Exploring the future of digital pathology in immuno-oncology and companion diagnostics

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DISCLOSURE

I am a full-time employee at Bristol-Myers Squibb
How can we treat cancer better?

Cancer cell

T cell

T cell approaches cancer cell.

T cell attacks cancer cell.

Cancer cell destroyed.
How can we treat cancer better?

• Immunotherapy is the most effective therapeutic approach in a variety of advanced and metastatic cancers that the cancer community saw in decades.

  MD Anderson immunologist Jim Allison awarded Nobel Prize

• Success of each of the agents is highly dependent on the choice of a specific approach for a specific patient population.
• Adoption of cancer immunotherapy requires the development and implementation of robust clinical-grade biomarkers.
• Once validated, there exists significant market access challenges including regulatory and reimbursement hurdles.
What is immuno-oncology?

- Immuno-oncology describes a range of therapies which utilize the immune system to treat cancer.
- Not all patients respond to therapy.
- Field is still working to understand:
  - Tumor microenvironment.
  - Key mechanisms that drive cancer-immune cell interaction.
What is PD-L1/PD-1 checkpoint blockade?

- Binding of PD-L1 to PD-1 leads to an immunosuppressive effect and allows the tumor to evade immune destruction.

PD-L1 is often expressed on the surface of tumor cells.

PD-1 is often expressed by T-cells and B-cells.
What is PD-L1/PD-1 checkpoint blockade?

- Nivolumab is a human IgG4 **anti-PD-1** monoclonal antibody
- Using **anti-PD1** antibodies to block **PD-1** allows those T-cells and B-cells to continue to attack tumor cells
Why do we need Companion Diagnostics?

Worrisomely High Ineffectiveness of Conventional Evidence-Based One Size Fits All Therapies Throws the Spotlight on Companion Diagnostics as Critical Tools in the Successful Administration of Personalized Medicine

% Patient Population Unresponsive to Mainstream Therapies by Category

1. Anti-Depressants
   - 39%
2. Asthma Drug
   - 42%
3. Diabetes Drug
   - 47%
4. Arthritis Drug
   - 55%
5. Alzheimer’s Drug
   - 74%
6. Cancer Drug
   - 80%

Heres What Companion Diagnostics (CDx) Brings to the Healthcare System

1. Reduced Side-Effects
2. Lower Healthcare Costs
3. Improved Patient Outcome
4. Benefits of Personalized Medicine
5. Higher Drug Development Success
6. Aids in Early Diagnosis & Treatment
7. Reduced Variability of Pharmacotherapy
8. Identification of the Most Effective Therapy
PD-L1 as a Companion Diagnostic

- PD-L1 positive cases should benefit
- PD-L1 negative cases should perhaps not receive anti-PD1 therapy
- PD-L1 negative cases may benefit from other treatment options
How is PD-L1 scored?

- PD-L1 CDx is currently implemented as an IHC assay
- 1% cutoff commonly used to determine PD-L1 positivity
- Scoring criteria and staining can vary depending on application (e.g. treatment type, indication)
Many antibodies for each drug target

- Same target, different antibodies
- Antibodies have varying amount of target specificity

### Assays for PD-L1 expression in UC

<table>
<thead>
<tr>
<th>Immunotherapy (IO)</th>
<th>Atezolizumab</th>
<th>Nivolumab</th>
<th>Pembrolizumab</th>
<th>Durvalumab</th>
<th>Avelumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection antibody</td>
<td>SP142</td>
<td>28–8</td>
<td>22C3</td>
<td>SP263</td>
<td>73–10</td>
</tr>
<tr>
<td>IHC platform</td>
<td>Ventana</td>
<td>Dako</td>
<td>Dako</td>
<td>Ventana</td>
<td>Dako</td>
</tr>
<tr>
<td>Cell types scored for UC</td>
<td>ICs</td>
<td>TCs</td>
<td>ICs + TCs</td>
<td>ICs + TCs</td>
<td>ICs + TCs</td>
</tr>
<tr>
<td>Cut-off definitions for UC</td>
<td>PD-L1+ (IHC 2/3) ≥5% of ICs PD-L1+</td>
<td>PD-L1+ ≥1% TC expression</td>
<td>PD-L1+ ≥10% TC and IC staining</td>
<td>PD-L1+ ≥25% of ICs and TCs with membrane PD-L1 staining</td>
<td>PD-L1+ ≥5% TC staining or ≥10% IC staining</td>
</tr>
<tr>
<td>Estimated PD-L1 prevalence in UC trials</td>
<td>~32%</td>
<td>~37%</td>
<td>~30%</td>
<td>~54%</td>
<td>~34%</td>
</tr>
</tbody>
</table>
Pathologists need to be trained for each assay

- Different staining (non-equivalent across assays, Rimm et al. 2017)
- Different scoring (ICs vs TCs vs IC + TC)
Many indications for each target

Share of patients displaying positive PD-L1 tumor stain varies by indication

Yarchoan et al. JCI 2019
Many biomarkers proposed to fill need for I-O CDx

CONCEPTUAL

Predictive Power
Biomarker Area Under the Curve (AUC)

Prevalence
Percent of patients positive by biomarker

MSI
PD-L1/2 Copy Number Variation
PD-L1 IHC TPS >50%
MuB High
CD3+/CD8+ TILs
PD-L1 IHC TPS >1%
Many more checkpoint targets to come
COMBINATORIAL EXPLOSION

Ipilimumab, the first approved checkpoint inhibitor, has been tested in dozens of clinical trials since 2001. And like many other drugs in its class, it is increasingly being tested in combination with other therapies.

US regulators approve ipilimumab for treatment of advanced melanoma.

Studies show improved survival in people with advanced melanoma.
Many potential therapeutic options

<table>
<thead>
<tr>
<th></th>
<th>FDA-Approved Cancer Drugs</th>
<th>All FDA-Approved Drugs (considering “off-label” use)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs</td>
<td>300</td>
<td>1000</td>
</tr>
<tr>
<td>Combinations of 2 Drugs</td>
<td>44,850</td>
<td>499,500</td>
</tr>
<tr>
<td>Combinations of 3 Drugs</td>
<td>4,455,100</td>
<td>166,167,000</td>
</tr>
</tbody>
</table>

https://www.curematch.com/blog-posts/numbers-combination-therapy-oncology/
More therapeutic options = More diagnostics

Patient population

Treatment

Standard approach

Tailored approach

Treatment A (effective in 20% of target population; 80% is waste)

Treatment A
Treatment B
Treatment C
Treatment D

Recap of challenges ahead in immuno-oncology

- Many antibodies for each drug target
- Many indications for each drug target
- Many new drug targets to come
- Combination trials introduce more therapeutic options
- More biomarkers needed for each therapy
- Trained pathologists needed to interpret each assay
What role does a pathologist play in determining treatment?

Pathologist will be asked to provide a diagnosis based on what they see through a microscope

• Presence of tumor
• Tumor grade/stage
• Perhaps 2 or 3 other markers
Potential Drawbacks

• Low throughput
  • Physical slides
  • Manual Inspection

• Low transparency
  • Inter-reviewer variability

• We have the opportunity to bridge the gap between what clinical care looks like today and shape what it can be
Digital Pathology: An Extremely Rich Data Source!

- Gigabyte sized images
- Millions of cells
- Limitless cell types once you include antibodies
- Pathology images are capable of capturing cells within the context of their tumor microenvironment
- May be easier to capture with aid of computational tools
Morphological Descriptors

- Automated image analysis used to glean morphological descriptors from histology
- Quantitative assessment of tissue
  - Architectural – Organization
  - Morphological – Shape
- Previous work Lee et al. 2016 in prostate cancer found histology features predictive of
  - Gleason grade
  - Biochemical Recurrence
Describing Patterns of Inflammation

- Saltz et al. (2016) developed a CNN based lymphocyte detection algorithm to TCGA histology slides
- Clusters of lymphocytes were used to describe different inflammation patterns
- Local TIL spatial structures correlated with survival
Artificial Intelligence targeting Digital Pathology

Precision histology: how deep learning is poised to revitalize histomorphology for personalized cancer care

Paige.AI nabs $25M, inks IP deal with Sloan Kettering to bring machine learning to cancer pathology

PathAI raises $60 million for AI pathology and diagnostic tools

Deep Lens collects another $13.7M for digital pathology

Proscia wraps up $8.3M Series A to expand digital pathology platform
Digital Pathology is ideally suited for I-O

1. Clearly identify cell phenotypes in tumor microenvironment
2. Capture spatial context of immune cell infiltration
3. Automation and standardization via computational image analysis
4. Explore hundreds or thousands of hypothesis from computationally derived biomarkers to identify best treatments for patients
How can digital pathology address challenges in I-O?

1. Tumor Profiling
2. OPAL Multiplex analysis
3. Proximity analysis
4. Tumor PDL1 scoring
5. Immune cell scoring
Preclinical assessment – a target worth pursuing?

Many IHC assays built for tumor assessment

Table 2. Distribution of validated IHC assays for each drug discovery phase

<table>
<thead>
<tr>
<th>Drug discovery phase</th>
<th>TV/LG</th>
<th>LO/preclinical</th>
<th>Phase I/II</th>
<th>Phase III</th>
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<tbody>
<tr>
<td>IHC-based biomarker</td>
<td>Early target/drug disease positioning and</td>
<td>PK/PD, development of clinical biomarker strategy</td>
<td>PoM biomarker deployment and patient selection</td>
<td>Feasibility assays for companion diagnostic</td>
</tr>
<tr>
<td>application</td>
<td>basic validation package</td>
<td>and further disease positioning</td>
<td>strategy build</td>
<td>development</td>
</tr>
<tr>
<td>Validated IHC assays (n)</td>
<td>41</td>
<td>60</td>
<td>25</td>
<td>4</td>
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IHC, immunohistochemistry; LG, lead generation; LO, lead optimisation; TV, target validation; PK/PD, pharmacokinetics/pharmacodynamics; PoM, proof of mechanism.

Smith and Womack. Journal of Pathology, 2013

• What does the treatment population look like in terms of the drug target?
IHC analysis

0

1+
HER2 Control Slides

2+

3+

Breast Tissue
IHC analysis

• Heavily used to test for presence of drug targets and drug response
• Pathologist must be trained to assess each assay
• If the goal is to develop a biomarker, must be concordant
• Image analysis could help with standardization
Quantitative scoring

Difficult and time consuming to score assays consistently manually.

Quantitative scoring may improve tasks of:
- Counting
- Stain Intensity
Location Matters

- Increasing interest of location of lymphocytes (TILs) relating to success in immunotherapies
- Co-localization of markers can paint complete picture of TME
- Multiple serial slides can present challenges for visual review

Ribas JEM 2016
How can digital pathology address challenges in I-O?

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OPAL Multiplex Analysis – What’s in the TME?

- CD4 = Green
- CD8 = Yellow
- CD20 = Red
- CD68 = Magenta
- CK = Cyan
- Foxp3 = Orange
Challenging to score

- OPAL allows for 4+ markers on single image
- Pathologists trained on IHC
- Co-localization of markers difficult to assess
- \(2^6 = 64\) possible phenotypes
- Necessitates image analysis

Parra et al. Nature Sci Reports 2017
Image analysis

• More consistent
• Higher throughput
How can digital pathology address challenges in I-O?

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Understanding the mechanisms for response

Many groups looking to characterize cell-cell proximity

- Feichtenbeiner 2014 - Mean nearest neighbor distance from FoxP3 to CD8 in gastric cancer – duplex IHC
- Feng 2016 - CD8 proximity with FoxP3/PD-L1 – Multi-spectral imaging
- Giraldo 2017 - Percent PD-L1 with proximal PD-1/CD-8 in Merkel Cell Carcinoma – serial monoplex IHC
Proximity Analysis

Register images → Annotate tumor region → Analyze → Generate spatial plots

Stain 1

Overall tumor region

Detect Stain1 Positive cells

Stain 2

Stain Positive tumor region

Detect Stain2 Positive cells*

*Stain2+ cells in this region includes both tumor cells and immune cells

Red indicates Stain1+ cell <30µm from Stain2+ cell

Proximity analysis

Plot Stain1 positive cells

Plot Stain2 cells

Merge layers
Percent engagement

Hypothesis is that engagement is predictive of response to I-O

%engagement = [# of Engaged Stain1+ cells]/[# of Total Stain1+ cells]

*Engaged if <30um cells
Note: 30µm distance selected based on prior internal analyses and review of external publications
Correlation between %engaged Stain1+ cells and manual %Stain2+ tumor

- Moderate correlation
- Most correlation is in high/high tumor samples
- Engagement score can help stratify cases with low %Stain2+ Tumor
How can digital pathology address challenges in I-O?

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Clinical assessment – can we stratify by response?

Implement CDx biomarker which can differentiate patients who will and will not respond to therapy.

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Smith and Womack. Journal of Pathology, 2013
PDL1+ Tumor Scoring

Fig. 1. Value of PD-L1 IHC test in gastric and GEJ cancer

PD-L1-positive gastric/GEJ cancer

This patient is more likely to respond to immunotherapy.

PD-L1-negative gastric/GEJ cancer

This patient is less likely to respond to immunotherapy.
PDL1+ Tumor Scoring

• Image analysis can help with standardization and improve precision/accuracy of readouts
• 0.83-0.88 Intra-class correlation for PD-L1 scoring in NSCLC (Rimm et al. 2017)
• Cut point concordance
  – Fleiss κ statistic: >50%, is 0.749, >1% is 0.537.
  – Kendall: >50% is 0.775, >1% is 0.612.
Precision Histology: AI-Based Image Analysis

- Built and validated a set of tissue region classification and cell classification models using commercial samples
Precision Histology: AI-Based Image Analysis

- Built and validated a set of tissue region classification and cell classification models using commercial samples.
- Once trained, can quickly generate thousands of features across a database of images.
Automated PDL1 Tumor Quantification is Consistent with Manual Scoring

<table>
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<tr>
<th>AUTOMATED</th>
<th>MANUAL</th>
<th>PDL1+ TUMOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>11%</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>35%</td>
</tr>
</tbody>
</table>

Agreement Overall = 78%
PPA = 80%
NPA = 76%

- Same number (prevalence) of PDL1+ patients (54%) using both methods.
- Only 80% of the positive patients are concordant between both methods.
Correlation of PDL1 Histologic Score
How can digital pathology address challenges in I-O?

1. Tumor Profiling
2. OPAL Multiplex analysis
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5. Immune cell scoring
PDL1+ Immune Cell Scoring

• Certain tumor types express low or no PDL1+, reducing the prognostic value of PDL1+ Tumor scoring
• Immune cells also express PDL1+, but immune cells more difficult to score

Table 2. ICC for the Pathologist Scores and Concordance Statistics

<table>
<thead>
<tr>
<th>Cells</th>
<th>Antibody, ICC (95% CI)</th>
<th>22c3</th>
<th>28-8</th>
<th>SP142</th>
<th>E1L3N</th>
<th>Summary, Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor cells</td>
<td>0.882 (0.873-0.891)</td>
<td>0.832 (0.820-0.844)</td>
<td>0.869 (0.859-0.879)</td>
<td>0.859 (0.849-0.869)</td>
<td>0.86 (0.02)</td>
<td></td>
</tr>
<tr>
<td>Immune cells</td>
<td>0.207 (0.190-0.226)</td>
<td>0.172 (0.156-0.189)</td>
<td>0.185 (0.169-0.203)</td>
<td>0.229 (0.211-0.248)</td>
<td>0.19 (0.03)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ICC, intraclass correlation coefficient.

*N = 90.

Rimm et al. JAMA 2017
AI is Capable of Distinguishing Tumor Cells vs Immune Cells vs Stromal Cells
AI Correlates with Consensus of Pathologists

Scoring Immune Cells not easy for pathologists

**All Immune Cells**

\[ R^2 = 0.85989, p = 9.9859e-28 \]

\[ y = 0.95x - 10 \]

**PDL1+ Immune Cells**

\[ R^2 = 0.70859, p = 3.927e-15 \]

\[ y = 0.33x - 0.46 \]

**PDL1- Immune Cells**

\[ R^2 = 0.83753, p = 4.357e-25 \]

\[ y = 1.7x + 3.5 \]
Tumor Associated Immune Cells

- TAICs have been implicated with improved response to immunotherapies
- AI-Powered Image Analysis can assist in distinguishing epithelial versus stromal immune cells
Tumor Associated Immune Cells
CD8 scoring

CD8 Scoring, Intra-epithelial

Correlation = 0.79

BMS-CD8 Score

0-Absent 1-Low 2-Mod 3-High

PathAl-CD8 Percentage
Concluding Remarks

- Digital Pathology and Image Analysis has the potential to become the platform for the next generation of companion diagnostics in I-O and beyond
- The future of precision medicine is going to be a lot more complex
- Computational tools will be necessary to mine image data for new biomarkers and match patients to treatments
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