APPLICATION OF NEOANTIGENS TO BLOOD MALIGNANCIES

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Disclosures

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2018: Critical Questions to Address

• How to increase fraction of patients with durable responses?

• How to minimize autoimmunity?
Studying Intratumoral Heterogeneity in CLL

A Inter-patient Heterogeneity

Chromosomal aberrations
- del(13q)
- Trisomy 12
- del(11q)
- del(17p)

Significantly mutated genes
- TP53
- ATM
- SF3B1
- NOTCH1
- MYD88
- IGHV

B Intra-sample Heterogeneity

Density

Capacity for evolution is all there already in the tumor samples at treatment initiation

Bacteria, Fungi, Viruses, Parasites
Pathogens: Prophylactic/Preventive Vaccines

Cancer  ———  Therapeutic Vaccines

Increased breadth and diversity of tumour-specific T cell response
Steering the Immune Response With a Vaccine

- Expand and broaden the T cell repertoire by inducing tumor-specific T cells
- Generate highly specific anti-tumor immunity with fewer side effects on vital tissues
Cancer vaccines have been around for awhile.

What’s different now?
1. 2012: Dramatic Clinical Responses After “Checkpoint Blockade Antibodies” for Solid Tumors

Cancer Care

- Surgery
- Radiotherapy
- Chemotherapy
- Immunotherapy
2. DNA Sequencing Across Cancers (n=>3000)

Somatic Mutations Have the Potential to Generate Neoantigens

Hitting the “Sweet Spot”

3.

Source material

- RNA
- DNA

Target prediction

- RNA-seq
- WES
- HLA typing

- Identification of somatic mutations
- Expression confirmation of mutated genes
- Prediction of personal HLA-binding peptides

Growing Compelling Evidence for Neoantigens as Effective Tumor Rejection Antigens

Disease: Melanoma
- Stage III/resectable
- Stage IV

Can a Personalized Cancer Vaccine Stimulate Anti-tumor Immunity in Humans?

Vaccine: Up to 20 Personalized Neoantigens as SLPs with Adjuvant (Poly-ICLC)

- DNA and RNA sequencing to identify tumor-specific mutations
- HLA-typing
- Prediction of personalized HLA-binding peptides

- 11 Enrolled
- 8 Vaccines generated
- 6 Dosed
Vaccine Induces T Cells Against Almost All Pools

20% of selected neoantigens induced CD8 T cell responses
>30% of selected neoantigens induced CD4 T cell responses
T Cells Recognize Mutated but Not Wild Type Epitopes
Enduring Complete Radiographic Responses After Neovax + α-PD-1 Treatment

After completion of vaccination

12 weeks after initiation of anti-PD-1 therapy

Pt. 2

After completion of vaccination

12 weeks after initiation of anti-PD-1 therapy

Pt. 6
Can Such a Vaccination Approach be Tested in Lower Mutation Rate Tumors?

**Disease: GBM**
- Rapidly fatal
- Cold tumor
- Blood-brain barrier?
- Impact of steroids?
Testing Neovax in a Lower Mutation Rate Tumor and Within Context of SOC Therapy: GBM

- 10 Enrolled
- 8 Vaccines dosed
Neovax: ‘Warming’ a Cold Tumor

Graphs showing CD4+ and CD8+ cell counts in initial and relapse phases with and without Dexamethasone treatment. Initial vs. relapse comparisons indicate significant differences ($p = 0.008$ and $p = 0.0006$) for CD4+ cells and CD8+ cells, respectively. (Pt. 5, Pt. 7, Pt. 8)
Neovax: ‘Warming’ a Cold Tumor

CD3+ CD45+ Selection
SMART-Seq2

CD4+ cells/mm²

CD8+ cells/mm²

Initial
Relapse

Initial
Relapse

+ Dexamethasone
- Dexamethasone

p = 0.006
p = 0.006

Pt. 5
Pt. 7
Pt. 8

CD3+ CD45+ Selection
SMART-Seq2
Pt 7: Strategy to Identify Intratumoral Neoantigen-specific T Cells

- Single cell transcriptome
- Complete CDR3 from TCRαβ
- TCR Reconstruction

Brain
- Disaggregation
- CD3+ selection

Single cell isolation

عظمى library

Smart-Seq2
Targeted TCR sequencing

Single cell transcriptome

TCR Reconstruction

Tumor infiltrating lymphocytes

- 39 singles
- 64 singles
- 103 singles

CD4 (n = 124)
CD8 (n = 70)
ND (n = 83)
Strategy to Identify Intratumoral Neoantigen-specific T Cells

- Disaggregation
- CD3⁺ selection

Single cell transcriptome

Complete CDR3 from TCRαβ

TCR Reconstruction

Tumor infiltrating lymphocytes

CD4 (n = 124)
CD8 (n = 70)
ND (n = 83)

Ex vivo, PBMC

weeks following vaccination initiation

CD4

CD8

Strategy to Identify Intratumoral Neoantigen-specific T Cells

![Diagram showing the strategy to identify intratumoral neoantigen-specific T cells.](image)
mutARHGAP35 Epitopes

<table>
<thead>
<tr>
<th>Variant</th>
<th>Sequence</th>
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<tr>
<td>WT</td>
<td>ARHGAP35 T</td>
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<tr>
<td>IMP12</td>
<td>HNLDLAEKDF<strong>M</strong> VNTVAGAMK</td>
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<tr>
<td>ASP35b</td>
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ARHGAP35-Specific T Cell Identified at Site of Disease

**Graphs:**
- **H02**:
  - IL-2 (pg/ml) for Mutant and Wildtype.
  - IL-2 levels are significantly higher in Mutant compared to Wildtype.
- **F10**:
  - Similar IL-2 (pg/ml) analysis for Mutant and Wildtype.
  - IL-2 levels are significantly higher in Mutant compared to Wildtype.

**Legend:**
- Mutant
- Wildtype
- OVA

**X-axis:**
- Logarithmic scale (10^3 to 10^7)
- ARHGAP35 peptide (pg/ml)

**Y-axis:**
- Logarithmic scale (0 to 200)
- IL-2 (pg/ml)
Summary

• Adverse impact of steroids with T cell priming
• Neoantigen-specific T cells can (variably) track to the site of tumor
• Neoantigen-specific T cells at the tumor site have variable transcriptional profiles
• On the horizon: combination with checkpoint therapy
Activation and proliferation of tumor-specific T cells

Migration to the site of tumor

Cytolysis of tumor
Active Disease: Targeting Indolent Lymphomas

- Advanced stage follicular lymphoma and chronic lymphocytic leukemia are incurable malignancies
  - CLL: Unmutated Ig heavy chain variable gene (IGHV) = aggressive biology and poor therapeutic response
  - No curative option exists for either disease
  - “Watch and wait” for asymptomatic disease

- Immune escape is central to lymphoma biology
  - T cell dysfunction widely reported
  - Immunotherapies are effective but low therapeutic index

Neoantigen vaccination *early in the disease course* can exploit
1) tumor specificity, 2) setting conducive to immune stimulation, and 3) *active disease setting allowing for study of evolving tumor-immune responses*
Neoantigen Load in CLL

• Nonselected, population-based patient cohort with WES, n=54
• 11 patients met criteria:
  • Unmutated IGHV
  • Untreated at time of sample and no treatment for at least 100 days
  • RNA seq
• 160 untreated CLL patients/yr; ~80 unmutated IGHV

54% ≥7 unique neoantigens

Patients with untreated CLL with unmutated IGHV

# Unique, predicted neoepitopes
Going Earlier in Disease.....

**Chronic lymphocytic leukemia**
- Unmutated *IGHV*, untreated
- NeoVax
- NeoVax + low-dose CTX
- NeoVax + PD1 blockade

**Follicular lymphoma**
- Following rituximab
- NeoVax + PD1 blockade

**Vaccinations:**
- Prime
- Boost
- Boost

**Weeks:**
- -5
- 0
- 5
- 10
- 15
- 20
- 25
- 30
- 35

**Primary safety endpoint**
**Primary immunological endpoint**

**Pembrolizumab**
(6 - 8 cycles)
### Future Directions: Combinations

**Obinutuzumab**
- **1980**: Unrelated donor allo-HSCT in patients with leukemia
- **1980**: DLI induces remissions in CML
- **1990**: GvL recognized as basis of allo-HSCT efficacy
- **2000**: NK cell alloreactivity impacts allo-HSCT outcomes
- **2010**: EBV-targeted CTls effective in B cell lymphomas
- **2014**: CAR-T cells induce remissions in CLL, B-ALL, DLBCL

**CAR-T Ibrutinib**
- **1980**: Personalized anti-idotype vaccine induces lymphoma regression
- **1996**: PR-3 identified as a leukemia-associated antigen
- **2000**: WT-1 identified as a leukemia-associated antigen
- **2009 and 2013**: Whole tumor cell GVAX effective in post-transplantation setting for AML and CLL
- **2014**: Systematic identification of neoantigens in CLL

**NeoVax**
- **1982**: Cure of B-cell lymphoma with anti-idotype mAb
- **1991**: CD20 discovered
- **2007**: Rituximab 1st mAb approved for cancer treatment
- **2014**: Blinatumomab approved for RRR B-ALL
- **2011**: Brentuximab approved for RR cHL/ALCL
- **2010**: Ofatumumab approved for RRR CLL

**Anti-PD1/CTLA4**
- **2003**: FcR polymerization predicts rituximab response
- **2014**: Pidilizumab + rituximab effective in RR FL
- **2013**: Pidilizumab (anti-PD-1 mAb) effective in RR DLBCL

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Summary

• Cancer-specific mutations can generate neoantigens that may drive immunotherapeutic responses

• Neoantigen vaccines offer a personalized approach to cancer immunotherapy and can induce cancer-specific immune responses

• Hematologic malignancies offer a unique platform for immunotherapeutic evaluation
## Acknowledgements

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