Validation of a Microsatellite Instability Assay by NGS

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VP R&D
Caris Life Sciences®

230,000+ tests performed in 2016

- Headquarters: Irving, Texas
- Laboratory: Phoenix, Arizona
  - 66,000 sq. ft. oncology-focused
  - ISO 15189, CLIA, CAP, NY State and CE Mark approved/certified
- Profiled 120,000+ patients
  - 7,000+ physicians
  - All 50 states
  - 63 countries
- Laboratory staff
  - Pathologists
  - Molecular Geneticists
  - Consulting Medical Oncologists
  - MD and/or PhD literature evidence review team
  - ~100 trained molecular & laboratory technicians
Comprehensive Technological Arsenal Fuels Invention Across a Broad Range of Molecular Biology

- **Comprehensive Technology Suite**
  - Sequencing (Next-Gen, Whole Exome, Sanger, Pyro)
  - Nanostring
  - PCR (ddPCR, nanoliter scale PCR, qPCR, RT-PCR)
  - Laser Capture Microdissection
  - *In Situ* Hybridization
  - Histology, special stains
  - Immunohistochemistry
  - Autostaining (DAKO, Ventana)
  - Slide imaging
  - Immunoprecipitation, immunoblotting
  - Cell culture
  - ELISA
  - Robotics
  - Protein cross-linking
  - Microarray
  - Mass Spec (Maldi TOF, Quantitative proteomics)
  - HPLC, FPLC
  - Polyligandhistochemistry
  - Luminex
  - Surface plasmon resonance
  - Dynamic Light Scattering
  - Fluorescent Activated Cell Sorting
  - *In vitro* pharmacology
  - Oligo synthesis (native or modified)
  - Nanosight
  - Exosome isolation and characterization
  - Liposomal formulations

- **Multi-Disciplinary Expertise**
  - Bioinformaticians
  - Mathematicians/Statisticians
  - Medical Oncologists
  - Molecular Biologists
  - Molecular Geneticists
  - Molecular Pathologists
  - Research Scientists
Caris Clinical Testing Menu

**Next Gen DNA Sequencing**  
*Illumina NextSeq System*

- 592 full genes sequenced
- Includes all SNVs and indels on guidelines
- Includes nearly all markers included in clinical trials

**Also reported**
- Total Mutational Load
- Copy Number Variation on 442 genes
- Micro Satellite Instability

**Next Gen RNA Sequencing**  
*Illumina MiSeq System*

- 53 genes sequenced
- Novel translocation detection independent of intronic breakpoint
- ALK translocation
- ROS1 translocation
- RET translocation
- NTRK 1-3 translocations
- CMET exon 14 skipping
- EFGFv3
- BRAF translocation
- RSPO3 translocation

**Protein Expression**  
*Ventana & Dako Immunohistochemistry*

- 25 lineage specific IHC
- Lung
  - PDL-1 (22c3)
  - ALK
  - PTEN
  - RRM1
  - TOPO1
  - TS
  - TUBB3
- CRC
  - MMR markers
- Breast
  - ER, PR, AR, Her2

Identifying key molecular features that bring value requires understanding the complexity of the system we are working within.
Caris CMI Testing for Immunotherapies

Identify Patients More Likely to Respond to Immune Checkpoint Inhibitors with Biomarker Testing from Caris Molecular Intelligence:

**PD-L1**
Immunohistochemistry

*Programmed death ligand-1 (PD-L1)* is among the most important checkpoint proteins that mediate tumor-induced immune suppression through T-cell downregulation. PD-L1 expression may indicate response to immune checkpoint inhibitors.

Caris has performed more than 30,000 PD-L1 tests across all lineages.

**MSI**
Fragment Analysis

*Microsatellite instability (MSI)* is caused by failure of the DNA mismatch repair (MMR) system. MSI-High correlates to an increased neoantigen burden, which may respond more favorably to immune checkpoint inhibitors.

MSI testing is included for all colorectal and endometrial cancers.

**TML**
Next-Generation Sequencing

*Total mutational load (TML)* measures the total number of non-synonymous somatic mutations identified per megabase of the genome coding area. Tumors with high TML likely harbor neoantigens and may respond more favorably to immune checkpoint inhibitors.

TML is reported for all solid tumors tested with Next-Generation Sequencing (592 genes).
The World just Changed – FDA approved pembro across all solid tumors that are MSI-High
MSI leads to an increase in the number of neoantigens and increases the likelihood of immune recognition.
Responses observed in a large proportion of patients

(Le et al. Science 2017)
Significant increase in survival was observed across MSI-High patients

(Le et al. Science 2017)
Traditional approach employs PCR to compare tumor to normal across 5 loci.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Panel</th>
<th>Marker</th>
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<tbody>
<tr>
<td>CTL</td>
<td>Promega MSI</td>
<td>BAT-25</td>
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Distribution of 27,039 polymorphic microsatellite markers across the human genome

MSI Validation Process

Compare NGS data to PCR (comparing cancer and normal tissue) on ~2200 patients

Algorithm Training: Maximize Sensitivity, Specificity, NPV, and PPV

Test on 200 Naïve patients and compare to MSI by PCR and MMR by IHC

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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</thead>
<tbody>
<tr>
<td>All (2196 Patients)</td>
<td>95.8%</td>
<td>99.4%</td>
<td>94.5%</td>
<td>99.2%</td>
</tr>
<tr>
<td>CRC (1198 Patients)</td>
<td>100.0%</td>
<td>99.9%</td>
<td>98.7%</td>
<td>99.6%</td>
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</tbody>
</table>
Distribution of in-dels across 5K patients

![Graph showing the distribution of in-dels across 5K patients. The x-axis represents the number of loci, ranging from 0 to 120, with a peak around 40 loci. The y-axis represents the frequency of patients, ranging from 0 to 300, with a peak around the 20-40 loci range. Red line indicates a threshold for MSI.](image)
Malignant Solitary Fibrous Tumor of the Pleura (MSFT)  
Esophageal and Esophagogastric Junction Carcinoma  
Lung Non-Small Cell Lung Cancer (NSCLC)  
- Non Epithelial Ovarian Cancer (non-EOC)  
- Lung Bronchioloalveolar carcinoma (BAC)  
Retroperitoneal or Peritoneal Carcinoma  
Gastrointestinal Stromal Tumors (GIST)  
- Extrahepatic Bile Duct Adenocarcinoma  
- Cell Lymphoma  
Ovarian Surface Epithelial Carcinomas  
Retroperitoneal or Peritoneal Sarcoma  
Head and neck Squamous Carcinoma  
Female Genital Tract Malignancy  
Lung Small Cell Cancer (SCLC)  
Liver Hepatocellular Carcinoma  
Acute myeloid leukemia (AML)  

Uveal Melanoma  
Thyroid Carcinoma  
Thymic Carcinoma  
Soft Tissue Tumors  
Small Intestinal Malignancies  
Retroperitoneal or Peritoneal Sarcoma  
Retroperitoneal or Peritoneal Carcinoma  
Prostatic Adenocarcinoma  
Pancreatic Adenocarcinoma  
Ovarian Surface Epithelial Carcinomas  
None Of These Apply  
Non Epithelial Ovarian Cancer (non-EOC)  
Nodal Diffuse Large B-Cell Lymphoma  
Neuroendocrine tumors  
Multiple Myeloma  
Melanoma  
Malignant Solitary Fibrous Tumor of the Pleura (MSFT)  
Male Genital Tract Malignancy  
Lymphoma  
Lung Small Cell Cancer (SCLC)  
Lung Non-Small cell lung cancer (NSCLC)  
Lung Bronchioloalveolar carcinoma (BAC)  
Low Grade Glioma  
Liver Hepatocellular Carcinoma  
Kidney Cancer  
Head and neck Squamous Carcinoma  
Glioblastoma  
Gastrointestinal Stromal Tumors (GIST)  
Gastric Adenocarcinoma  
Female Genital Tract Malignancy  
Extrahepatic Bile Duct Adenocarcinoma  
Esophageal and Esophagogastric Junction Carcinoma  
Colorectal Adenocarcinoma  
Cholangiocarcinoma  
Breast Carcinoma  
Bladder Cancer  
Acute myeloid leukemia (AML)
Relationship between MSI, TML, PDL-1

All Lineages

- TML
- MSI
- PDL1

N=8952

3% of Patients are MSI unstable

17.6% of Patients are PDL-1 positive

8.1% of Patients are TML high

408

194

93

228

46

9

1417

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MSI is highly correlated to TML in Colorectal Cancer Patients
MSI-NGS vs. TML – relationship depends on lineage

![Scatter plots comparing MSI as altered microsatellite (MS) loci determined by NGS to TML per megabase for colorectal adenocarcinoma (n = 1267), endometrial cancer (n = 667), NSCLC (n = 964), and melanoma (n = 175). The horizontal line indicates 46 altered MS and the vertical line indicates 17 mutations/Mb.](image)

**Figure 4.** Scatter plots comparing MSI as altered microsatellite (MS) loci determined by NGS to TML per megabase for colorectal adenocarcinoma (n = 1267), endometrial cancer (n = 667), NSCLC (n = 964), and melanoma (n = 175). The horizontal line indicates 46 altered MS and the vertical line indicates 17 mutations/Mb.
Relationship of other immunotherapy markers to MSI-NGS
Frequency of MSI across lineages

(Le et al. Science 2017)
Thank you!