IMMUNOTHERAPY FOR GI MALIGNANCIES
LEARNING FROM ZEBRAS

Robert A. Anders, M.D., Ph.D.
Associate Professor of Pathology
Co-Director, Tumor Microenvironment Laboratory
Bloomberg-Kimmel Institute for Cancer Immune Therapy
Johns Hopkins School of Medicine
rander54@jhmi.edu

Roger C. Haggitt Gastrointestinal Pathology
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Disclosures

• Bristol Myers Squibb
• Merck
• Adaptive Biotechnologies
• Five Prime Therapeutics
• FlxBio
• Stand up 2 Cancer
• National Institutes of Health
• Roche Diagnostics

Goals

• Human tissue as a biomarker
  – Prognostic
  – Predictive
• PD-L1 expression as a predictive biomarker
• Results of PD-1 / PD-L1 axis blockade in colon cancer clinical trial
• Histology of adverse events
• Expanding immune based cancer therapy to microsatellite stable patients

Human Tissue as a Prognostic Biomarker

Patient Prognosis
Colon Cancer

Dukes 1932
TNM 1988
Galton 2006

Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

20 SEPTEMBER 2009 VOL 313 SCIENCE
Immune Contexture and Prognosis

Human Tissue as a Predictive Biomarker

Predictive Tissue Biomarkers

- 1st generation
  - Type and origin of cancer
    - Lymphoma vs. small cell carcinoma
    - Breast vs. colorectal carcinoma
- 2nd generation
  - HER2/neu
    - Herceptin therapy
    - Breast and gastric carcinoma
- 3rd generation
  - Immune contexture
    - Immune checkpoint inhibitors
    - Cytotoxic lymphocytes
    - Inhibitory immune cells

Predictive Biomarkers in Immune Based Cancer Therapy

CTLA-4 and PD-1
Distinct Inhibitory Immune Checkpoints

PD-L1 Expression as a Predictive Biomarker

134 published articles on 20 different cancer types


Her2/neu stain

Sunshine and Taube
Current Opin in Pharm. 2016
PD-L1 Expression is an Imperfect Biomarker for Response to PD-1/L1 blockade

Should Colorectal Cancer Even be Considered for PD-1 / L1 Blockade?

ONE Complete Response 21+ months

PD-1 Blockade in Colon Cancer Clinical Trial

**Study Design**

<table>
<thead>
<tr>
<th></th>
<th>Colorectal Cancers</th>
<th>Non-Colorectal Cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort A</td>
<td>Deficient in Mismatch Repair (n=25)</td>
<td></td>
</tr>
<tr>
<td>Cohort B</td>
<td>Proficient in Mismatch Repair (n=25)</td>
<td></td>
</tr>
<tr>
<td>Cohort C</td>
<td>Deficient in Mismatch Repair (n=21)</td>
<td></td>
</tr>
</tbody>
</table>

- Anti-PD1 – 10 mg/kg every 2 weeks
- Primary endpoint: immune-related 20-week PFS rate and response rate
- Mismatch repair testing using standard PCR-based test for detection of microsatellite instability

**Clinical Trial**

<table>
<thead>
<tr>
<th></th>
<th>MSI Colon Ca</th>
<th>MSS Colon Ca</th>
<th>MSI Non-Colon Ca</th>
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<tbody>
<tr>
<td>N</td>
<td>13</td>
<td>25</td>
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- Objective Response Rate
- Disease Control Rate
Clinical Trial Results

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<tr>
<td>N</td>
<td>13</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Objective Response Rate</td>
<td>62%</td>
<td>0%</td>
<td>60%</td>
</tr>
<tr>
<td>Disease Control Rate</td>
<td>92%</td>
<td>16%</td>
<td>70%</td>
</tr>
</tbody>
</table>

Rationale for PD-1 Blockade in Colon Cancer

- Antigens
- Lymphocytes

MUTATIONAL DENSITY IN COLON CANCER

- Antigens
- Mutations
- Neo-epitopes
Mutations Associated Neo-epitopes
Somatic mutations resulting in new amino acid
- Neo-antigen
- HLA haplotyping MHC-I
- Epitope prediction algorithm
  Lundegaard / Nielsen

Mutational Burden is Associated with anti-PD-1 Treatment Efficacy
Clinical Trial Data

Mutational Burden
- MSI
- MSS
- Objective Response
- Stable Disease
- Progressive Disease

T-CELL DENSITY IN COLON CANCER

Anti-CD8 Stained Colorectal Carcinoma

Lymphocytes
Annotated “tumor” regions extracted

335 CD8+ cells / mm²

CD8 Analysis
Correlation with Microsatellite Status

- Density of CD8+ Cells within the Tumor
- P = 0.10
- Density of CD8+ Cells at Invasive Front
- P = 0.04

CD8 Analysis
Correlation with Clinical Response

PD-L1 Expression in Gastric Adenocarcinoma

Elizabeth Thompson M.D., Ph.D.
PD-L1 Expression in Mismatch Repair Deficient Colon Cancer

Tumor Associated Macrophages Expressing PD-L1 in Colon Cancer

PD-L1 Expression Shield Effect

PD-L1 Expression Shield Effect

PD-L1 Analysis Clinical Trial Data

Rationale for PD-1 Blockade in Colon Cancer
**PD-L1 Expression**

*Inflammatory vs Tumor cells*

- Adenocarcinoma
- Melanoma

**PD-L1 on myeloid cells**

**PD-L1 on tumor cells**

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**EXPANSION OF PD-1 / L1 BLOCKADE**

**CLINICAL TRIAL**

**Trial Design**

- Phase II
- 86 metastatic mis-match repair deficient tumors
- Germline sequencing of *MSH2, MSH6, PMS2* and *MLH1*
- 12 different cancer types
- 10 mg/kg of anti-PD-1 every two weeks for up to 2 years
- Primary goal: Objective response based upon RECIST 1.1 criteria

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**Secondary Goal**

*Tissue Analysis*

- Pre- and post-therapy samples

- Next generation sequencing
- RNA Seq
- T-cell receptor beta deep sequencing
- Multiplex real time RT-PCR
- Proteomics
- Cy-TOFF
- "something my lab is really good at measuring"
- "You’ll need to sign this confidentiality agreement in order to discuss"

**ROUTINE H&E HISTOLOGY**

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**Secondary Tissue Analysis**

*Pre- and post-therapy samples*

- Metagenomic
- "something my lab is really good at measuring"
- "You’ll need to sign this confidentiality agreement in order to discuss"

**FIVE DOLLARS / SLIDE**

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**Metastatic Lesions After PD-1 Blockade**

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3/24/2017
**Histology of MSI CRC tumors**

**MSI CRC Histology**

*Evidence of immune mediated regression?*

**Regression**

**Adverse Events**
- Autoimmunity / Inflammatory Injury
  - Skin
  - Gastrointestinal
  - Respiratory
  - Hepatobiliary
  - Musculoskeletal
  - Endocrine

**Adverse Events**
- Autoimmune Cardiomyopathy

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PD-L1 Regulates a Critical Checkpoint for Autoimmune Myocarditis and Pneumonitis in MRL mice


1 Laboratory of Molecular Autoimmune Disease, Clinical Division, Brigham and Women's Hospital, Boston, MA 02115, USA
2 Department of Pathology, Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115, USA
Adverse Events

Anti-CTLA-4 Associated Colitis

- Retrospective study
- Endoscopic mucosal biopsies
- 22 patients with anti-CTLA4 induced colitis +/- steroids
- 12 patients with first presentation of IBD
- 5 normal controls
- Immunohistochemical stains for T- and B-cell markers
- Digital image analysis to report cell densities

Maryam Kherad-Pazhouh M.D.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal Controls (N=17)</th>
<th>Normal plus anti-CTLA4 (N=10)</th>
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<tbody>
<tr>
<td>Macrophages</td>
<td>38 (0.96)</td>
<td>8 (0.15)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>3 (0.06)</td>
<td>1 (0.02)</td>
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CD4 Density (#/mm²)

- Ipi-AC
- Ipi
- Ipi-AC--
- e-I
- BD

CD8 Density (#/mm²)

- Ipi-AC
- Ipi-AC--
- e-IBD

FOXp3 Density (#/mm²)

- Ipi-AC
- Ipi-AC--
- e-IBD

After steroid treatment
What is the critical number of CD 20 or CD 138 cells to count to make the diagnosis?

Adverse Events
Anti-CTLA-4 Associated Colitis

Absence of basal plasmacytosis

CURRENT EFFORTS

Interrogating Atypical MSS CRC

Clinical Trial

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Improved quantitation methods

Atypical MMS CRC

Increased T-cell Density

A. Immunohistochemistry: T cell infiltration
B. Gene expression profile
   Laser capture microdissection (LCM) on FFPE tissue section and Taqman PCR RNA expression

Interrogating Atypical MSS CRC

A. Immunohistochemistry: T cell infiltration
B. Gene expression profile
   Laser capture microdissection (LCM) on FFPE tissue section and Taqman PCR RNA expression
C. Flow cytometry: PD-1 / IFN-γ co-expression

Atypical MSS CRC

Unique mRNA Profile

Laser capture microdissection (LCM) on FFPE tissue section
Conclusions

- Pathology has a key role in prognostic and predictive biomarkers in immuno-oncology
- PD-L1 expression is a predictive biomarker
- Better biomarkers are likely to include a combination of several parameters
- The success of PD-1 / L1 blockade in mismatch repair deficient (MSI) colon cancer
Conclusions

• Pathology has a key role in prognostic and predictive biomarkers in immuno-oncology
• PD-L1 expression is a predictive biomarker
• Better biomarkers are likely to include a combination of several parameters
• The success of PD-1 / L1 blockade in mismatch repair deficient (MSI) cancer
• Mucinous regions in colorectal cancer maybe indicators of immune mediated cancer regression
• Histology of adverse events associated with checkpoint blockade
• Expanding immune based cancer therapy to mismatch repair proficient colorectal cancer

Thank you
Clinical trial patients

Collaborators

Anders lab members
- Qingfeng Zhu
- *Lan Luan
- *Andrew Lyman
- Jenny Chung
- Mahdi Khan
- Hao Bai
- Hai-Xiang Sun
- Mina Behni
- Ying Li
- Mariana Gayyed
- Angela Stierholtz
- Paul Markowski
- Suresh K. Nayar

* current