New Horizons in Immunotherapy
For Head and Neck Cancer – 2015
Andrew Weinberg, PhD

Defining Phenotype and Function of T cells within H&N Tumors

- Immunologic Paradigm as it relates to cancer
- Tumor infiltrating lymphocytes in Head and Neck Cancer
- Expression of OX40, PD-1, and CTLA-4 in H&N Cancer
- OX40 agonist and their effects on Tregs
- Visualizing TCR engagement within the tumor:
  Can immunotherapy influence TCR function and repertoire?
IMMUNOLOGICAL PARADIGM

The major function of the immune system is to recognize and eliminate harmful entities within the body without destroying “self” tissue.

Tumor Ags Drain to LNs
Prime T and B cell responses
Clonal Expansion of CD8 Tumor Infiltrating Lymphocytes in Head and Neck Cancer Patients
Flow cytometry-based expression of OX40, CTLA-4, and PD-1 on T cell subsets; Tumor vs PBL (Head and Neck Cancer Patient).
Treg percentage of total CD4 cells in Blood vs TIL Head and Neck Cancer patients
OX40 expression on Treg, CD4, CD8 T cells in Head and Neck Cancer Patients

- **Tregs**
  - Percent OX40+: <.0001

- **Conventional CD4s**
  - Percent OX40+: <.0001

- **CD8s**
  - Percent OX40+: 0.0168

**OX40 Mean Fluorescent Intensity**

- Blood
- Tumor

![Graphs showing OX40 expression on Treg, CD4, CD8 T cells in Head and Neck Cancer Patients]
PD1 expression on Treg, CD4, CD8 T cells in Head and Neck Cancer Patients

- Tregs
  - $<0.0001$

- Conventional CD4s
  - $<0.0001$

- CD8s
  - $<0.0001$
Intracellular CTLA-4 expression on Treg, CD4, CD8 T cells in Head and Neck Cancer Patients

Tregs

Conventional CD4s

CD8s

<0.0001

<0.0001

<0.0001
Co-expression of OX40, CTLA-4 and PD-1 on Treg, CD4, CD8 T cells in Head and Neck Cancer Patients

Tregs

<0.0001

Conventional CD4s

<0.0001

CD8s

<0.0001
# Isolating Tregs TIL (FoxP3-RFP mouse) 4 days after OX40 agonist Treatment Gene Array Assessment

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OX40 engagement depletes intratumoral Tregs via activating FcγRs, leading to antitumor efficacy.
Nur77<sup>GFP</sup> Transgenic Mice Assess T cell Receptor Signaling in Tumor Models

**In vivo – Spleen**
OT-I/Nur77<sup>GFP</sup> T cells, infect with Listeria-oval

**In vitro**
OT-I/Nur77<sup>GFP</sup> stimulated with SIINFEKL alter peptide ligands

Assessing TIL in the Nur77-GFP Model

GFP Upregulated in an Antigen-Specific Manner in the Tumor

Inoculate flank MCA205OVA vs MCA205WT

1X10⁶ OT-I/Thy1.1/Nur77GF

10d

7d

Harvest tumor, spleen

MCA205-OVA

MCA205-WT

OTI/Thy1.1/Nur77GFP

CD69 CD44 PD1 Nur77GFP

NS NS

Fold change CD69 MFI (WT/OVA expressing)

Fold change CD44 MFI (WT/OVA expressing)

Fold change PD1 MFI (WT/OVA expressing)

Fold change GFP MFI (WT/OVA expressing)

p<.0001

WT (Tumor) Host CD8+ (Spleen)

OVA (Tumor) WT (Tumor)

10d 1X10⁶ OT-I/Thy1.1/Nur77GF

7d

Harvest tumor, spleen
Effect of Immunotherapy on TCR Engagement in the Tumor
(MCA205 tumor model: Assess Nur77GFP 7 days post-αOX40)

Rat IgG
α-OX40

Fold difference CD8+GFPhi

NS

% GFPhi of CD4+Foxp3

p=.0008

% GFPhi of CD8+

p=.0027

Fold difference CD8+GFPhi

Rat IgG
α-OX40
α-CTLA4
α-PD1
α-PDL1
Sorting GFP positive and negative CD8 T Cells from Tumor: anti-OX40 vs rat Ig
TCR repertoire of CD8s invading MCA205 Tumor in mice treated with anti-OX40 GFPhi vs GFPlo?

Expanded Clones TCR CDR3 Sequence: CASSQEGDGYEQYF and CASSQEGDGHEQYF
Are there qualitative differences in Nur77-GFP high and low T cells in Tumor?

**TCR CDR3 Sequencing: Adaptive Biotech**

**Anti-OX40/anti-PDL-1 Combo**

- 1. Rat Ig
- 2. Anti-OX40
- 3. Anti-PDL-1
- 4. Anti-OX40/anti-PDL-1
Combo Therapy: Analyses of TCR Clones in GFP High and Low CD8 T Cells

**Top 100 Clones by Sample**

**Tumor**
- **GFP$_{hi}$**
  - Clone 1: 15.83
  - Clone 2: 9.08
  - Clone 3: 2.14
  - Clone 4: 7.38
- **GFP$_{Low}$**
  - Clone 1: 2.23
  - Clone 2: 1.80
  - Clone 3: 7.21
  - Clone 4: 1.36

**Spleen**
- **GFP$_{hi}$**
  - Clone 1: 0.14
  - Clone 2: 0.04
  - Clone 3: 0.01
  - Clone 4: 0.08
- **GFP$_{Low}$**
  - Clone 1: 1.94
  - Clone 2: 1.91
  - Clone 3: 0.31
  - Clone 4: 1.47
Clonal Expansion of CD8 Tumor Infiltrating Lymphocytes in Head and Neck Cancer Patients
Providence Cancer Center
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Patients
Healthy volunteers
Patient Coordinators