Novel Clinical Applications of Cancer Genomics

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Disclosure Information

I have the following financial relationships to disclose:

Founder of Pagerbox, Inostics, Personal Genome Diagnostics, Inc.

Consultant for Spectrum Pharmaceuticals and Amgen.

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.
CANCER

INITIATION

- Infection
- DNA Damaging Agents
- Family History
- Bad Luck/Random
- Time/Age
Infection
DNA Damaging Agents

Family History
Bad Luck/Random

Time/Age

MUTATIONS

INITIATION
Normal Pre-malignant Invasive Metastatic

**Pathways**
- **APC**
  - Normal colonic epithelium
  - Small adenoma
  - Large adenoma
  - Carcinoma
  - Patient age (years): 30-50, 40-60, 50-70

- **RAS**
  - Cell Cycle/Apoptosis
  - TGF-β

**PROGRESSION**

**TIME**
Human Cancer Exomes Sequenced
Non-synonymous Mutations per tumor
Cancer Mutations
September 2014

Coding Mutations
1,712,998
Genes
>20,000
Clinically Meaningful
91 Genes
Cancer Mutations
September 2014

91 Genes

Eligibility for active clinical trials (55/91; world-wide)

Associate with FDA approved therapies
Colorectal Cancer Genetics
pre-2006

Pathways

APC

RAS

PI3K Cell Cycle/Apoptosis TGF-β

Normal colonic epithelium
Small adenoma
Large adenoma
Carcinoma

Patient age (years) 30-50 40-60 50-70

TIME
Mutation Spectra in Colorectal Adenocarcinomas from Whole Genome Studies
Mutation Spectra in Colorectal Adenocarcinomas from Whole Genome Studies

- **APC (80%)**
- **P53 (60%)**
- **KRAS (43%)**
- **TTN (31%)**
- **PIK3CA (18%)**
- **PBXW7 (11%)**
- **SMAD4 (10%)**
- **NRAS (9%)**
- **Beta-Catenin (5%)**
- **BRAF (<5%)**
- **IDH (<5%)**
Mutation Spectra in Colorectal Adenocarcinomas from Whole Genome Studies
Mutation Spectra in Colorectal Adenocarcinomas from Whole Genome Studies
Colorectal Cancer - Oncogenes and Tumor Suppressor genes per tumor

**Oncogenes**

Gain of Function

- KRAS (43%)
- PIK3CA (18%)
- BRAF (<5%)

Number of driver gene mutations per tumor

Fraction of cases (%)
Colorectal Cancer - Oncogenes and Tumor Suppressor genes per tumor

**Loss of Function**

APC (80%)

P53 (60%)
Colorectal Cancer - Oncogenes and Tumor Suppressor genes per tumor

**Oncogenes**

- Number of driver gene mutations per tumor
- Fraction of cases (%)

**Tumor Suppressor Genes**

- Number of driver gene mutations per tumor
- Fraction of cases (%)

[Chart showing distribution of driver gene mutations for Oncogenes and Tumor Suppressor Genes]
Oncogenes and Tumor Suppressor genes per tumor

Fraction of tumors (%) vs. Number of driver gene mutations per tumor.

- Red bars: Oncogene Mutations
- Blue bars: Oncogene + Tumor Suppressor Gene Mutations

Cancer Types: Medulloblastoma, Pancreatic Cancer, Glioblastoma, Colorectal Cancer, Breast Cancer
Lack of Oncogenes

Few activating mutations in CRC

Tumor suppressors dominate

Need to learn how to target Tumor Suppressors

Alkylating agents in BRCA or PALB2 related tumors (breast and pancreas)
Genetic Heterogeneity

**INTRATUMORAL heterogeneity** within a primary tumor

**INTER-METASTATIC heterogeneity** between two metastases

**INTRA-METASTATIC heterogeneity** within a metastatic lesion

**INTER-PATIENT heterogeneity**
Genetic Heterogeneity

Random Mutations

Selective Pressure

Time

Passenger Mutations

Driver Mutations
Colorectal Cancer Genetics

1. Number of mutations depends on etiology (MMR-deficient or sporadic)

2. Point Mutations are the most common form genomic alteration

3. Majority of alterations occur as loss of function in Tumor suppressor genes

4. Tumors are genetically heterogeneous and this governs molecular resistance
Clinical Application of Cancer Genetics

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

Tumor Tissue
- FFPE
- Frozen tissue

Blood & other bodily fluids
- Cell-free DNA
- Circulating tumor cells (CTCs)
Liquid Biopsy

**Plasma**
- Water 91%
- Proteins 7%
- Metabolites (trace)
- Cell-free DNA (trace)

**Cellular Components**
- White Blood Cells 2-3%
- Platelets 2-3%
- Red Blood Cells 90%
- Circulating tumor cells (trace)
Source circulating cell-free DNA

- Bone Marrow
- GI Tract
- Skin
- Fetal DNA

Pool of Cell-free DNA
Source circulating cell-free DNA

Bone Marrow

GI Tract

Skin

Transplanted Tissue DNA

Pool of Cell-free DNA
Source circulating cell-free DNA

Bone Marrow

GI Tract

Skin

Tumor DNA

Pool of Cell-free DNA
Mutations are highly specific

- Pre-Cancer Cell: Mutations
- Cancer Cell: Mutations
- Normal Cells: No Mutations
Molecular Analysis

![Diagram of molecular analysis process]

- Tumor DNA
  - Direct Sequencing
    - Mutation: e.g. APC 1338 C > T

- Plasma DNA
  - real-time PCR
  - BEAMing
    - Total DNA Concentration: e.g. 11,500 DNA fragments per sample
    - % Mutations: e.g. 0.27%
    - Mutant DNA Concentration: e.g. 31 mutant DNA fragments per sample

Before Surgery
Day 0

After Surgery
Day 1

CT scan negative
After Surgery
Day 42

CT scan positive
After Surgery
Day 244

13.4 % 0.015 % 0.11 % 0.66 %

Percent Mutant DNA

Circulating tumor DNA

Somatic mutations can be effective biomarkers largely because of specificity.

Digital Genomics has improved sensitivity and throughput sufficient for real clinical application.

Applications will include genotyping, monitoring tumor burden, tracking molecular evolution (i.e. resistance), minimal residual disease and early detection.

Session at ESMO GI on this topic tomorrow.
Clinical Application of Cancer Genetics

- Prognostic Markers
- Somatic Cancer Genome Data
- Dynamic Biomarkers
- Immune Antigens
- Predictive Markers
Response to PD-1 Blockade
Melanoma

Patient with Melanoma

Topalian et al., NEJM 2012
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)

First defined by Papadopoulos, Kinzler and Vogelstein in early 1990s.
Mutations per tumor

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

Melanoma and Lung Cancers
Mutations per tumor

- Mismatch-repair proficient colon cancers
- Mismatch-repair deficient colon cancers

- Liquid Tumors
- Pediatric Tumors
- Sporadic Adult Solid Tumors
- Mutagen Associated tumors
- Mismatch repair tumors
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><strong>Cohort A</strong></td>
<td><strong>Cohort C</strong></td>
</tr>
<tr>
<td>Deficient in</td>
<td>Deficient in</td>
</tr>
<tr>
<td>Mismatch Repair</td>
<td>Mismatch Repair</td>
</tr>
<tr>
<td>(n=25)</td>
<td>(n=21)</td>
</tr>
<tr>
<td><strong>Cohort B</strong></td>
<td></td>
</tr>
<tr>
<td>Proficient in</td>
<td></td>
</tr>
<tr>
<td>Mismatch Repair</td>
<td></td>
</tr>
<tr>
<td>(n=25)</td>
<td></td>
</tr>
</tbody>
</table>

- Anti-PD1 (Pembrolizumab) - 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
## Baseline Demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MMR-deficient CRC n=13</th>
<th>MMR-proficient CRC n=25</th>
<th>MMR-deficient non-CRC n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median Age – years</strong></td>
<td>46</td>
<td>62</td>
<td>59</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>100%</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>Ampullary/Biliary</td>
<td>0</td>
<td>N/A</td>
<td>40%</td>
</tr>
<tr>
<td>Endometrial</td>
<td>0</td>
<td>N/A</td>
<td>20%</td>
</tr>
<tr>
<td>Small bowel</td>
<td>0</td>
<td>N/A</td>
<td>20%</td>
</tr>
<tr>
<td>Prostate</td>
<td>0</td>
<td>N/A</td>
<td>10%</td>
</tr>
<tr>
<td>Gastric</td>
<td>0</td>
<td>N/A</td>
<td>10%</td>
</tr>
<tr>
<td><strong>&gt; 2 Prior Therapies</strong></td>
<td>100%</td>
<td>100%</td>
<td>90%</td>
</tr>
<tr>
<td><strong>Lynch Syndrome</strong></td>
<td>85%</td>
<td>0%</td>
<td>40%</td>
</tr>
</tbody>
</table>
Biochemical Responses
## Objective Responses

<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>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>
Target Lesions

% Change from Baseline SLD

-100 -50 0 50 100

MMR-proficient CRC
MMR-deficient CRC
MMR-deficient non-CRC
Mismatch repair deficient Colorectal Cancer

Baseline

Week 20

Tumor Markers
Progression-Free Survival

**All Cohorts**
- Mismatch-repair proficient
- Mismatch-repair deficient

**CRC Cohorts**
- Mismatch-repair proficient
- Mismatch-repair deficient
Overall Survival

All Cohorts
- Mismatch-repair proficient
- Mismatch-repair deficient

CRC Cohorts
- Mismatch-repair proficient
- Mismatch-repair deficient
## Adverse Events

### All Grades

<table>
<thead>
<tr>
<th>Event-no. (%)</th>
<th>All Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Any</strong></td>
<td>14 (34)</td>
</tr>
<tr>
<td><strong>Generalized Symptoms</strong></td>
<td>3 (7)</td>
</tr>
<tr>
<td><strong>Pancreatitis</strong></td>
<td>6 (15)</td>
</tr>
<tr>
<td><strong>Pneumonitis</strong></td>
<td>1 (2)</td>
</tr>
<tr>
<td><strong>Endocrine Disorders</strong></td>
<td>5 (12)</td>
</tr>
<tr>
<td><strong>Rash/pruritus</strong></td>
<td>7 (17)</td>
</tr>
<tr>
<td><strong>Thrombocytopenia</strong></td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

*Up through Jan 2015*
Baseline PD-L1 Expression and CD8 T Cell Infiltration

- dMMR CRC
- dMMR non-CRC
- pMMR CRC

H&E | PD-L1 | CD8
--- | --- | ---

T | T | T
N | N | N
T | T | T
N | N | N
T | T | T
N | N | N

Invasive Front

TIL
Mutation Burden is Associated with Efficacy

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

• Mismatch repair deficient tumors are highly responsive to checkpoint blockade with anti-PD1.

• Clinical benefit is noted across tumors with mismatch repair deficiency including cancers of the colon, uterus, stomach, pancreas, prostate, duodenum and bile ducts.

• Biochemical response correlates with radiographic response as well as with PFS and OS.

• Mismatch repair deficient tumors are highly mutated and are rich in CD8⁺ T cells and PD-L1 expression at the tumors’ invasive front.
Response assessment: Every 9 weeks
Primary endpoint: ORR per RECIST v1.1
Secondary endpoints: Duration of response, disease control rate, PFS, OS, safety
Conclusion

• This is the first study to use a tumor’s genetics to guide immunotherapy

• Mismatch repair deficiency is represented in \(~4-5\%) of cancers of the colon, rectum, endometrium, stomach, bile duct, pancreas, prostate and brain

• 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
Summary

Somatic mutations can be effective biomarkers & therapeutic targets largely because of specificity.

Somatic mutations can have alternative applications (circulating tumor DNA and immunogenic antigens).

Future therapeutics that target somatic alterations need to target non-traditional targets (i.e. tumor suppressors, immunogenic mutations, multiple agents).

Future applications will focus on unmet clinical needs.
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