2015 Society for Immunotherapy of Cancer (SITC) Biomarkers Task Force-Progress and Challenges

Magdalena Thurin, Ph.D., NCI
Society for Immunotherapy of Cancer (SITC) Biomarkers Task Force 2015 - Focus on Predictive Markers
Steps in biomarker development and registration

1. Biobanking
2. Biomarker discovery
3. Technical / analytical validation
4. Quality control accreditation
5. Clinical validation
6. FDA / EMA approval

Source: © 2011 BMJ Publishing Group Ltd & European League Against Rheumatism
Society for Immunotherapy of Cancer (SITC)
Biomarkers Task Force

Focus and responsibilities: review the state of art existing assays and technologies, identify challenges for further success and provide recommendations.

4 Working Groups
- WG1-validation of candidate biomarkers (Thurin M and Masucci G)
- WG2- identification of the most promising technologies (Yuan J)
- WG3- testing of high throughput immune signatures (Stroncek D)
- WG4- investigation of the pre-treatment tumor microenvironment (Gnjatic S)

Steering Committee: Butterfield L, Disis N, Fox B, Khleif S and Marincola F

Expected Outcome: One-pagers, a series published monthly describing the technology, platform, level of evidence. Meeting-April 1st, 2016, Bethesda, MD

Publication:
WG1 chairs: Thurin (NCI) and Masucci (Department of Pathology, Karolinska Institute)

- vβ TCR repertoire - Lanny Kirsch (Adaptive Biotechnologies, Inc.)
- PD-L1 IHC - John Alvarez (Janssen Pharmaceutical of J&J)
- Nanostring - Alessandra Cesano (Nanostring Technologies, Inc.)
- Single Cell Network Profiling (SCNP) - Alessandra Cesano and Rachael Hawtin (Nodality, Inc.)
- Enzyme-Linked ImmunoSpot (ELISPOT) - Sylvia Janetzki (Johannes Gutenberg University, Meinz) and Jenny Zhang (Mayo Clinic)
- Immunoscore - Sylvan Senthamil (Omni Array Biotechnology)
- Myeloid Derived Suppressor Cells (MDSC) - Paul Robbins (Medimmune)
- Mutational load/neoepitopes - Howard Streicher (NCI)
- Statistical issues - Kevin Dobbin (University of Georgia)

**Expected Outcome:**
- White paper - Validation of Biomarkers for response to immunotherapy, manuscript in preparation
- One-pagers: method and clinical use: TCR immunosequencing, EliSpot, Nanostring, SCPN, Clinical Validation; in preparation: PD-L1, Immunoscore, MDSC, NGS
### Analytical validation steps

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analytical validation</strong></td>
<td>Analytical validation of the test demonstrates how accurately the test measures the analyte of interest in the patients specimen</td>
</tr>
</tbody>
</table>
| **Pre-analytical validation** | Tissue acquisition, specimen handling, size, ischemic time  
Shipping conditions  
Fixation, conditions, fixative formula, timing  
Decalcification type and time  
Dehydration and clearing  
Paraffin embedding, storage, sectioning  
Antigen retrieval method, time, temperature  
Drying  
Storage, time to analysis |
| **Analytical validation** | Antibody selection, validation, controls  
Assay, staining method  
Controls, reference standards, external, internal  
Manual vs. computerized digital image analysis  
Image acquisition, whole tissue sections vs. snapshot images  
Reproducibility, repeatability, precision, accuracy |
| **Post-analytical validation** | Data interpretation  
Scoring/quantification/interpretation  
Cutoff value, dichotomous vs. continuous variables  
Multiplex assays  
Software |
## Clinical validation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
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<tbody>
<tr>
<td>The clinical validation of the biomarker is the process linking a biomarker to a clinical endpoint.</td>
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<td>Sensitivity of the biomarker, referred to as the ability of a biomarker to predict a meaningful change in clinical endpoint. Specificity of the biomarker, referred to as the ability of a biomarker to distinguish patients who are responders from non-responders.</td>
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<tr>
<td>A graphical approach for showing accuracy across the entire range of biomarker concentrations. ROC curve is essentially a plot that captures true positive rate against false positive rate of an assay. AUC value close to 1 indicates good discrimination, whereas an AUC of 0.5 provides no useful information regarding the likelihood of response.</td>
<td></td>
</tr>
<tr>
<td>ROC is used to set cut points; the sensitivity and specificity of the assay must be demonstrated through robust ROC curves that provide support for the cut points established to identify responders vs. non-responders.</td>
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Common study designs for biomarker studies
(Ziegler A et al. Human Genetics 2012)
Challenges for successful biomarker validation

- “Fit for purpose” biomarker must have clinical utility (diagnostic, prognostic, or predictive).
- Analytical validation - biospecimens should be collected from cohort with defined clinical endpoints using rigorous standard operating procedures (SOPs).
- Sample sets must be sufficiently sized to statistically power the study, and the data should reflect a robust system capable of generating meaningful results (e.g., community cohort).
- Biomarkers must be confirmed in a fully independent test and validation cohorts.
- The steps will include the first retrospective validation of collected specimens followed by a prospective validation or in a retrospective prospective design trials.
- To establish "cut points" for the assay, which are required by physicians to make clinical decisions (a ROC that is a plot that captures true positive rate against the false positive rate of an assay is used to set cut points).
- An important aspect of determining cut-points to support clinical decision making is determining the positive and negative predictive values for the assay i.e., the likelihood of a false positive or false negative result, and what does that ultimately mean in terms of impact.
Figure 1. The basis of the immunosequencing assay. Multiple V, D and J gene segments exist in the germline genome. Initial receptor diversity is generated by recombination of V, D and J segments; and additional non-templated diversity is introduced at the junc...
Figure 5. Quantitative sequencing of TCRB in patients with melanoma. Progressors (blue) were associated with both a lower number of TILs (shown as percentage TIL infiltrate) and a lower TIL clonality than responders (orange). Adapted from Tumeh et al. (2014)

Ilan Kirsch, Marissa Vignali, Harlan Robins

Molecular Oncology, 2015, Available online 15 September 2015

http://dx.doi.org/10.1016/j.molonc.2015.09.003
Genetic basis for Immunotherapy in cancer

(Champiat, S. et al. 2014)
PD-L1 IHC methods currently in testing/technical challenges

- A multiple PD-L1 IHC antibodies have been utilized, including 28-8, 5H1, MIH1, and 405.9A11 (17 reported) and PD-L1 companion diagnostic has been developed.
- PD-L1 is a labile antigen sensitive to antigen retrieval method. Optimal method for epitope retrieval method for each tissue type is needed.
- The lack of a clear definition of "positive" tumor PD-L1 staining by IHC. Lack of calibrators, controls.
- The pattern of expression can be cytoplasmic, membranous or none.
- Scoring: intra-tumoral PD-L1 (TC), invasive margin PD-L1, T cells (IC) in both locations or combination of the above (TC+IC).
- Some systems include intensity into the staining algorithm (H and Allered) or proportional method.
- Cut-off points for a positive result ranging from >1% to >50% based on percent tumor cells stained.
- PD-L1 negative patients who respond: i) assay sensitivity (below detection level of IHC); ii) tumor sampling, heterogeneity; iii) other ligands (PD-L2).

<table>
<thead>
<tr>
<th>mAb clone</th>
<th>JHU</th>
<th>BMS</th>
<th>Merck</th>
<th>Roche</th>
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</thead>
<tbody>
<tr>
<td>Automated</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Staining location scored</td>
<td>Membrane</td>
<td>Membrane</td>
<td>Membrane, any intensity</td>
<td>Membrane</td>
</tr>
<tr>
<td>Cell type(s) scored</td>
<td>Tumor cells</td>
<td>Tumor cells</td>
<td>Tumor and/or infiltrating immune cells, APS scoring method</td>
<td>Infiltrating immune cells</td>
</tr>
<tr>
<td>Cutoff</td>
<td>&gt;5%</td>
<td>&gt;5%</td>
<td>&gt;1%</td>
<td>&gt;1%</td>
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A Blueprint Proposal for Companion Diagnostic Comparability


- **Blueprint Proposal** developed by four pharmaceutical companies (Bristol-Myers Squibb Company, Merck & Co. Inc., AstraZeneca PLC, and Genentech, Inc.) and two diagnostic companies (Agilent Technologies, Inc./Dako Corp and Roche/Ventana Medical Systems, Inc.)

- The goal of the proposal is to characterize the performance of the various PD-1/PD-L1 companion diagnostic tests developed by Dako and Ventana for NSCLC. NSCLC was chosen as the first disease for study because all the pharmaceutical companies involved in developing the blueprint proposal have active drug development programs in NSCLC, each with its own specific companion diagnostic.

- Having multiple drug-diagnostic pairs in the pipeline raises the very real possibility that multiple PD-1 inhibitors may be approved by the FDA for a single indication like NSCLC.
PD-L1 IHC pharmDx

PD-L1 IHC 22C3 pharmDx
• PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA® (pembrolizumab).
• PD-L1 IHC 22C3 pharmDx is a qualitative immunohistochemical companion diagnostic assay using mAb Anti-PD-L1, Clone 22C3
• EnVision FLEX visualization system on Autostainer Link 48.
• Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining is used for scoring.
• The specimen should be considered PD-L1 positive if TPS ≥ 50% of the viable tumor cells exhibit membrane staining at any intensity.
• PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA® (pembrolizumab).

PD-L1 IHC
• PD-L1 IHC 28-8 pharmDx is not indicated as an aid in identifying NSCLC patients for treatment with OPDIVO® (nivolumab).
• PD-L1 IHC 28-8 pharmDx is a qualitative immunohistochemical “complementary” diagnostic assay using mAb Anti-PD-L1, Clone 28-8.
• EnVision FLEX visualization system on Autostainer Link 48.
• Any staining is used for scoring.
• The specimen should be considered PD-L1 positive at ≥ 1% of any staining at any intensity.
• PD-L1 IHC 28-8 pharmDx is not indicated as an aid in identifying NSCLC patients for treatment with nivolumab.
Questions/challenges

- Biopsy are not representative of the tumor because of the heterogeneity.
- Does the PD-L1 assay support its use to select patients for treatment with anti-PD-1 inhibitor?
- Is the co-existence of PD-L1 and TILs a prerequisite to the response to anti-PD-1 therapy?
- What phenotype will capture ‘inflamed’ phenotype, most effective T cells? Suppressor cells (Treg, MDSC); Frequency and TCR Vβ repertoire?
- How to better characterize effector T cells beyond PD-1/PD-L1? Tumor or antigen specific T cells? Activation markers e.g., CD25, OX40, granzyme B/degranulation marker CD107a?
- Need for Multiplex assays ???
- Novel approaches- RNA and quantitative assay for PD-L1???
- Is mutational burden and TCR clonality informative and sufficient for clinical decision making? At what cut-point?
- How should these assays be improved?? Epitope prediction, functional assays?
Linking T-cell receptor sequence to functional phenotype at the single-cell level (Han A et al., 2015 Nature)