Immunity and Therapeutic Efficacy

Brad Nelson, PhD
BC Cancer, Victoria BC
#SITCWinterSchool
January 25 2022
Disclosures

• Advisory Boards/Consulting: Virogin
• Contracted Research: Zymeworks, Innovakine Therapeutics
• Co-Founder, CEO: Innovakine Therapeutics
Tumor evolution gives rise to intratumoral heterogeneity

Clonal Theory (Nowell 1976)

Time

- Normal/Healthy Cell
- Tumor Population 1
- Tumor Population 2
- Tumor Population 3
- Clonal Mutation (exist in all cancer cells)
- Subclonal Mutations (exist in a subset of cancer cells)
How does the immune system contend with tumor evolution?

Clonal Theory (Nowell 1976)

Time

- Normal/Healthy Cell
- Tumor Population 1
- Tumor Population 2
- Tumor Population 3
- Clonal Mutation (exist in all cancer cells)
- Subclonal Mutations (exist in a subset of cancer cells)
High-Grade Serous “Ovarian” Cancer (HGSC)
TCGA data mining reveals a correlation between mutation load and CD8+ TIL.

- HGSC has an intermediate point mutation load.
- POLE
- MSI
- Cervical
- Endometrial
- Ovarian (HGSC)
HGSC has extraordinarily high copy number alterations

Ciriello et. al. Nat Gen 2013
HGSC tumors often exhibit robust TIL responses

Killer T cells (CD8)
Helper T cells (CD3)
B cells (CD20)
Plasma cells (CD79a)
Macrophages (CD68)
Tumor cells (panCK)
TIL are strongly associated with survival in HGSC

Dense CD8+ TIL

Sparse CD8+ TIL

BCCA/VGH cohort
high-grade serous (HGSC)
optimally de-bulked
n = 200
p = 0.0008

Clarke, B. et al. 2009
Milne, K. et al. 2009
Conundrum

• Hot tumors are common and favorably prognostic in HGSC
• Yet response rates to current immunotherapies are low:
  – checkpoint blockade: 10-15% OR
  – TIL/CAR-T therapy: best response is stable disease
Three requirements for a successful immune response:

1. Robust TIL responses
2. Killer T cells (CD8)
3. Helper T cells (CD3)
4. B cells (CD20)
5. Plasma cells (CD79a)
6. Macrophages (CD68)
7. Tumor cells (panCK)
Three requirements for a successful immune response

- **Antigens**
- **Access**
- **Activity**
Extensive spatial profiling of 212 primary tumors from 38 HGSC patients

Whole genome sequencing
→ clonal architecture & mutation signatures

Multi-colour IHC
→ TIL patterns: T cells, B cells, plasma cells

Nanostring profiling
→ molecular subtype, immune gene expression

TCR & BCR sequencing
→ T cell and B cell clonal distributions

Allen Zhang, Rob Holt, Sohrab Shah, et al. Cell 2018
Tumor evolution leads to intratumoral heterogeneity

Clonal phylogeny in ovarian cancer (patient 4)

A McPherson...S Shah, Nature Genetics 2016
Patients show a range of TIL “temperatures”

*Immunohistochemistry data for 4 TIL subsets*

Density (cells/HPF)

TIL:
- CD8+ T cell
- CD4+ T cell
- CD20+ B cell
- Plasma cell

Allen Zhang, Rob Holt, Sohrab Shah, et al. Cell 2018
TIL densities can vary widely within a patient

CD8+ TIL densities at 10 anatomical sites, 20 high-powered fields each

Allen Zhang, Rob Holt, Sohrab Shah, et al. Cell 2018
TIL are negatively associated with intratumoral heterogeneity. Polyclonal tumors tend to be cold, while monoclonal tumors tend to be hot.

Clonal phylogeny in ovarian cancer (patient 4)

Monoclonal tumors tend to be hot

Polyclonal tumors tend to be cold

Allen Zhang, Rob Holt, Sohrab Shah, et al. Cell 2018
T cell clones track spatially with tumor clones

Example from ovarian cancer patient #2

Tumor clone phylogeny:

TCR sharing at 4 tumor sites:

Allen Zhang, Rob Holt, Sohrab Shah, et al. Cell 2018
Hot tumors show signs of immune editing

Example from ovarian cancer patient #15

**HLA allelic loss:**

Loss of:
- HLA-A*24:02
- HLA-B*13:01
- HLA-C*03:04

**Neoantigen depletion:**

Observed/expected subclonal neoantigen rate

Allen Zhang, Rob Holt, Sohrab Shah, et al. Cell 2018
Lung cancer: immune evasion is linked to prognosis

- Non-small-cell lung cancer: 88 cases and 258 specimens
- Hot tumors show decreased clonal diversity and increased immune editing (neoantigen and/or HLA loss)
- The extent of immune evasion was key to prognosis

![Diagram showing immune-evasion capacity]

**Immune-evasion capacity**
- Low immune evasion
  - High immune infiltration or no immune escape
  - No immune editing
  - No HLA LOH
  - No antigen-processing defect
- High immune evasion
  - Low/mixed immune infiltration and immune escape
  - Immune editing /
  - HLA LOH /
  - Antigen-processing defect

**Figure**
- Disease-free survival
- **HR = 0.27 (0.12–0.61)**
- log-rank $P = 9 \times 10^{-4}$

*Rosenthal R…Swanton C. Nature 2019*
Checkpoint blockade can select for a variety of immune evasion mechanisms.
Checkpoint blockade can select for a variety of immune evasion mechanisms

Number of cases showing a given resistance mechanism

- B2M (n=9)
- Other (n=10)
- JAK (n=3)
- Neoantigen depletion (n=4)
- Tumor-mediated immunosuppression (WNT, n=2; PTEN, n=6)
- Additional inhibitory checkpoints (VISTA, n=8; LAG3, n=5; TIM3, n=5)
- Unknown (n=19)
Tumor-specific CD8+ T cells can emerge & then disappear during tumor progression

T cell clones:
- Vβ13.6
- Vβ5.2
- Vβ1

Spencer Martin et al, Oncoimm 2017
Only a small minority of CD8+ TIL are tumor reactive in HGSC

Scheper, Kelderman...Schumacher, Nat Med, 2018
Despite their prognostic benefit, hot tumors can exhibit:

- Antigen loss
- MHC loss
- Loss of tumor-reactive T cells over time
- Multiple immune suppressive factors
- High proportion of bystander T cells
- Mixture of hot & cold sites in individual patients
- Progression toward colder tumors at end stage

What is the phenotype of tumor-reactive TIL and how can we help them?
Defining the phenotype of tumor-reactive CD8 TIL in HGSC

Co-expression of CD39, PD1 and CD103
A small subset of CD8+ TIL co-express CD39, PD1 & CD103

% of intraepithelial CD8+ TIL

Cell phenotype:
- Triple-positive
- Other
- Triple-negative

CD39: + + + + - - - - - -
CD103: + + - - + + - - - -
PD-1: + - + + - + - + -
Co-expression of CD39, CD103 & PD-1 defines the most prognostically favourable CD8 TIL

Céline Laumont, Maartje Wouters, Julian Smazynski et al. Clin Can Res 2021
Single-cell profile of CD8 TIL co-expressing CD39, PD1 & CD103

Highly activated, complex phenotype:
- **Mean** = 25 TCRs per patient
- **Granzyme B** – killing
- **PD-1, CD39, TIGIT, LAG-3, TIM-3** – inhibitory
- **CXCL13** – B cell recruitment

Céline Laumont, Maartje Wouters, Julian Smazynski et al. Clin Can Res 2021
The hottest tumours contain T cells, B cells, plasma cells & macrophages.
The hottest tumours contain tertiary lymphoid structures

- Killer T cells (CD8)
- Helper T cells (CD3)
- Plasma cells (CD79a)
- Dendritic cells (CD208)
- FDCs (CD21)
- HEVs (PNAd)

T cell zone
GC
Follicle
HEV

100 μm
Some hot-ish tumours contain T cells and macs but not B cells

- Killer T cells (CD8)
- Helper T cells (CD3)
- B cells (CD20)
- Plasma cells (CD79a)
- Macrophages (CD68)
- Tumor cells (panCK)
TIL-B responses are associated with stronger cytolytic signatures.

TCGA ovarian cancer dataset

- **CD8A**
  - P < 0.0001
  - ****

- **IFNG**
  - P < 0.0001
  - ****

- **GZMB**
  - P < 0.0001
  - ****

- **PRF1**
  - P < 0.0001
  - ****

Key:  
- **no B cells**  
- **B cells**  
- **PC**

Tumor-infiltrating T cells and B cells show a combined effect on survival.

Ron deLeeuw et al. Can Imm Res 2015
Wouters & Nelson SITC 2017
Abundance and prognostic significance of B cells and plasma cells across cancers

**B cell and plasma cell abundance:**

![Color-coded heatmap showing abundance of B cells and plasma cells across various cancer types.]

**Prognostic associations:**

![Color-coded heatmap showing associations between B cells and plasma cells across various cancer types.]

Céline Laumont et al, in revision
B cells are associated with survival and immunotherapy response in sarcoma
Florent Petitprez…Wolf H. Fridman, Nature Jan 23 2020

Tertiary lymphoid structures improve immunotherapy and survival in melanoma
Rita Cabrita…Goran Jonsson, Nature Jan 23 2020

B cells and tertiary lymphoid structures promote immunotherapy response
Beth A. Helmink…Jennifer A. Wargo, Nature Jan 23 2020

Mature tertiary lymphoid structures predict immune checkpoint inhibitor efficacy in solid tumors independently of PD-L1 expression
Lucile Vanhersecke…Antoine Italiano, Nature Cancer Aug 2021
B cells & T cells have complementary definitions of ‘self’

Céline Laumont et al, in revision
B cells can promote antigen spreading, a holy grail of immunotherapy

Céline Laumont et al, in revision
B cells and plasma cells have an impressive anti-tumor armamentarium.
B cells and plasma cells have an impressive anti-tumor armamentarium

Laumont and Nelson
Cancer Cell 2021

New therapeutic opportunities
Take home messages

• Tumors evolve under numerous selective pressures, including the immune response
• Like all treatments, immunotherapy can shape tumor evolution significantly
• T cell responses are important yet fragile
• The strongest, most prognostic, durable TIL responses involve T cell, B cells and macrophages
• B cells and antibodies use diverse effector mechanisms which are orthogonal to T cells
• A more holistic understanding of TIL cell types and mechanisms will inspire new approaches to immunotherapy
### Nelson Lab:
- Alex Rodriguez, PhD
- Phineas Hamilton, PhD
- Céline Laumont, PhD
- Julian Smazynski
- Allyson Banville
- Monica Fuss
- Shreena Kalaria
- Megan Fuller
- Alexi Pearson-Lund
- Mitchell Adamson
- Sam Preshaw

### Key Alumni:
- Maartje Wouters, PhD
- David Kroeger, PhD
- Nicole Little, MSc
- Darin Wick
- Ron deLeeuw, PhD
- Charlotte Lo
- Spencer Martin
- Kwame Twumasi-Boateng, PhD

### MCIC (Histo Core):
- Katy Milne
- Bronwyn Gibson-Wright
- Heather Derocher
- Sonya Laan
- Stacey LeDoux
- Hannah McCarter
- Chanel Ghesquiere
- Daniel Kos
- Talia Gooyear

### Collaborators - Victoria:
- Peter Watson, MD
- Julian Lum PhD
- Sindy Babinsky

### Collaborators - Ottawa:
- John Bell, PhD
- Harry Atkins, MD
- Natasha Kekre, MD

### Collaborators - Toronto:
- Pam Ohashi, PhD
- Linh Nguyen, PhD
- Marcus Butler, MD

### Collaborators - Montréal:
- John Stagg, PhD
- Réjean Lapointe, PhD
- Anne-Marie Més-Masson, PhD

### Collaborators - Vancouver:
- Anna Tinker, MD
- Blake Gilks, MD
- David Huntsman, MD
- Jessica McAlpine, MD
- Dianne Miller, MD
- Michael Anglesio, PhD
- Christian Steidl, MD
- Liz Chavez
- Lauren Chong

### Collaborators - MOCOG/OTTA/AOCS:
- Leigh Pearce, PhD
- Malcolm Pike, PhD
- Susan Ramus, PhD
- David Bowtell, PhD
- Dale Garsed, PhD
- Anna DeFazio, PhD

### Funding:
- BC Cancer Foundation
- CCSRI
- CIHR
- TFI
- US DOD
- CRS
- Genome BC
- BioCanRx NCE
- Conconi Family
- CFI
- MSFHR
- Innovakine Therapeutics

---

**Special thanks to our patients**