ASCP Scientific Updates: Liquid Biopsies: Current Limitations and Potential Applications

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• MD Anderson Cancer Center and Guardant Health have a formal strategic relationship involving the clinical implementation and use of liquid biopsies.
Alexander Lazar MD/PhD

- Professor, Pathology & Genomic Medicine, MDACC
- UT Southwestern (MD/PhD)
- BWH / DFCI / MGH / Harvard – AP, Soft Tissue, Dermatopathology
- ~400 papers and book chapters on molecular diagnostics and multi-omic analysis of solid tumors, targeted and immunotherapies in sarcoma and melanoma

Roadmap

- Immunotherapy in melanoma
- Potential biomarkers to inform immunotherapy in melanoma
- General considerations in liquid biopsies
- Can liquid biopsies in guide immunotherapy decisions in melanoma (and other solid tumors)?

We have made major advances in the treatment of melanoma with targeted therapy and immunotherapy

**FDA-approved agents for stage IV melanoma**

- Dacarbazine (1976)
- High-dose IL-2 (1998)
- Ipilimumab (2011)
- Vemurafenib (2011)
- Dabrafenib (2013)
- Trametinib (2013)
- Dab+Tram (2014)
- Vem + Cobi (2015)
- TVEC (2015)
- Nivolumab + Ipilimumab (2015)
- Pembrolizumab (2014)
- Nivolumab (2014)

Data: National Cancer Institute, 'Clinical Trials of Immunotherapy for Melanoma'

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These advances are associated with improved survival

1-year survival rates for stage IV melanoma

30–35%1,2 47%4 56%5 69%6 73%7 74%8 74%9

1990 2001 2002 2003 2004 2005 2006 2007 2008 2009

Can we improve response rates even further, and bring these therapies to more patients?

Adapted from slide of Georgina Long

Melanoma Biomarkers

• Immune microenvironment
  – PD-1, PD-L1 expression
  – CD8 and others
  – Clonality
• Mutational Load
  – Total
  – Specific
  – MSI / MMRD
  – CNA burden

• Oncogenic Pathways
  – ERK
  – PTEN/PI3CA/AKT
  – Wnt/b-catenin
• Serum factors
• Microbiome
• Assay Timing
  – Tissue-based
  – Radiology
• Ready for the Clinic?
Material and Methods

- Example of PD-L1 labeling
  - Membranous labeling
    (Complete circumferential/Partial)

**PFS by PD-L1 Expression Level (1%)**

<table>
<thead>
<tr>
<th>PD-L1 ≥1%*</th>
<th>NIVO + IPI</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIVO + IPI</td>
<td>12.4</td>
<td>0.44</td>
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<tr>
<td>IPI</td>
<td>13.9</td>
<td>--</td>
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</table>

<table>
<thead>
<tr>
<th>PD-L1 &lt;1%*</th>
<th>NIVO + IPI</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIVO + IPI</td>
<td>2.8</td>
<td>0.67</td>
</tr>
<tr>
<td>IPI</td>
<td>2.8</td>
<td>--</td>
</tr>
</tbody>
</table>

*Per validated PD-L1 immunohistochemical assay based on PD-L1 staining of tumor cells in a section of at least 100 evaluable tumor cells.

How can we best predict responses to immune checkpoint blockade?

**Distribution of CD8+ T cells**

Immune differences seen in responders and non-responders to PD-1 therapy (namely, CD8+ cells at invasive margin in responders before treatment and in tumor while on therapy)

Mutational Load

How can we best predict responses to immune checkpoint blockade?

Genomic factors

- Mutational load and neoantigens may help explain varied response to therapy

Recurrent SERPINB3 and SERPINB4 mutations in patients who respond to anti-CTLA4 immunotherapy

Oncogenic Pathways

Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity

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Role of Oncogenic Signaling Pathways: PI3K-AKT Pathway

- PTEN Loss → ↓ T-cell infiltration, ↓ sensitive to T-cell killing, and ↓ outcomes with checkpoint inhibitors (in mice and pts)
- Identified actionable strategies to overcome resistance
  - VEGF & PI3Kβ inhibitors
  - PI3Kβi (GSK) + Pembrolizumab

Peng et al., Cancer Discovery, 2016 (Cover)

Serum Factors

Figure 1: Outcomes with elevated levels of serum LDH, A. PD-1 for patients with normal (black line) and elevated (red line) serum LDH. B: C), C桅 Cord saliva.

Copyright © 2018 American Society for Clinical Pathology.
Bacteria within the gut of patients with cancer can modulate responses to therapy

Evidence for the role of the microbiome in animal models of melanoma published in Science 2015

Higher diversity of gut microbiome observed in responders to PD-1 blockade
### PIC-0014: A randomized trial to evaluate the impact of gut microbiome modulation in patients going on to treatment with immune checkpoint blockade

<table>
<thead>
<tr>
<th>Day</th>
<th>Event</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Day-14</td>
<td>Administer FMT from complete responders (CD3, CD8+ T-cells)</td>
</tr>
<tr>
<td>0</td>
<td>Day-0</td>
<td>Administer FMT from complete responders (CD3, CD8+ T-cells)</td>
</tr>
<tr>
<td>7</td>
<td>Day-7</td>
<td>Administer FMT from complete responders (CD3, CD8+ T-cells)</td>
</tr>
<tr>
<td>0</td>
<td>Day-0</td>
<td>ADP-4+ aCTLA4 (anti-TNF drug)</td>
</tr>
<tr>
<td>14</td>
<td>Day-14</td>
<td>Administer FMT from complete responders (CD3, CD8+ T-cells)</td>
</tr>
<tr>
<td>42</td>
<td>Day-42</td>
<td>Administer FMT from complete responders (CD3, CD8+ T-cells)</td>
</tr>
</tbody>
</table>

**All patients:** CT scans with RECIST week 12

**Primary endpoint:** safety and tolerability

**Secondary endpoints:** engraftment, response and correlation studies

**During modulation in patients going on to treatment with immune checkpoint blockade (anti-CTLA4)***

- **Assay Timing:**
  - **Day 0:** Baseline blood and fecal sampling
  - **Day 7:** Blood and fecal sampling
  - **Day 14:** Blood and fecal sampling
  - **Day 42:** Blood and fecal sampling

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### These approaches may also be helpful in treating immunotherapy toxicity

**Wang et al., Nature Medicine, 2018**

1. **Stool**
   - **Day 0:** Stool sample
   - **Day 1:** Stool sample
   - **Day 2:** Stool sample

2. **Colony**
   - **Day 1:** Colony sample
   - **Day 2:** Colony sample

**Two patients** treated with immune checkpoint blockade (anti-CTLA4, anti-PD1) with severe symptoms of immunotherapy toxicity:

- **Resolution of all symptoms**
- **Healthy donor**
- **Complete responders**

**All patients** treated with FMT from a healthy donor and had complete resolution of all symptoms

**Day 7:** Blood and fecal sampling

**Day 14:** Blood and fecal sampling

**Day 42:** Blood and fecal sampling

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### Assay Timing
Understanding Resistance to Checkpoint Inhibitors

- Longitudinal collection and analysis of biospecimens from patients receiving CTLA4 → PD1
- Responders versus Non-Responders, Anti-PD1
- Baseline: Statistically significant but overlapping CD8, CD3
- On-Treatment: Marked difference in intratumoral inflammation

Need to increase infiltration/inflammation in non-responding tumors

Peng,... Wargo, Cancer Discovery, 2016

We may have acceptable predictive biomarkers at present but may simply be looking at the wrong time point

Liquid Biopsies
Terms & Definitions

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ctDNA</td>
<td>Free DNA polymer found in circulating blood and urine that can be used to identify cancer-specific mutations.</td>
</tr>
<tr>
<td>ctDNA assay</td>
<td>Assay designed to detect ctDNA in plasma or urine, using either liquid biopsy methods or next-generation sequencing.</td>
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</tbody>
</table>

Comparing ctDNA & Tissue Assays

<table>
<thead>
<tr>
<th>Comparison</th>
<th>ctDNA Assay</th>
<th>Tissue Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection</td>
<td>Plasma/urine</td>
<td>Tissue sample</td>
</tr>
<tr>
<td>Processing</td>
<td>Liquid biopsy</td>
<td>Formalin-fixed, paraffin-embedded (FFPE) tissue</td>
</tr>
<tr>
<td>Analysis</td>
<td>NGS</td>
<td>IHC, immunohistochemistry</td>
</tr>
<tr>
<td>Clinical utility</td>
<td>Early detection, minimal invasive, and sensitive to minimal residual disease</td>
<td>Sensitivity limited to detectable areas, local therapy monitoring, and surgical planning</td>
</tr>
</tbody>
</table>

Table 1: Terms and Definitions

Table 2: Comparison of ctDNA Versus Tissue Testing
Table 2. Summary of Key Findings on the Use of ctDNA Analysis in Patients with Cancer

| Key Findings |  
| --- | --- |
| **Pre-analytic** |  
| Need to understand how collection and handling procedures affect results. |  
| **Analytical Validity** |  
| Need to understand the relevant performance characteristics of each assay. |  
| **Interpretation & Reporting** |  
| Discordance between tissue and liquid biopsies is not unexpected |  
| Clonal hematopoiesis of indeterminate potential (CHIP) |  

Published in: Jason D. Merker; Geoffrey R. Oxnard; Carolyn Compton; Maximilian Diehn; Patricia Hurley; Alexander J. Lazar; Neal Lindeman; Christina M. Lockwood; Alex J. Rai; Richard L. Schilsky; Apostolia M. Tsimberidou; Patricia Vasalos; Brooke L. Billman; Thomas K. Oliver; Suanna S. Bruinooge; Daniel F. Hayes; Nicholas C. Turner; Journal of Clinical Oncology 2018, 36, 1631-1641. DOI: 10.1200/JCO.2017.76.8671 Copyright © 2018 American Society of Clinical Oncology
# Key Findings: Clinical Validity & Utility

## Table 2. Summary of Key Findings on the Use of ctDNA Analysis in Patients with Cancer

**Key Findings:**
- Lots of emerging data here

### Melanoma Biomarkers
- Immune microenvironment
  - PD-1, PD-L1 expression
  - CD8 and others
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- Mutational Load
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- Assay Timing
  - Tissue-based
  - Radiology
- Ready for the Clinic?

## Conclusions
- Liquid biopsies are here to stay.
- They are useful in multiple cancer types.
- Later stage oncological management is much better established than population screening and diagnosis in cancer.
- Becoming a preferred method for documenting resistance to targeted therapies.
- Best use cases in solid tumors will be for mutations associated with immunotherapy response, MSI and TMB.
- Beyond DNA: RNA, methylation, etc...