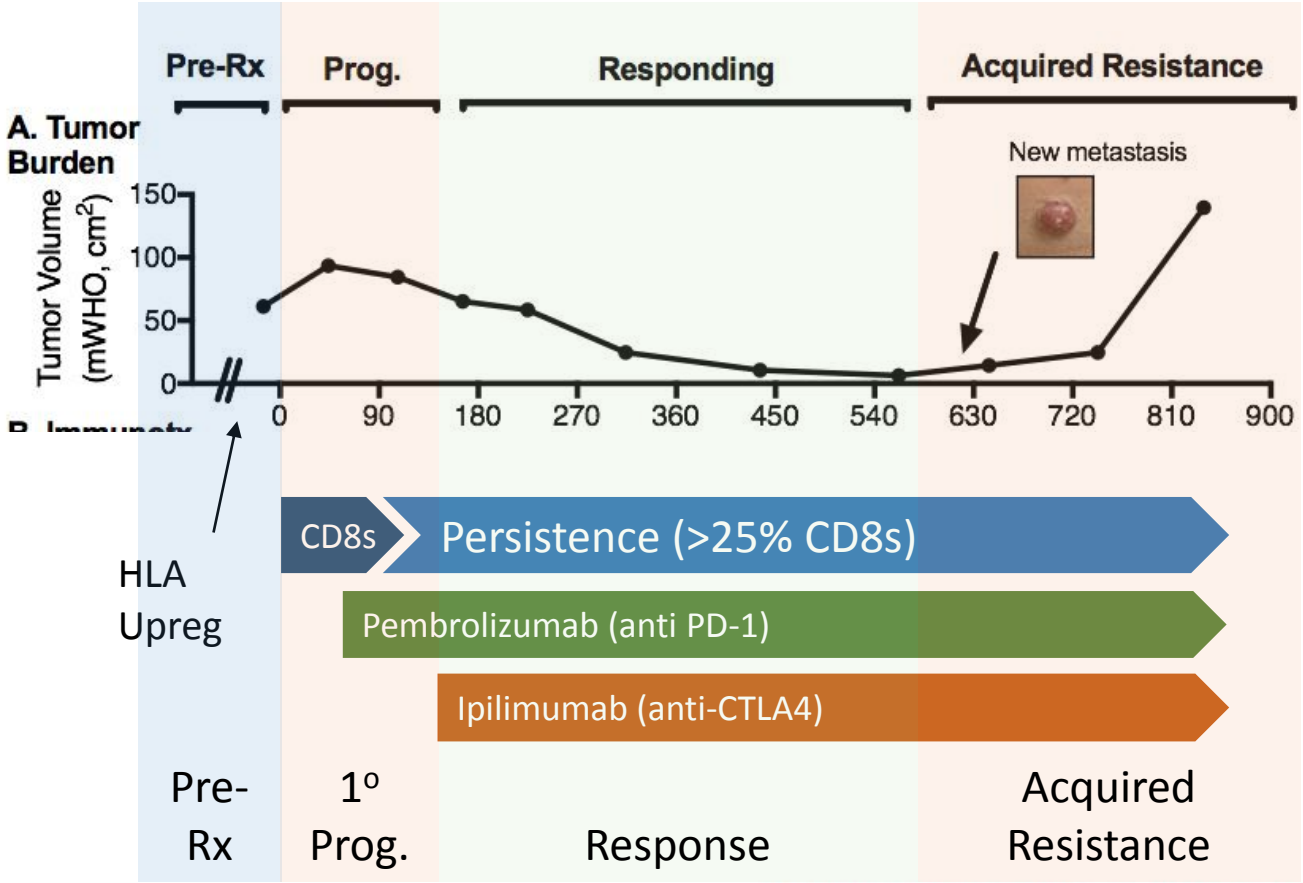
- Endogenous autologous MCPyV specific T cells
- Expanded ex vivo from peripheral blood and reinfused

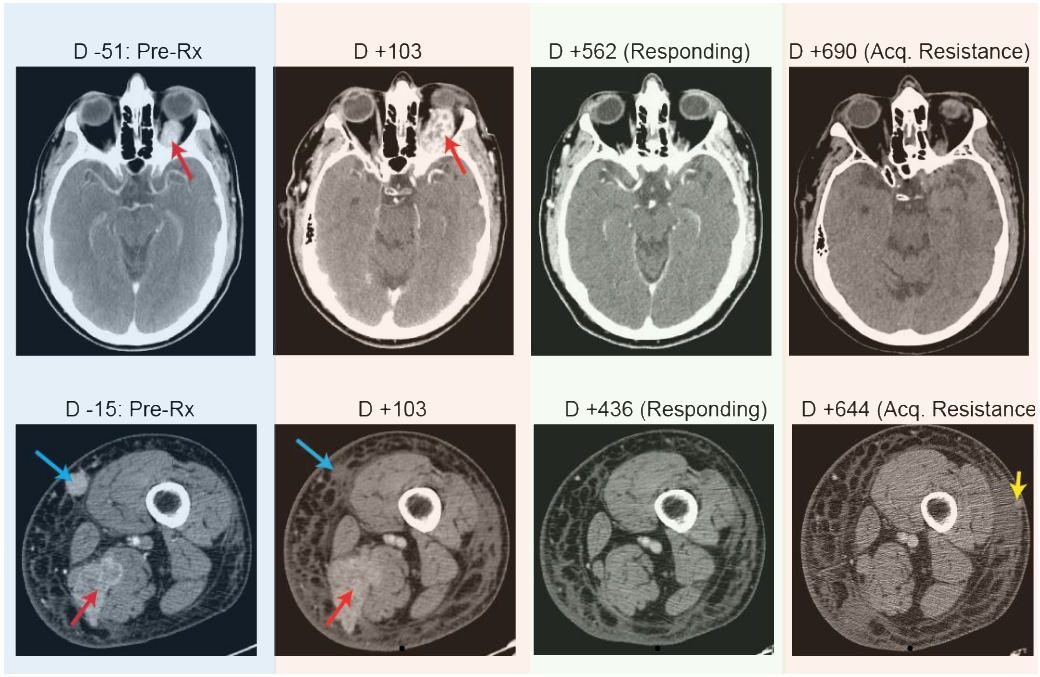



Dr. Aude Chapuis

NCT01758458

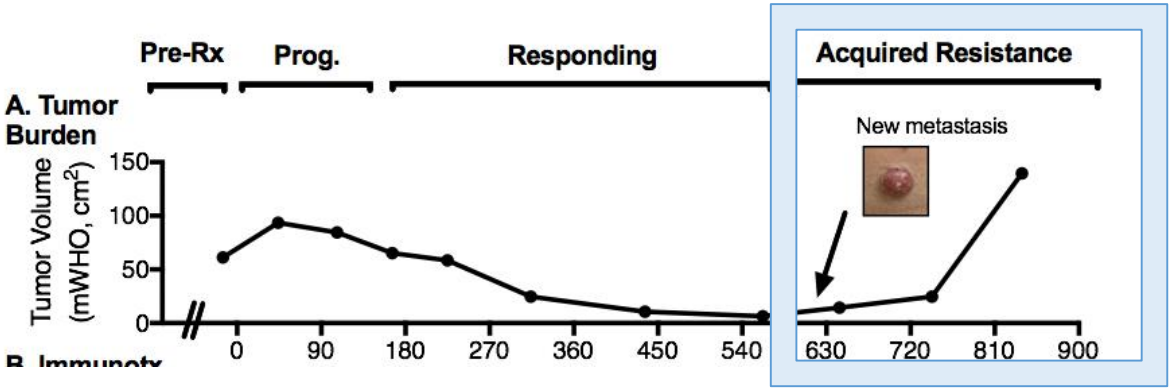
Case Study: Acquired Resistance After HLA-B Restricted Endogenous MCPyV-Specific CD8+s

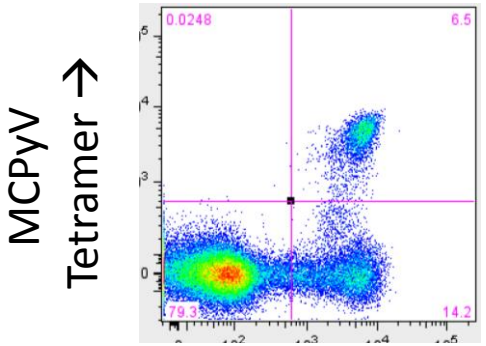




59 y/o M w/widely metastatic MCC refractory to multiple prior tx's including pembrolizumab

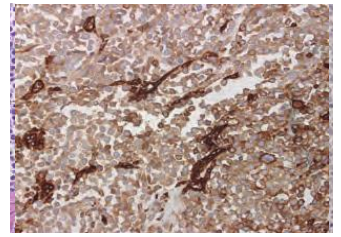
Acquired Resistance Mechanism Unclear by Comprehensive Standard Approaches



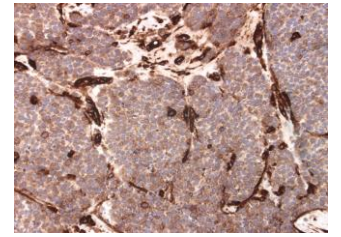


α-HLA-ABC

Before

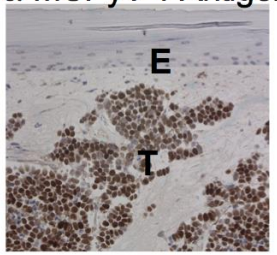


Acquired Resistance

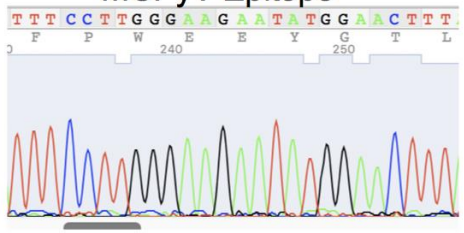


- Infused **T cells persisted**
- **Tumor expressed class I** MHC
- Tumor **expressed viral antigens** and epitope sequence unmutated
- Exome sequencing unrevealing

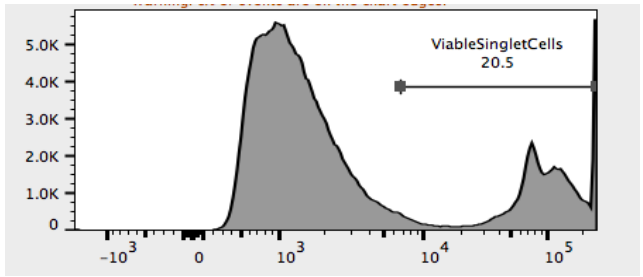
α-MCPyV T Antigen



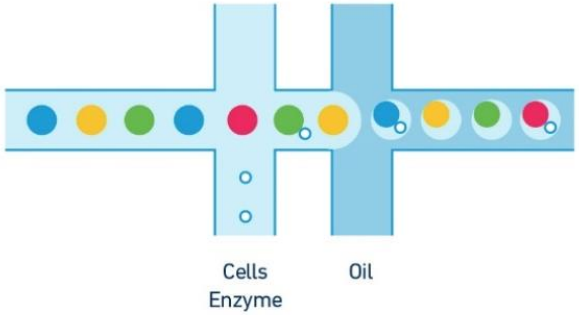
Sequence of Targeted MCPyV Epitope



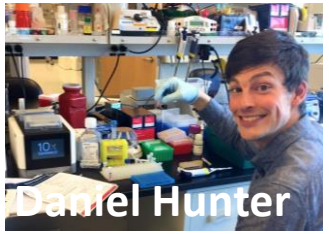
Single Cell Transcriptomics by scRNAseq



Viably frozen single cell product:
Mixed tumor, TIL, and stromal populations



Cell labelling
(up to 10K cells/sample)



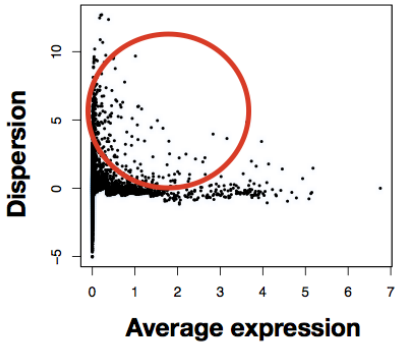
Sequencing

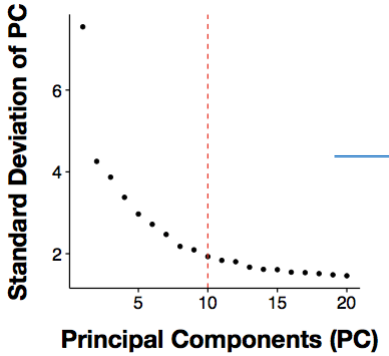
Cell ID	Transcript ID	Sequence (100-200nt 3')	Sample ID

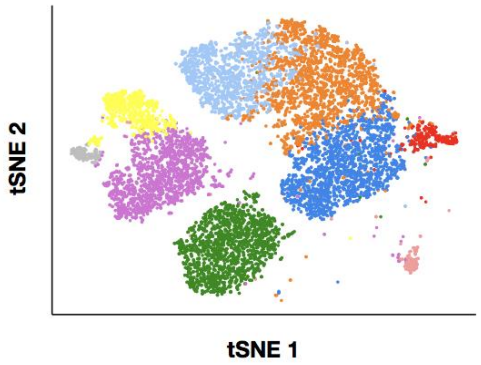
Oligo dT based RT & Library Prep

Zheng G.X. et al., Nat. Communications. 2016

ScRNAseq: Data Analysis





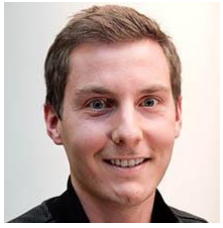


1. **Normalization**
(corrects for library size)

2. **Gene filtering**
(ID most variable genes)

3. **Principal Component Analysis**
(clusters cells by like gene expression)

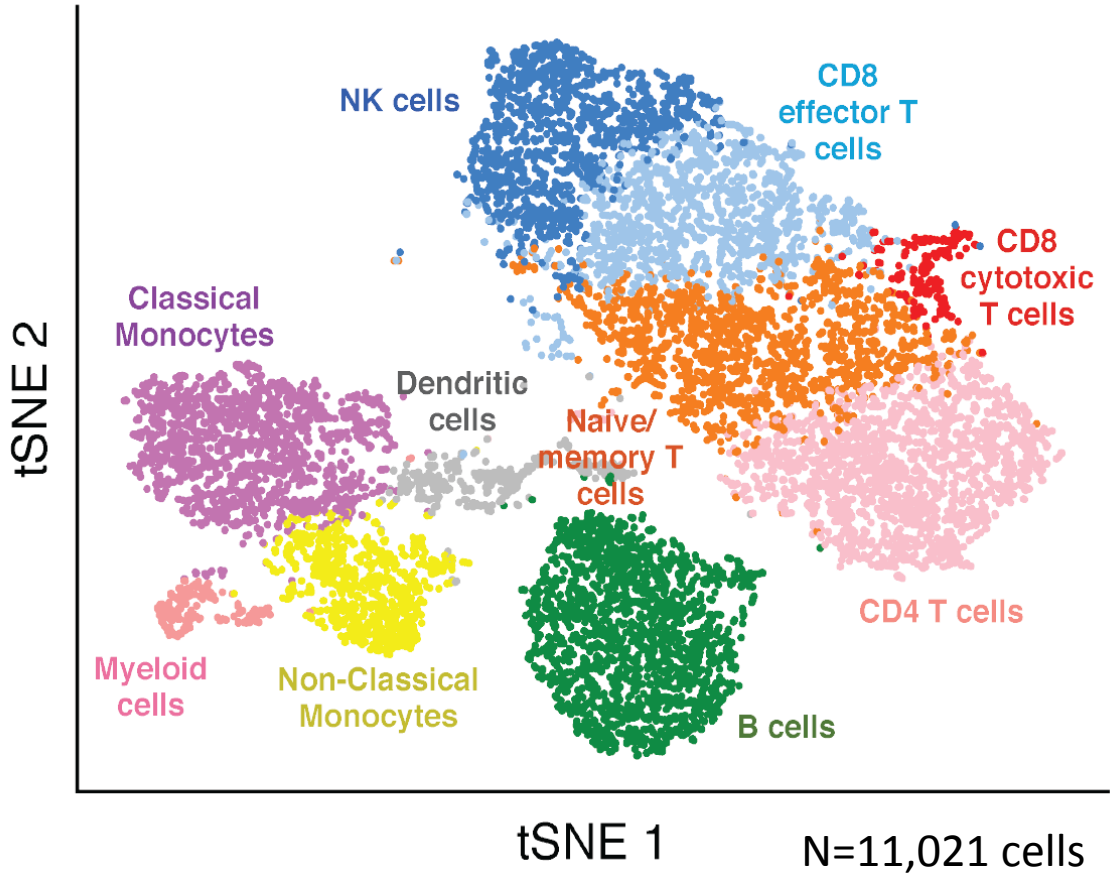
4. **tSNE**
(visualization)


Dr R. Gottardo

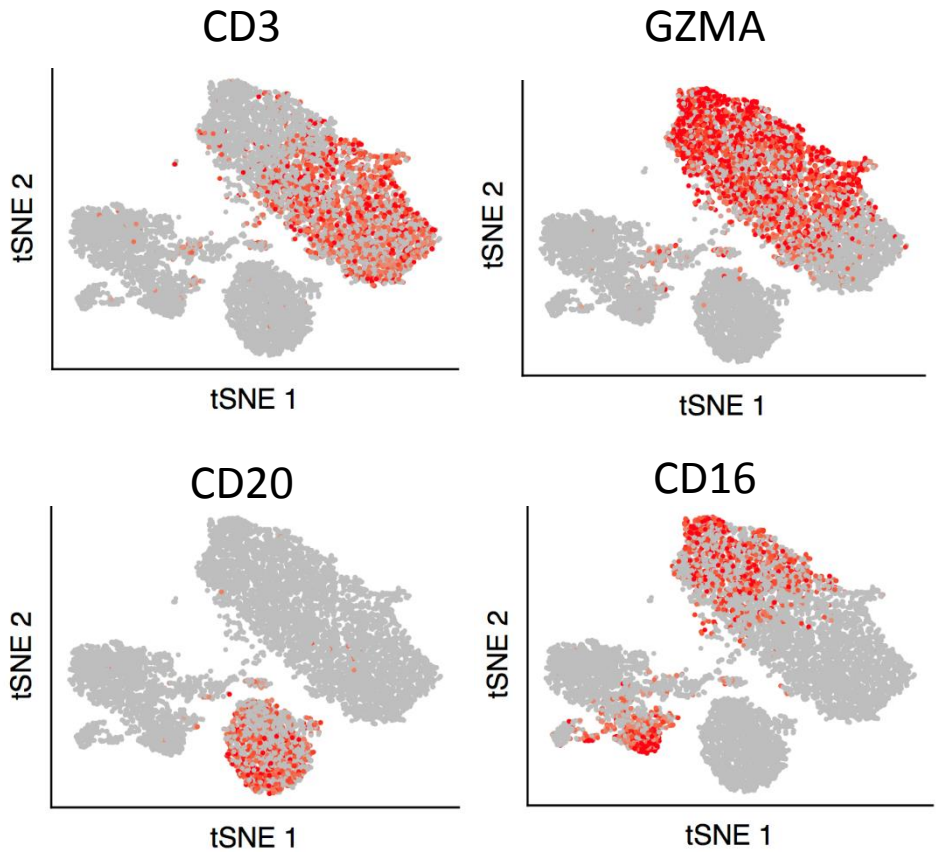

Dr V. Voillet

Satija R. et al., Nat. Biotech. 2015
Finak et al, Genome Biol, 2015

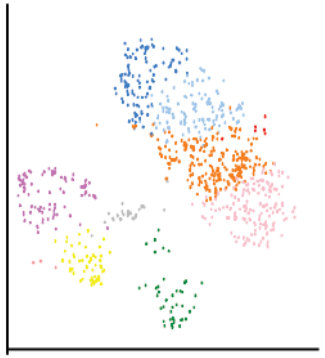
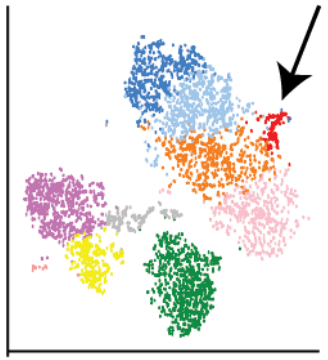
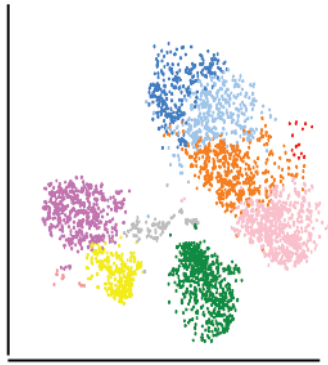
PBMC: What Cells Mediated Immune Response?



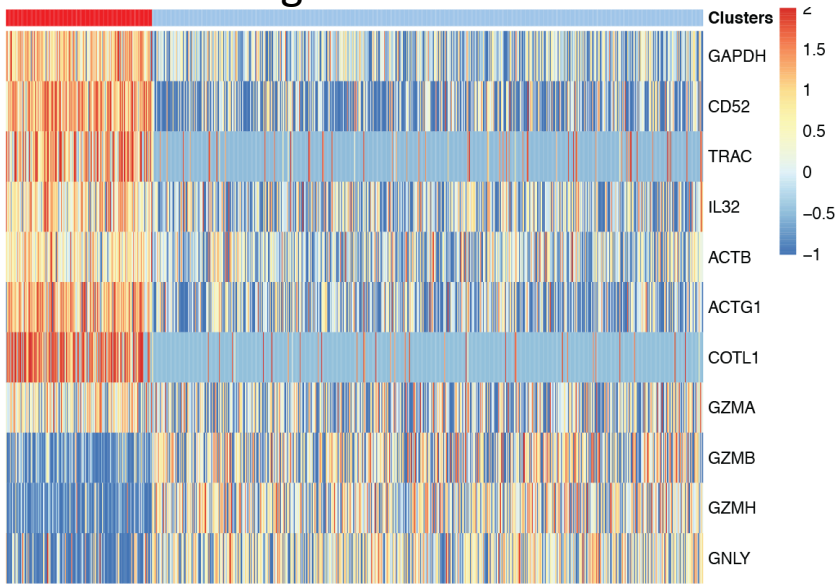
N=11,021 cells



Activated CD8+s: Mediators of Immune Response

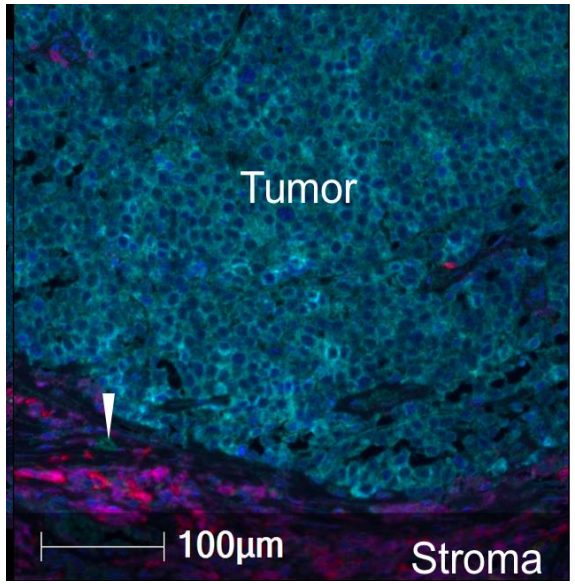
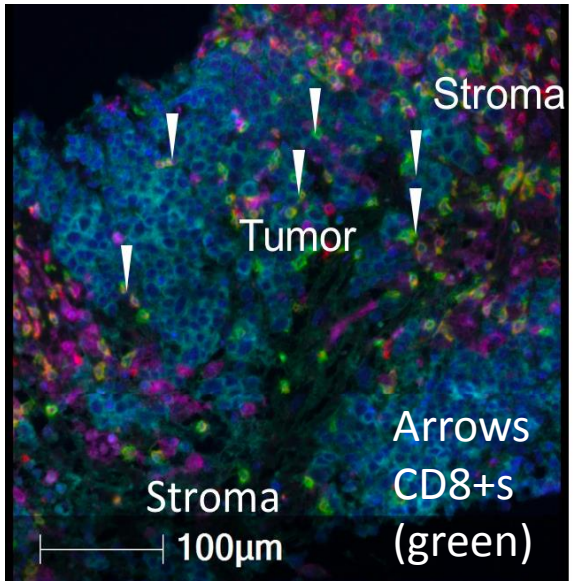
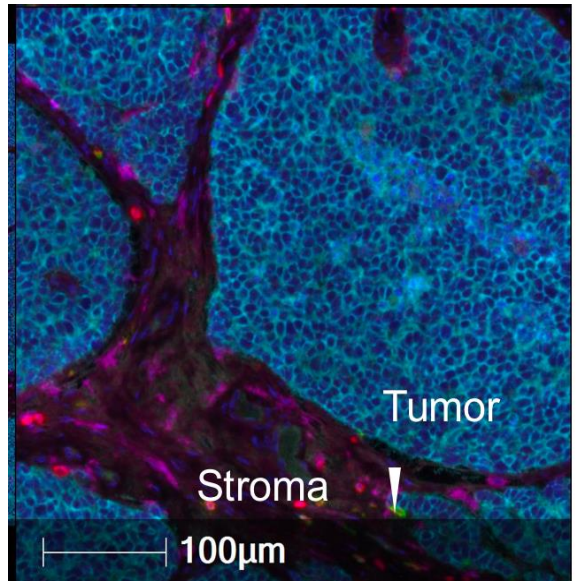
Pre-treatment	Early post-rx (D+27)	Responding (D+376)	Acquired Resistance (D+614)
			
% CD8+s in red cluster: 7		2	15	2

CD8+ CTL Cluster:
Red Light Blue



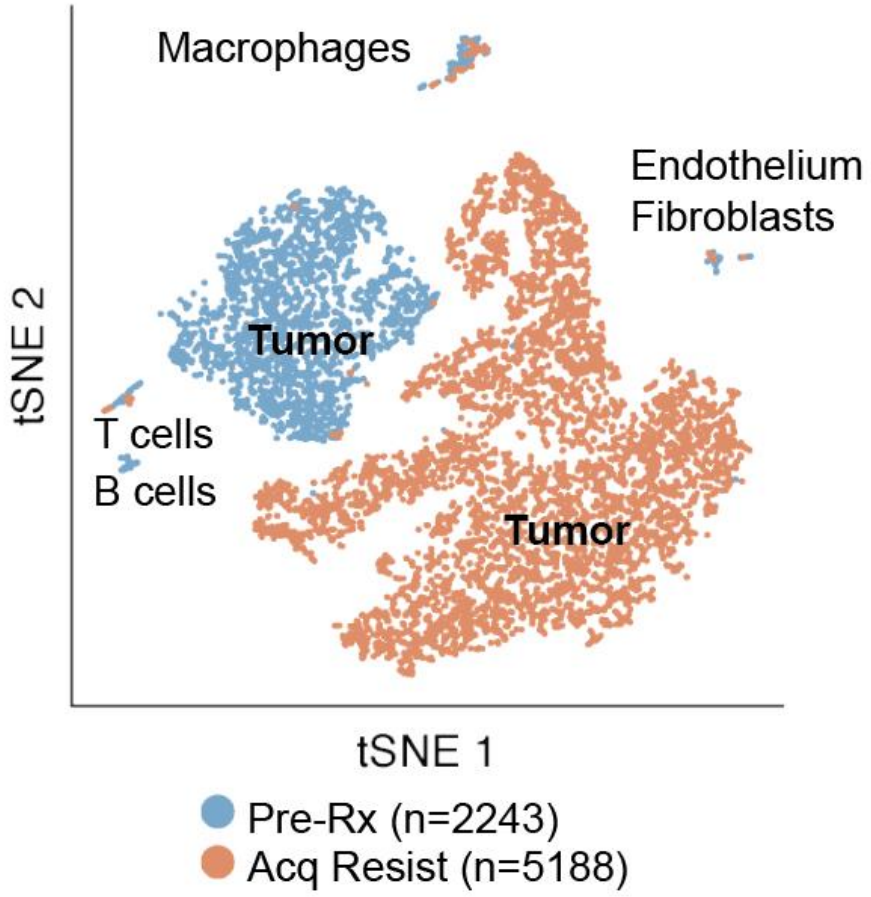
Red activated cluster: CD8+ T cells overexpressed activation, cell division, & glycolysis genes

Activated CD8+s Infiltrated Regressing Tumor

	Pre-Rx	Responding	Acquired Resistance
			
	2/mm²	328/mm² P = 0.01	56/mm²
Detection of infused cells by beta-seq?	No	**Yes**	

Dr. Rob Pierce

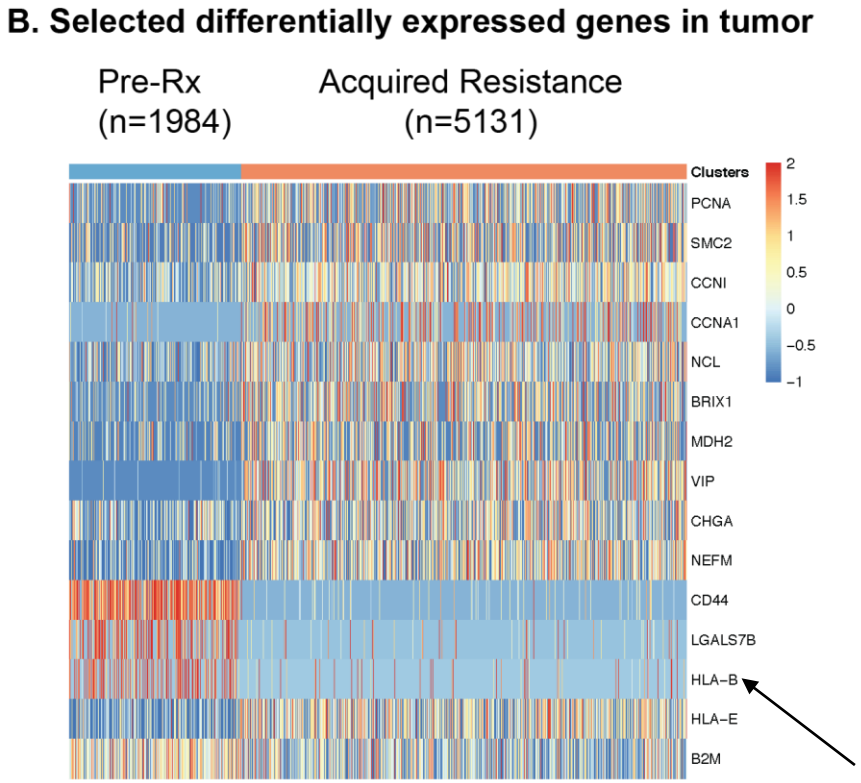
Tumor Cells Drive Acquired Resistance

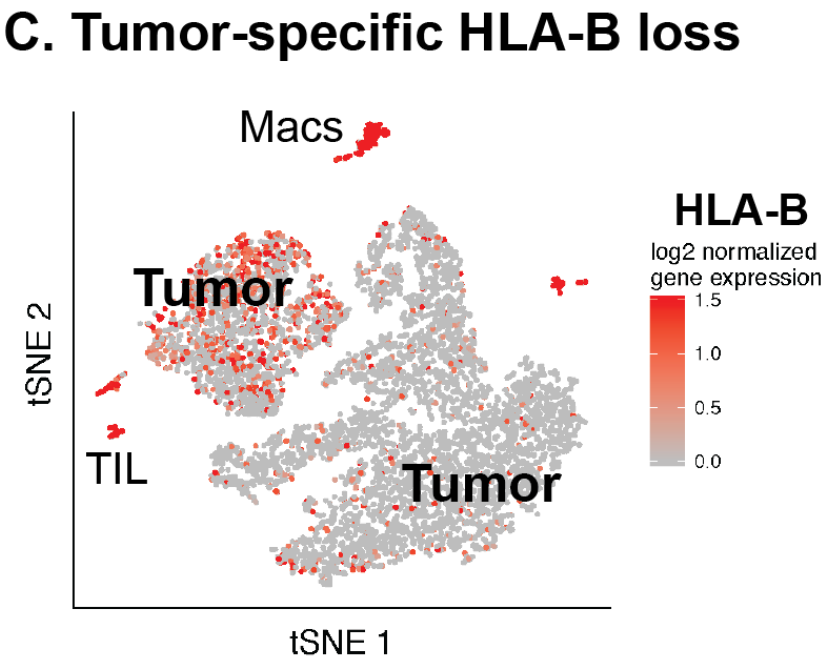


- TILs, TAMs, fibroblasts, endothelial cells superimposable pre-RX and AR
  - NOT drivers of acquired resistance

- Instead, huge expression shifts were seen in **tumor cells**

Mechanism 1: HLA-B Loss under T Cell Pressure

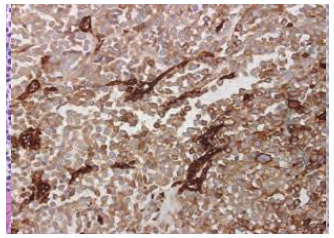




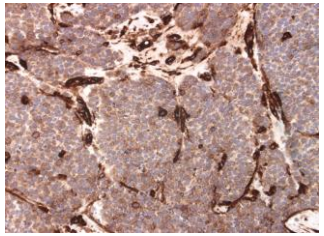
- Patient received HLA-B*3502 restricted CD8s targeting MCPyV

HLA-B Loss is Specific, Reproducible & Reversible

Before

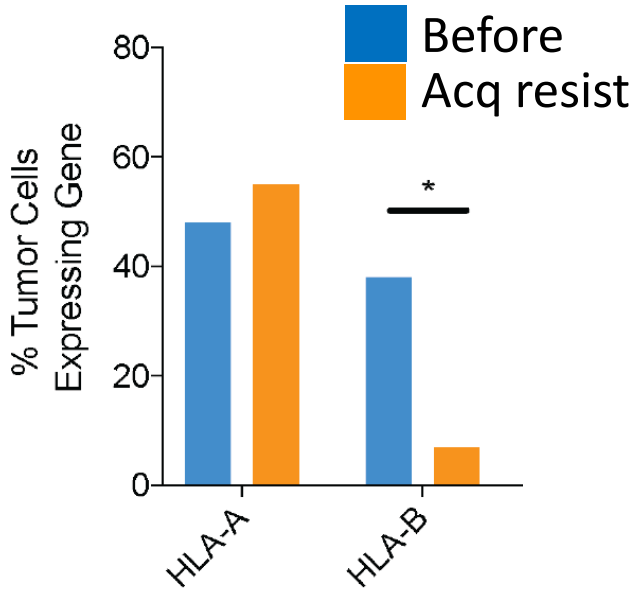


Acquired Resistance

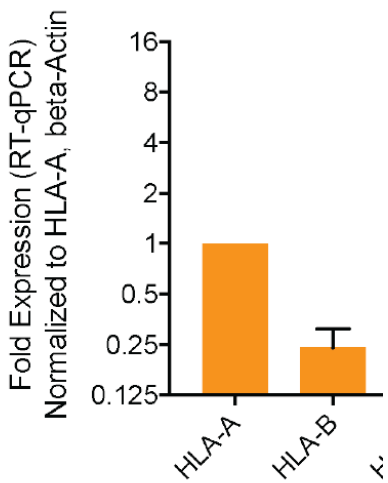


Maintained HLA-A Expression explains unrevealing IHC

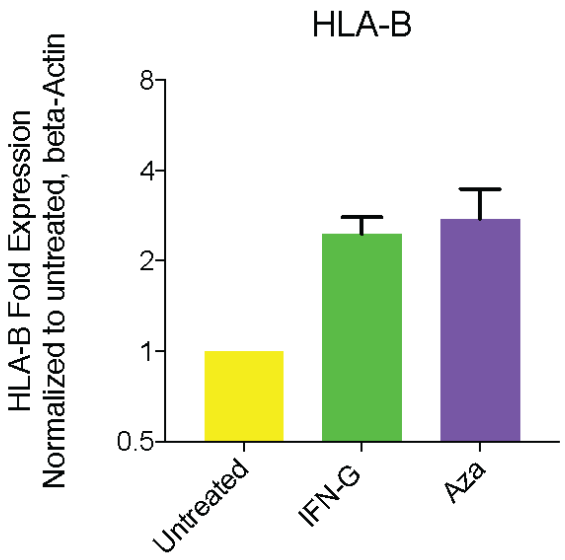
scRNAseq



qPCR of re-bx acq. resistance



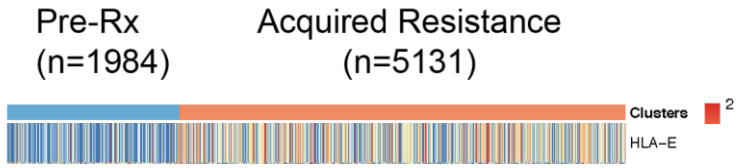
Rx w/5-Aza



Mechanism 2: HLA-E Upregulation to avoid NKs

- HLA-E Suppresses NK cells via NKG2A

- MCC tumor highly expressed HLA-E pre-treatment and further overexpressed this at relapse
  - This suggests a mechanism of NK resistance



Braud et al, Nature 1998

Lessons and Take Home Messages

- Cellular therapy trials offer insight into mechanisms of acquired resistance through defined antigen/T cell interactions
- scRNAseq on small specimens provides simultaneous information on thousands of cells from tumor, TIL, microenvironment. It is thus poised to maximize yield from immunotherapy studies.
- Data from our lab and others provide strong rationale for transfer of T cells of multiple specificities
  - Tran et al NEJM 2016

Thank You!

Dr. Aude Chapuis
Dr. Megan McAfee
Dr. Maurizio Perdicchio
Felecia Wagener
Daniel Hunter
Marcus Lindberg
Miranda Lahman
Erika Kiem

Dr Paul Nghiem
Dr. Natalie Vandeven
Dr. Candice Church
Dr. Rima Kulikauskas
Dr. Jayasri Iyer
Dr. Aric Colunga
Hannah Thomas
Krista LaChance
Dr. Natalie Miller

Dr. Philip Greenberg
& Lab

Dr Raphael Gottardo
Valentin Voillet PhD
Samson Koelle

Dr. David Koelle

Dr. Jason Bielas
BJ Valente, PhD

Dr. Shailender Bhatia

Dr. Rob Pierce

Dr. Cassian Yee

FHCRC Core Facilities
- IIRC
- Genomics
- Immunohistochemistry
- Flow cytometry
- CPF/GMP

Clinical Team
Toni-Ann Lupinacci
Kieu-Thu Bui
Cari Morin/Ana Radu
Susan Lemmon
Melanoma & skin RNs
Immunotherapy RNs

UW/FHCRC fellowship &
Dr. John Thompson

MCC Patients and their families
Without whom this research would not be possible

Funding:
NIH NATIONAL CANCER INSTITUTE
DAMON RUNYON CANCER RESEARCH FOUNDATION
FRED HUTCH CURES START HERE
UNIVERSITY OF WASHINGTON 1861
MCC Gift fund
Travel Support: SITC & WSMOS