Engineering immunity against cancer
Traditional strategies to treat cancer

- Surgery, radiation therapy, chemotherapy
- Targeted drugs

Braf inhibitor in metastatic melanoma:


Why immunotherapy?

• Immune system is driven by molecular recognition – the ultimate targeted drug
• …yet able to target many tumor-specific molecules – avoid resistance
• Self-amplifying
• memory
**Cancer immunotherapy – misunderstandings, false starts and false leads**

<table>
<thead>
<tr>
<th>Early beliefs/findings</th>
<th>Later clarifications</th>
</tr>
</thead>
</table>
| Tumors are “self”, therefore any tumor-specific T-cells would be deleted during development (Burnett theory of tolerance 1949) | • Many autoreactive T-cells escape deletion in the thymus  
• Mutations in tumors can create “neoantigens” not subject to tolerance |
| Athymic nude mice have same frequency of chemically-induced tumors as normal mice | Athymic mice retain a substantial residual population of functional T-cells and NK cells |
| Immune system would not have evolved to protect against tumors as they happen late in life | Steadily accumulated evidence that the immune system “edits” tumors – thus acting even when the response is not successful |

<table>
<thead>
<tr>
<th>1970’s</th>
<th>1980’s</th>
<th>1990’s</th>
<th>2000’s</th>
</tr>
</thead>
</table>
| Discovery of MHC restriction | Discovery of the T cell receptor | Discovery of key costimulatory and co-inhibitory receptors  
Discovery of “danger sensor” receptors | Discovery of factors governing T cell persistence and function |

A key issue: immune suppression by tumors

- regulatory T-cells
- hypoxia
- galectins
- IL-10
- adenosine
- TGF-β
- arginase
- myeloid-derived suppressor cells
- tumor-associated macrophages
The breakthroughs: “checkpoint blockade”

Pivotal anti-CTLA-4 trial:


The breakthroughs: “checkpoint blockade”

The second checkpoint: PD-1

Immuno-oncology comes of age


Cancer immunotherapy comes of age

Ira Mellman, George Coukos & Glenn Dranoff

REVIEW
Why does immune oncology need engineers?

- How do we dissect the detailed cellular and molecular events occurring during immunotherapy? – advanced computational analyses
- How do we genetically engineer greater function and control directly into immune cells? – synthetic biology
- How do we do better than native immunoregulatory molecules? – protein engineering
- How do we safely get the full potency of immunotherapy drugs?
Building on the success of checkpoint blockade

Does not respond to anti-PD-1 therapy

- Failure of T cells to enter tumor?
- T cells not primed in the patient?

Does respond to anti-PD-1 therapy

- Provide inflammatory cues to the tumor
- Activate T cells with a vaccine

Spranger, Gajewski J. Immunother. Cancer 2013
Creating a T cell pool with therapeutic vaccines

Injection site

Vaccine = peptide antigen + adjuvant (inflammatory cue)

draining lymph nodes

Tumor-specific peptide

Antigen presenting cell

tumors

T cell

TCR

MHC-I
Cancer vaccines in the clinic

“CD8 T cells were stimulated once with T-cell–depleted PBMC pulsed with assay OLP and 10 to 13 days later, IFN-g–producing NY-ESO-1–specific CD8 T cells were enumerated by ELISPOT…”

“…we also tested purified unstimulated CD8 T cells for NY-ESO-1 tetramer staining and failed to detect clear evidence of positive cells from any of the samples tested…”
Improving one key component – vaccines

Antigen accumulation in draining lymph nodes by whole-tissue imaging:

1. s.c. injection at tail base
2. Resect draining LNs

- axillary
- inguinal

Peptide fluorescence

Positive control – direct injection ex vivo

uninjected
A clinically-validated strategy for LN targeting: sentinel lymph node mapping


Physiology of solute transport in tissues

Cumulative recovery in peripheral lymph (% of dose) vs. molecular weight (kDa)

albumin

Hypothesis: target albumin, target lymph nodes

- **Haipeng Liu**

- **molecular adjuvant – CpG DNA**

- **antigen**

- **cargo**

- **lipophilic tail**
  - albumin binding

- **polar block**
  - promote solubilization

- lymph node

- vaccine amphiphiles

- endogenous albumin
Designing for albumin binding promotes lymph node targeting

Lymph node targeting amphiphiles as potent peptide vaccines

S = Soluble vaccine
A = Amphiphile vaccine

therapeutic anti-tumor vaccination–B16F10 melanoma model:

Gp100 variant peptides

Liu et al. Nature 2014
Back to overcoming tumor-induced suppression of immunity...

- regulatory T-cells
- hypoxia
- arginase
- galectins
- IL-10
- adenosine
- TGF-β
- myeloid-derived suppressor cells
- tumor-associated macrophages
Meanwhile, our neighbors were exploring strategies to invoke innate immunity against tumors:

Response dependent on:
- CD8 T-cells
- NK cells
- Neutrophils
- Eosinophils

Zhu et al. *Cancer Cell* 2015

Eric Zhu/Dane Wittrup
Defining the requirements for the endogenous immune response to eradicate large immunosuppressive tumors

“adaptive-centric” therapy: amph-vaccines (Trp2, gp100, …)

“innate-centric” therapy: Extended half-life IL-2 (IL-2-albumin fusion)

Checkpoint blockade Abs (anti-PD-1, anti-CTLA-4)

Support adaptive immune response

T-cells

B16F10 melanoma

NK cells

neutrophils

macrophages

Dane Wittrup/Cary Opel/Kelly Moynihan
A simplified model

- **B16F10 melanoma**
- **T-cells**
- **NK cells**
- **Macrophages**
- **Neutrophils**

**Amph-vaccine** rapidly expands massive cohort of anti-tumor T-cells

**Checkpoint blockade** keeps T-cells functional in tumor

**Extended-PK IL-2** brings leukocytes to the tumor

**Ab-mediated tumor cell killing** leads to antigen uptake by cross-presenting DCs

**DCs prime additional T-cells in LNs and tumor**

**Antibody promotes killing of tumor by innate immune cells**
Why does immune oncology need engineers? – challenges still to be met

- Activating immunostimulatory molecules specifically in tumors
- Can we model the events leading to immunosuppression vs. tumor rejection? - generate hypotheses
- New tools needed to measure the state and function of the immune response in humans – “How’s my immune system?”
Acknowledgments

Kelly Moynihan  
Talar Tokatlian  
Kavya Rakhra  
Tyson Moyer  
Andrew Zmolek  
Talar Tokatlian  
Eric Dane  
Jacob Martin

Li Tang  
Yiran Zheng  
Archana Boopathy  
Mariane Melo  
Sabrina Yang  
Anasuya Mandal  
Yuan Zhang  
Sudha Kumari

Dane Wittrup  
Naveen Mehta  
Marcela Maus  
Ana Castano

Funding:  
NIH

Former lab members:  
Matthias Stephan  
Haipeng Liu  
Bonnie Huang  
Brad Jones  
Greg Szeto

Scripps Center for HIV/AIDS Vaccine Immunology & Immunogen Discovery