FROM MOLECULE TO PATIENT

ASCPT 2019 ANNUAL MEETING
Basic immunology for clinical pharmacologists: application to cancer therapy

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What do you know? True or False

• Transgenic T cells only recognize intracellular antigens?
• Therapeutic mononclonal antibodies are only used to block checkpoint inhibitor molecules?
• Only dendritic cells are antigen presenting cells?
• Adaptive immunity only recognizes mutations in tumor cells?
• Immunohistochemistry is the most useful way to monitor anti-tumor immunity?
Fundamental components of immunity

- Rapid response
- Pattern recognition
- Scavenger receptors
- Cytokines and other instructive molecules
- Direct response for host defense
  - Phagocytosis
  - Anti-microbial activity
- Slow response
- Increasing affinity receptors
- Memory
  - Recirculation
  - Self-renewal
  - Qualitative changes

Pattern recognition molecules drive innate inflammation

Inflammation provides context for adaptive immune response

Adaptive immunity of primarily composed of T cells and B cells

T cell receptor

T cell subtypes:
- CD8 = cytotoxic
- CD4 = helper

Antigen receptors:
- Highly diverse due to recombination during lymphocyte development
- 2.5e7 possible combinations
- Clonal selection drives specificity

B cell receptor

Antibody isotype influences activity

IgA
IgE
IgG
- IgG1, IgG2, IgG3, IgG4
IgM
IgD
Antibody and TCR structure

The T cell antigen receptor

- Antigen combining site
- Resembles an Ig Fab fragment
- Domain structure: Ig gene superfamily
- Monovalent
- No alternative constant regions
- Never secreted
- Heterodimeric, chains are disulfide-bonded
- Very short intracytoplasmic tail
- Positively charged amino acids in the TM region
- Antigen combining site made of juxtaposed Vα and Vβ regions
- 30,000 identical specificity TcR per cell

https://www.slideshare.net/rajud521/t-cell-antigen-receptor
Generation of diversity in lymphocyte antigen receptors

- 2.5e7 possible combinations!
- Can’t be germline encoded (compare with PRR and NK-R)
- Allelic exclusion: 1 antigen receptor per cell
- Fitness of receptor is defined by positive and negative selection

- Class switching
- Affinity maturation
- Somatic hypermutation

http://www.biology-pages.info/A/AgReceptorDiversity.html

adapted from Janeway CA et al. 2001 (26)
Functions of antibodies

Different Ab isotypes have different activities

 Functions and properties of immunoglobulin

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Figure 4-17 part 2 of 2 Immunobiology, 6/e, © Garland Science 2005
Agonist antibodies mimic ligands; role for cross-link via FcR binding

T cells are the major mediators of tumor immunity due to their effector activities. The production of these molecules and cytolytic activity are commonly monitored to examine “functional” immunity.
Why Immunotherapy for Cancer?

• Immune system is exquisitely specific: chemo- and radiation therapy are not; even TKIs can be off-tumor.
• Immune system spreads to many areas of the body and is quick to respond upon re-exposure to antigen.
• Immune system remembers. Responses are durable.
• Immunity can be engineered and personalized with synthetic biology.
T cells in tumor are a good prognostic indicator

What are these immune cells; how did they get here and why are they relevant?

Central P = 0.0358

Peripheral P = 0.0661
Today’s goal is to help you understand this!
T cells recognize degraded proteins presented at the surface of cells: they peer inside cells (MHC restriction)

CD4 T cell: MHC class II

CD8 T cell: MHC class I

Dendritic Cell

MHC II = pAPC
MHC I = all nucleated cells

Tumor cell
Antigen processing

-Antigen can be acquired by multiple different receptor systems
-Macrophages and B cells can presented antigen acquired exogenously on MHC class II molecules
-Only DC can present exogenously acquired antigen on MHC class I molecules.

Nature Reviews Immunology 12, 813–820, copyright 2012.
T cells require multiple “signals” for support....

- **TCR: Signal 1**
- **Costimulation: Signal 2**
- **Cytokines: Signal 3?**
- **Proliferation and Survival**
Tolerance

Mechanism to prevent the diversity of receptors generated during recombination from attacking the “self” proteins.

Peripheral tolerance is context dependent—

inflammation (PRR...)

Resting DC shut-off T cells.

How is tumor antigen presented?
Environmental conditions dictate T cell differentiation and functions with canonical transcription factors.

A role for adjuvants in vaccines
Clonal selection

Naïve population

Primary response (effectors)

Memory

Lymph Node

Metabolic, transcriptional and epigenetic changes = memory T cells are “poised”
Clonal diversity can be estimated by TCR sequencing
-similar process for B cells; memory and plasma cell generation
Trafficking: how to get off the Beltway! Zipcodes and parking permits.
Resistance Mechanisms: Darwinian or Newtonian?

- Inflammation in the TME, commonly type I or type II IFN, drive responses designed to limit immunopathology.

Tumor cell intrinsic:
- PDL1
- MHC

Extrinsic:
- Treg
- MDSC
- M2 macrophages
- granulocytes
- CAFs

Environmental:
- nutrient availability
- hypoxia
- acidification

Primary vs Adaptive Resistance
Target rich environment for immunologists
Targeting resistance mechanisms works

Primary vs Adaptive Resistance
- JAK
- STK11/LKB1
- neoantigen loss

Need on-trial analysis to understand resistance

Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma

Anti-PD-1 + Anti-CTLA4

Anti-PD-1

Anti-CTLA4

Progression-free Survival (%)

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Months

If the tumor contains no T cells...

A “cold” “desert” environment

http://polarsoils.blogspot.com/2016/08/what-biome-is-antarctica.html
If the tumor contains no T cells...

- Side step host immunity by providing T cells.
CAR-T and synthetic biology
Cancer vaccines

- Prophylactic (preventative) or therapeutic
- Seeking clonal expansion and tumor infiltration
- Happy hunting ground of materials engineers!
- Generation of durable memory

Cell-based vaccines
Protein based
Vector based
Adjuvants!


Rationale for combination therapies

- Chemotherapies
- Radiation therapy
- Oncolytic viruses
- Targeted therapies
- Immunotherapies
- Epigenetics
  - Immunogenic cell death
    - Release of antigen in the correct context (inflammation)
    - ATP/NAD...”find me”
    - Cell surface calreticulin “eat me”
    - HMBG1/HSP/mitoDNA...”get an upset stomach from me”


Also, disrupt TME immunosuppression “wipe and reset”?
What are prognostic and diagnostic biomarkers with immunotherapy?

- How to monitor immunity?
- How to monitor resistance?
- Is enumerating CD8s sufficient?
  - Functional state
    - Cytokine production (Elispots)
    - Proliferative capacity

Static markers at initial diagnosis:
- Genomic analysis (WES, targeted seq)
- IHC for molecular & immune markers
- Flow/CyTOF for phenotyping
- RNA seq for profiling the transcriptome
- Single cell (TCR seq, RNA seq)

Dynamic markers during therapy:
- Genomic analysis at progression
- IHC for molecular & immune markers
- Flow/CyTOF for phenotyping
- RNA seq for profiling the transcriptome
- Single cell (TCR seq, RNA seq)

Analysis of germline SNPs
- Flow/CyTOF for phenotyping (best if paired with tumor)
- Cytokine profiling in serum
- Exosome analysis (WES, RNAseq, best if paired with tumor)
- Single cell (TCRseq, RNAseq)

Flow/CyTOF for phenotyping (best if paired with tumor)
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Emerging Technologies used for biomarkers

Beyond H&E/IHC

• Multispectral
• MIBI
• Flow cytometry/Cytof (low numbers?)
• Sequencing (sc?) with algorithms such as CIBERSORT
• Nanostring
• Epigenetic analysis (ATACseq)

• Intratumoral vs systemic?
• Liquid biopsies.

• Immunoscore
• CD8:Treg
• Inflammatory gene signatures
• TCR profiling
How are we intervening in tumor immunology?

- **Blocking Ab:**
  - Checkpoint molecules
  - Chemokines/receptors
  - Cytokines
- **Stimulatory Ab**
  - Receptors
- **Targeting/delivery**
  - ADC
- **Immunomodulatory SMI**
  - Metabolic enzymes
  - Transcription factors
  - Epigenetic modification
- Generally not targeting tumor directly, but the IS

Access to tissue and on-target activity
What do you know? True or False

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