Mutations as Immune Antigens: PD-1 Blockade in Tumors with Mismatch Repair Deficiency

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Ludwig Center for Cancer Genetics and Therapeutics
Disclosure Information

I have the following financial relationships to disclose:

Founder and shareholder in Pagerbox, Papgene, LLC and Personal Genome Diagnostics, Inc.

Consultant for Merck.

Under separate licensing agreements between Inostics, Personal Genome Diagnostics and the Johns Hopkins University, Dr. Diaz is entitled to a share of royalty and milestone payments received by the University on sales of products related to research described in this presentation.
Human Cancer Exomes Sequenced

- Glioblastoma (35)
- Head and Neck Cancer (66)
- Non-Hodgkin Lymphoma (74)
- Lung Cancer (Non-Small Cell) (147)
- Lung Cancer (Small Cell) (163)
- Breast Cancer (33)
- Esophageal Adenocarcinoma (57)
- Esophageal Squamous Cell Carcinoma (79)
- Hepatocellular Cancer (39)
- Gastric Cancer (53)
- Colorectal Cancer (66)
- Ovarian Cancer (42)
- Endometrial Cancer (49)
- Pancreatic Cancer (45)
- Prostate Cancer (41)
- Chronic Lymphocytic Leukemia (12)
- Melanoma (135)
- Acute Myeloid Leukemia (8)
- Glioblastoma (14)
- Medulloblastoma (8)
- Rhabdoid Cancer (4)
- Neuroblastoma (12)
- Acute Lymphocytic Leukemia (11)
Non-synonymous Mutations per tumor
Clinical Application of Cancer Genetics

- Prognostic Markers
- Somatic Cancer Genome Data
- Immune Antigens
- Predictive Markers
- Dynamic Biomarkers
Cancer Mutations

Coding Mutations
1,712,998 Genes
>20,000 Clinically Meaningful
91 Genes
Cancer Mutations

91 Genes

Associate with FDA approved therapies

Eligibility for active clinical trials
(55/91; world-wide)
Clinical Application of Cancer Genetics
• DNA fragments of 180-200bp with half life of ~2 hours

• Specific to tumor

• Real-time, non-invasive, multi-lesions, potentially cheaper (considering cost of biopsies)

• Often very low amount of ctDNA in the sea of wild type DNA - ”Needle in a farm”
Monitoring response to checkpoint inhibitors using ctDNA

cDNA levels increased initially as lymphadenopathy progressed by examination, but then became undetectable 3 weeks prior to clinical improvement.

Clinical Application of Cancer Genetics

- Prognostic Markers
- Somatic Cancer Genome Data
- Immune Antigens
- Predictive Markers
- Dynamic Biomarkers
Mutations per tumor

- Mismatch repair tumors
- Mutagen Associated tumors
- Sporadic Adult Solid Tumors
- Pediatric Tumors
- Liquid Tumors

Melanoma and Lung Cancers

Mutations per tumor
Mutations per tumor

Mismatch-repair proficient colon cancers

Mismatch-repair deficient colon cancers

Malignant Tumors

Liquid Tumors

Pediatric Tumors

Sporadic Adult Solid Tumors

Mutagen Associated tumors

Mismatch repair tumors

Mutations per tumor
Hypothesis

- Mutations have been shown to encode proteins that can be recognized and targeted by the immune system.

- Average tumor has dozens of somatic mutations; Mismatch repair deficient tumors harbor thousands of mutations.

- Immune augmentation with PD-1 blockade may be highly effective in mismatch repair deficient tumors.
Mismatch Repair Deficiency

**Microsatellite instability** in tumor cells is due to deficient DNA mismatch repair:

- **germline** (Lynch syndrome) and/or **sporadic** mutations (MLH1, MSH2, MSH6, PMS2, EpCAM)
- **epigenetic silencing** (MLH1 hyper-methylation)
Associated tumor types

**Colorectal cancer**
- Associated with hereditary nonpolyposis colorectal carcinoma (HNPCC)
- 15% of sporadic colorectal carcinomas (3-5% of advanced disease)
- stage II CRC: associated with better prognosis, no benefit from 5FU alone
- stage IV CRC: associated with worse prognosis

**Other tumor types:**
Endometrial, gastric, small bowel, ampullary, cholangiocarcinoma, pancreatic, sarcoma, prostate, gliomas and others at similar frequencies.
PD-1 Pathway and Pembrolizumab

- Binding of PD-1 to its ligands PD-L1 and PD-L2 inhibits effector T-cell function\(^1\)
- PD-L1 expression on tumor cells and macrophages suppresses immune surveillance, permitting neoplastic growth\(^2\)
- Pembrolizumab is a humanized, IgG4 monoclonal antibody that
  - Binds to PD-1 with high affinity, preventing pD-1 from binding to PD-L1 and PD-L2
  - Has demonstrated robust antitumor activity and manageable toxicity in multiple advanced cancers\(^a\)

\(^a\)FDA approved for the treatment of unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF\(^{V600}\) mutant, a BRAF inhibitor.
Study Design

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

- Anti-PD1 (Pembrolizumab) – 10 mg/kg every 2 weeks
- Primary endpoint: immune-related 20-week PFS rate and response rate
## Objective Responses (Sept 2015, N=63)

<table>
<thead>
<tr>
<th></th>
<th>MMR-deficient CRC</th>
<th>MMR-proficient CRC</th>
<th>MMR-deficient non-CRC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>20</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td><strong>Objective Response Rate</strong></td>
<td>55%</td>
<td>0%</td>
<td>55%</td>
</tr>
<tr>
<td><strong>Disease Control Rate</strong></td>
<td>90%</td>
<td>16%</td>
<td>72%</td>
</tr>
</tbody>
</table>
Biochemical Responses

![Graph showing biochemical responses over time for MMR-proficient CRC, MMR-deficient CRC, and MMR-deficient non-CRC.](image)

% Tumor Marker Change vs. Time (days)

- Red: MMR-proficient CRC
- Green: MMR-deficient CRC
- Black Dotted: MMR-deficient non-CRC
Target Lesions: CRC Cohorts
Duration of Response

![Graph showing duration of response for different groups: MMR-proficient CRC, MMR-deficient CRC, and MMR-deficient non-CRC. The graph plots % change from baseline SLD against days.](image-url)
Survival Curves (October 2015)

Overall Survival

Progression-Free Survival
Mismatch repair deficient Colorectal Cancer

Baseline

Week 20

Tumor Markers

% Tumor Marker Change

Days

CEA
Baseline PD-L1 Expression and CD8 T Cell Infiltration

- **H&E**: Hematoxylin and Eosin staining for histological examination.
- **PD-L1**: Expression of programmed death-ligand 1, indicating immune checkpoint blockade.
- **CD8**: Presence of cytotoxic T cells.

**Legend**:
- **T**: Positive staining
- **N**: Negative staining

**Diagnosis Types**:
- **dMMR CRC**: Mutations in DNA mismatch repair genes associated with colorectal cancer.
- **pMMR CRC**: Wild-type DNA mismatch repair in colorectal cancer.
- **dMMR non-CRC**: Mutations in DNA mismatch repair genes in non-colorectal cancers.

**Invasive Front and TIL**:
- **Invasive Front**: The boundary between the tumor and normal tissue.
- **TIL**: Tumor-infiltrating lymphocytes, indicating immune cell infiltration.
Invasive Front PD-L1 Expression and CD8 T Cell Infiltration

Invasive Front CD8⁺ T cells

Invasive Front PD-L1 Expression

Tumor Front PD-L1 Expression
Mutation Burden is Associated with Efficacy

- MMR-deficient tumors
- MMR-proficient tumors
- Objective Response
- Stable Disease
- Progressive Disease

P=0.007
P=0.02
Summary

• Radiographic changes may not be tumor progression.

• Patients with mixed responses (e.g. brain mets) may derive benefit.

• Mismatch repair deficiency is easily determined using an existing commercially available test.

• Suggests genomics more influential than histology for mismatch repair deficient tumors treated with anti-PD1
Future Directions

• Primary Resistance
Primary resistance to anti-PD1 in MSI-H tumors

- Non-responders with >1500 mutations per genome
- No difference in PD-1 expression
- Comprehensive evaluation ongoing
Future Directions

• Primary Resistance

• Secondary Resistance (if any)?
Future Directions

- Primary Resistance
- Secondary Resistance (if any)?
- Subpopulations
Response or Stable Disease in MSS CRC cohort

25 MSS CRC patients
<100 mutations across all 25 tumors

Broad exploration of microenvironment and neoantigens in these tumors
Future Directions

- Primary Resistance
- Secondary Resistance (if any)?
- Subpopulations
- MSI-H in context of other tumor types
Where do MSI-H tumors fit into the checkpoint inhibition paradigm?

Anti-PD-1 Responsive tumor types

Low mutational burden
- Kidney
- Bladder
- Gastric
- Lymphoma

High mutational burden
- Melanoma
- NSCLC
- MSI-H Tumors
- POLE and POLD
Where do MSI-H tumors fit into the checkpoint inhibition paradigm?

Anti-PD-1 Responsive tumor types

Low mutational burden

Endogenous Immunogen?
Virus?
Recurrent highly immunogenic and addicted neoantigens?

High mutational burden

DNA Damaging Toxins

Germline DNA repair defects
Clinical Trials

- KEYNOTE – 164: 3rd line MSI-H CRC
- KEYNOTE – 177: 1st line MSI-H CRC
- FDA Breakthrough Therapy Designation
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