ASCP Immuno-Oncology Scientific Updates Webcast:
Tumor Mutational Burden

Faculty

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Objectives

Summarize ongoing investigational efforts regarding TMB and its ability to predict response to checkpoint inhibitor therapy

Discuss the current advantages and limitations to the testing and interpretation of TMB
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Course Outline

Overview
Current Evidence Surrounding TMB
Calculating, Interpreting, and Reporting TMB Measurements
Conclusion and Additional Resources

Overview

Overview: Current IO Therapy Biomarker Landscape

- PD-L1 IHC
- Microsatellite instability-High (MSI-H) or Mismatch repair deficient (dMMR)
- Tumor mutational burden (TMB)
- Gene expression profiling
- Multiplex immunohistochemistry/immunofluorescence
- Immune cell repertoire diversity
- Many others...

Current Clinical Practice
Emerging
Investigational

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Overview: TMB’s Role as an Emerging Predictive Biomarker

- Measurement of the number of mutations that exists within a tumor
- Has been proposed to be a useful biomarker in predicting response to immune checkpoint inhibitors in several tumor types

Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer

Kombucha plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden

TMB mutational load predicts survival after immunotherapy across multiple cancer types

Cancer Cytopen. 2019 Dec;127(12):735-736

TMB Across Tumor Types

Nature Biotechnology 34, 9019–9024 (2016)
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**Overview: TMB's Relation to MSI and PD-L1**


Current Evidence Surrounding TMB
Current Evidence Surrounding TMB: Evidence and Indications from Current Trials

- Data from trials are contradictory
- CheckMate 227
  - Nivo+ipi versus chemotherapy in 1st line NSCLC
  - High TMB (>10 mut/Mb) ICI associated with longer PFS (irrespective of PD-L1)
  - Did not hold for OS
- KEYNOTE-021, 189, 407
  - Pembrolizumab plus platinum-based chemotherapy for metastatic NSCLC
- KEYNOTE-010, 042
  - Pembrolizumab monotherapy in PD-L1 positive advanced NSCLC

Evidence and Indications from Current Trials, continued

KEYNOTE-010: phase 1/2, 2L+
KEYNOTE-042: phase 3, 1L, PD-L1x1%
KEYNOTE-021: phase 1/2, 1L, NSQ
KEYNOTE-189: phase 3, 1L, NSQ
KEYNOTE-407: phase 3, 1L, SQ

LBA79: Association between tissue TMB (ITMB) and clinical outcomes with pembrolizumab monotherapy (pembro) in PD-L1-positive advanced NSCLC in the KEYNOTE-010 and 042 trials – Herbst RS, et al

- Study objective
  - To evaluate the association between ITMB levels and outcomes in the KEYNOTE-010 and KEYNOTE-042 trials

- Key patient inclusion criteria
  - Advanced NSCLC
  - PD-L1 positive
  - PD-L1 TP53
  - ≥1 tumor samples

- ITMB assessment by whole exome sequencing of tumour tissue
  - OS, PFS, ORR association with ITMB assessed

- Conclusion: Previously treated or unselected patients with PD-L1+ NSCLC, high ITMB was associated with improved clinical outcomes in those receiving pembro monotherapy

*Pre-specified cut-points at 175 mut/exome; assessed as continuous log_{2} transformed variable


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LBAIE: Pembrolizumab (pembro) plus platinum-based chemotherapy (chemo) for metastatic NSCLC: tumor TMB (TMB) and outcomes in KEYNOTE-021, 189, and 467 – Paz-Ares L, et al

- Study objective
  - To evaluate the association of TMB with efficacy of pembrolizumab + platinum-based chemotherapy vs. platinum-based chemotherapy alone

  Key patient inclusion criteria:
  - Untreated stage IV NSCLC, squamous or non-squamous NSCLC
  - ALK or EGFR negative
  - ACGO PS 0-1

  TMB assessment by whole exome sequencing of tumour tissue

  Outcomes (OS, PFS, ORR) association with TMB assessed

  Conclusion: No association between TMB and efficacy in all studies. Pembro + chemo had OS, PFS, and ORR benefits in both low and high TMB groups (≥175 or <175 mut/exome)


Calculating, Interpreting, and Reporting TMB

Calculating, Interpreting, and Reporting TMB: Assays Used

Gold standard: matched tumor-normal whole exome sequencing

Targeted panels: either matched tumor-normal or tumor only targeted cancer gene panels
- Caris SureSelect XT
- Foundation One CDx
- Illumina TruSeq
- MSK-IMPACT
- NeoGenomics Neotype
- PGDx elio tissue complete
- QIAGene TMB panel
- ThermoFisher Oncomine
- TMLA
- Numerous academic labs

* not a comprehensive list
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Friends and QuIP Objectives

- Identify variation between TMB assessed by WES and targeted panels
- Create TMB reference standards to facilitate alignment between various assays
- Assess interassay/interlab variability and identify sources of variation
- Develop recommendations to minimize variation in methods of TMB estimation and reporting
- Inform and advise best practices for prospective clinical studies

Factors Influencing TMB Calculation

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Factors Influencing TMB Calculation: Recommendations

<table>
<thead>
<tr>
<th>Factor</th>
<th>Parameter</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-analytical</td>
<td>Sample processing</td>
<td>• Standardize sample processing protocols&lt;br&gt;• Minimize interlaboratory variability</td>
</tr>
<tr>
<td>Sequencing parameters</td>
<td>Genomic region covered</td>
<td>• Select gene panels that screen for actionable mutations or biomarkers&lt;br&gt;• Select panels with larger genome coverage (ideally &gt;1 megabase or greater)</td>
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<tr>
<td>Bioinformatics</td>
<td>Standardization of workflow</td>
<td>• Align panel-derived TMB values to a WES analysis-derived reference standard to ensure consistency regardless of the assay&lt;br&gt;• Standardize bioinformatic algorithms used for mutation calling and filtering</td>
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<tr>
<td>Comparison of results</td>
<td>Calibration of outputs</td>
<td>• Ensure reporting consistency by developing templates for clinically meaningful reporting (e.g., report TMB as mutations per megabase)&lt;br&gt;• Allow calibration of results from different studies</td>
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Outline of QuIP TMB Harmonization Study

20 FFPE tumor samples with matched WES data available

Comparative Analysis:
- TMB level and correlations
- Bridging from pTMB to aesTMB
- TMB classification
- Inter-lab comparisons of the identified variants
- Germline mutation filtering


Pre-analytic Factors


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**Assay Clustered Together Independent of Operating Lab**

**2-tier vs. 3-tier Classification**

- 2-tier classification: 3-tier and 5-tier tests
- 3-tier classification: 3-tier and 5-tier tests

- Misclassification: 25%
- Weak misclassification: 22%
- Strong misclassification: 1.5%

**Interlaboratory Reproducibility**

- False-positive variants:
  - Higher in assays without duplicate removal (deduplication) (CTML)
  - Particularly in specimens with highly fragmented, low-quality DNA samples with over-calling of C>T or A fixation artifacts
  - Low-frequency variants close to the VAF cut-point contributed considerably to panel TMIs calling variation between assays/labs.

- False-negative calls:
  - Associated with insufficient depth of coverage at the respective positions and DNA input

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Germline Filtering and Panel Size
- Germline Filtering
  - Performance of filtering using SNP databases for LUAD sample
    - Sensitivity (PPV) for calling mutation as somatic:
      - 97% (90%) → gnomAD
      - 90% (90%) → ExAC
      - 70% (91%) → dbSNP
    - Limiting to more common SNPs (minor allele frequency >0.001 in gnomAD)
    - Increased sensitivity to 98% but decreased PPV to 81%
- Panel Size
  - At least 1 Mbp in size (coding)
  - Even with large panels, variability of TMB score is expected due to probabilistic nature
  - Simulating 5 commercial panels in WES data, only 17-28% additional error occurred on top of probabilistic error, demonstrating that sufficient panel size is more critical than particular panel content

Study Summary
- Overall low influence of specific laboratory performing the analysis
- Commercial labs were in range of respective TMB scores determined by hospital labs
- Most panels had moderate to strong correlations with TMB measured by WES
  - r(64)=0.84

FOCR Phase I: In Silico Analysis
- TCGA data from 32 tumor types
  - Theoretical variability of panel-derived TMB estimates relative to common, standardized WES-derived TMB across various panels
- 11 labs took WES data from TCGA and calculated TMB from their panel subset of exome using their own pipelines (panel TMB)
- Reference TMB value was created from WES data using a uniform agreed upon bioinformatics pipeline

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### Assays Used in FOCR In Silico Study: Results Blinded

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Panel name</th>
<th>A panels</th>
<th>Used sample median (IQR)</th>
<th>TMB median (IQR)</th>
<th>Published references, methodology, etc.</th>
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### Regression for Panel TMB as a Function of WES TMB

A) All Cancers

B) 73% of assays had slope values >1, indicating overestimation of TMB

Due to blinded nature, contributing factors couldn’t be analyzed

When limiting analysis to 8 cancer types (stratum 1), only 50% of assays had slopes >1

### Correlation by Tumor Type

All 11 labs overestimated bladder cancer TMB

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Consensus Recommendations

- Accuracy
  - Should be determined by validating against reference TMB values
    - Comparable companion diagnostic with FDA clearance/approval
    - WES w/ validated performance and standardized TMB calculation method
  - At least 30 samples over range of TMB (0-40 mut/Mb)
  - TMB: continual vs. categorical have specific metrics

- Precision
  - Performed using several samples with analyses as outlined in accuracy studies
  - Evaluated as composite score (mut/Mb)
  - TMB: continual vs. categorical have specific metrics

Consensus Recommendations, continued

- Sensitivity
  - Impact of tumor purity on TMB categorical call should be evaluated using multiple samples
    - 6-10 undiluted samples where each sample is diluted to at least 5 levels of tumor purity w/ 10 replicates at each level

- QC
  - QC metrics: median exon coverage, coverage uniformity, etc
  - Identify % of tests passing TMB QC metrics in routine testing
FOCR Phase 2B: Alignment to Clinical Samples

FOCR Phase 2B: Calibration Approaches

FOCR Phase 2B: Alignment to Clinical Samples, continued

- Variability across labs/assays was similar when testing FFPE-derived tumor samples to that observed in TCGA samples and cell lines

- Calibration approaches using TCGA data were more robust than using a small number of cell line samples
  - May be a viable approach to align across panel TMB scores
  - Additional work needed
Conclusions and Future of TMB

- Clinical utility data is still emerging
- TMB may have more significance in certain tumor types and in certain therapeutic settings
- Increasing data and recommendations for laboratories calculating and reporting TMB
- FOCR, QuIP → further phases and publications forthcoming
- Recommendations for validating against other FDA cleared/approved assays or against a reference standard WES

Additional Resources

- Friends of Cancer Research
  - focr.org/tmb
- Detailed validation recommendations: