TMB in liquid biopsy as a predictive biomarker for immunotherapy

Enriqueta Felip, Vall d’Hebron Hospital
Evolution of biomarker testing in NSCLC: past, current & future

1. Histomorphological Diagnosis:
   - Archival FFPE tumor specimens

2. Molecular Diagnosis:
   - Archival cancer specimens
   - Macro- or Micro-dissection of Tumors
   - Extract tumor nucleic acids: DNA and RNA

   **Representative technologies:**
   - Single Biomarker Tests:
     - Sanger DNA Sequencing
     - RT-PCR
     - FISH
     - IHC
   - Multiplex, Hot Spot Mutation Tests:
     - PCR-based SNapShot
     - PCR-based Mass Array SNP
     - Sequenom
   - Initial High-Throughput Technologies:
     - SNP/CNV DNA microarray
     - RNA microarray
   - Next Generation Sequencing (NGS):
     - Whole Genome or Exome capture Sequencing (DNA)
     - Whole or Targeted Transcriptome Sequencing (RNA)
     - Epigenetic profiling

   **Current approach (target-based therapy V1.0):**
   - Single gene molecular testing for decision-making in individual patients

   **Evolving approach (target-based therapy V2.0):**
   - Multiplexed molecular tests with increased sensitivity & output for decision-making in individual patients

   **Near-future approach (patient-based therapy):**
   - Genomic profiling by high throughput next generation sequencing for decision-making in individual patients

   \(\rightarrow\) Plasma ct DNA by NGS

Adapted from Li, Gandara JCO 13
Immunotherapy in NSCLC

- Essential treatment in 2L / 1L / unresectable stage III
- PDL1 predictive biomarker
- TMB a potential biomarker
  - immune checkpoints inhibitors typically active in tumours with high TMB

Schumacher & Schreiber Science 15
Nonsynonimous mutations determines sensitivity to pembrolizumab in NSCLC
PFS by tumor mutation burden subgroup
CheckMate 026 TMB analysis

**High TMB**

<table>
<thead>
<tr>
<th>Nivolumab</th>
<th>Chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 47</td>
<td>n = 60</td>
</tr>
<tr>
<td>Median PFS, months</td>
<td>9.7</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(5.1; NR)</td>
</tr>
<tr>
<td>HR</td>
<td>0.62 (95% CI: 0.38, 1.00)</td>
</tr>
</tbody>
</table>

**Low/medium TMB**

<table>
<thead>
<tr>
<th>Nivolumab</th>
<th>Chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 111</td>
<td>n = 94</td>
</tr>
<tr>
<td>Median PFS, months</td>
<td>4.1</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(2.8, 5.4)</td>
</tr>
<tr>
<td>HR</td>
<td>1.82 (95% CI: 1.30, 2.55)</td>
</tr>
</tbody>
</table>
Total exome mutations vs genes in FoundationOne panel
CheckMate 026 TMB analysis
CheckMate 227 part 1 study design

Key Eligibility Criteria
- Stage IV or recurrent NSCLC
- No prior systemic therapy
- No known sensitizing EGFR/ALK alterations
- ECOG PS 0–1

Stratified by SQ vs NSQ

N = 1189

≥1% PD-L1 expression

R 1:1:1

N = 550

<1% PD-L1 expression

R 1:1:1

Nivolumab 3 mg/kg Q2W
Ipilimumab 1 mg/kg Q6W
n = 396

Histology-based chemotherapy
n = 397

Nivolumab 240 mg Q2W
n = 396

Nivolumab 3 mg/kg Q2W
Ipilimumab 1 mg/kg Q6W
n = 187

Histology-based chemotherapy
n = 186

Nivolumab 360 mg Q3W + histology-based chemotherapy
n = 177

Nivolumab + ipilimumab
n = 396

Chemotherapy
n = 397

Nivolumab + ipilimumab
n = 139

Chemotherapy
n = 160

Patients for PD-L1 co-primary analysis

Patients for TMB co-primary analysis

Co-primary endpoints: Nivolumab + ipilimumab vs chemotherapy
- OS in PD-L1–selected populations
- PFS in TMB-selected populations

Database lock: January 24, 2018; minimum follow-up: 11.2 months

\(^a\)NCT02477826 \(^b\)NSQ; pemetrexed + cisplatin or carboplatin, Q3W for ≤4 cycles, with optional pemetrexed maintenance following chemotherapy or nivolumab + pemetrexed maintenance following nivolumab + chemotherapy; SQ: gemcitabine + cisplatin, or gemcitabine + carboplatin, Q3W for ≤4 cycles; \(^c\)The TMB co-primary analysis was conducted in the subset of patients randomized to nivolumab + ipilimumab or chemotherapy who had evaluable TMB ≥10 mut/Mb

Hellmann AACR 18, NEJM 18
TMB and tumor PD-L1 expression identify distinct and independent populations of NSCLC

TMB (number of mutations/Mb)

0 20 40 60 80 100 120 140 160

TMB ≥10 mut/Mb

<1% 29%

≥1% 71%

TMB <10 mut/Mb

<1% 29%

≥1% 71%

Hellmann AACR 18, NEJM 18
Is TMB a relevant biomarker for patient selection?

Updated descriptive analysis:
HR for OS with nivolumab + ipilimumab vs. chemotherapy in patients with TMB ≥10 mut/Mb
= 0.77 (95% CI: 0.56, 1.06)

Exploratory analysis in patients with TMB <10 mut/Mb:
HR for OS with nivolumab + ipilimumab vs. chemotherapy
= 0.78 (95% CI: 0.61, 1.00)
## PFS in patients with high TMB (≥10 mut/Mb) by tumor PD-L1 expression

### ≥1% PD-L1 expression

<table>
<thead>
<tr>
<th></th>
<th>Nivo + ipi (n = 101)</th>
<th>Chemo (n = 112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median PFS, mo&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.1</td>
<td>5.5</td>
</tr>
<tr>
<td>HR</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>0.44, 0.88</td>
<td></td>
</tr>
</tbody>
</table>

1-y PFS = 42%

1-y PFS = 16%

Nivolumab + ipilimumab

Chemotherapy

### <1% PD-L1 expression

<table>
<thead>
<tr>
<th></th>
<th>Nivo + ipi (n = 38)</th>
<th>Chemo (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median PFS, mo&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.7</td>
<td>5.3</td>
</tr>
<tr>
<td>HR</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>0.27, 0.85</td>
<td></td>
</tr>
</tbody>
</table>

1-y PFS = 45%

1-y PFS = 8%

Nivolumab + ipilimumab

Chemotherapy

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*HR* (Hazard Ratio)

95% CI: nivo + ipi (5.5, 13.5 mo), chemo (4.3, 6.6 mo); 95% CI: nivo + ipi (2.7 mo, NR), chemo (4.0, 6.8 mo)
Pan-tumor genomic biomarkers for PD-1 checkpoint blockade–based immunotherapy

Razvan Cristescu1*, Robin Mogg1†, Mark Ayers1, Andrew Albright1, Erin Murphy1, Jennifer Yearley1, Xinwei Sher1, Xiao Qiao Liu1, Hongchao Lu1, Michael Nebozhyn1, Chunsheng Zhang1, Jared K. Lunceford1, Andrew Joe1, Jonathan Cheng1, Andrea L. Webber1, Nageatte Ibrahim1, Elizabeth R. Plimack2, Patrick A. Ott3, Tanguy Y. Seiwert4, Antoni Ribas5, Terrill K. McClanahan1, Joanne E. Tomassini1, Andrey Loboda1, David Kaufman1†

Programmed cell death protein–1 (PD-1) and programmed cell death ligand–1 (PD-L1) checkpoint blockade immunotherapy elicits durable antitumor effects in multiple cancers, yet not all patients respond. We report the evaluation of >300 patient samples across 22 tumor types from four KEYNOTE clinical trials. Tumor mutational burden (TMB) and a T cell–inflamed gene expression profile (GEP) exhibited joint predictive utility in identifying responders and nonresponders to the PD-1 antibody pembrolizumab. TMB and GEP were independently predictive of response and demonstrated low correlation, suggesting that they capture distinct features of neoantigenicity and T cell activation. Analysis of The Cancer Genome Atlas database showed TMB and GEP to have a low correlation, and analysis by joint stratification revealed biomarker-defined patterns of targetable-resistance biology. These biomarkers may have utility in clinical trial design by guiding rational selection of anti–PD-1 monotherapy and combination immunotherapy regimens.
≈ 30% of patients with NSCLC have inadequate tumour tissue for testing at diagnosis *(Lim C, et al. Ann Oncol, 2015)*

- 58% of all randomized patients had **TMB- evaluable samples**

*Randomized patients include those from all treatment arms in part 1 (nivolumab + ipilimumab, nivolumab, chemotherapy, and nivolumab + chemotherapy arms). The FoundationOne CDx™ assay employs comprehensive QC criteria, including the following critical characteristics: tumor purity, DNA sample size, tissue sample size, library construction size, and hybrid capture yields.*

*Helimann AACR 18*
Hypermutated Circulating Tumor DNA: Correlation with Response to Checkpoint Inhibitor-Based Immunotherapy

Yulian Khagi\(^1\), Aaron M. Goodman\(^{1,2}\), Gregory A. Daniels\(^3\), Sandip P. Patel\(^1\), Assuntina G. Sacco\(^3\), James M. Randall\(^3\), Lyudmila A. Bazhenova\(^3\), and Razelle Kurzrock\(^1\)

Abstract

**Purpose:** Tumor mutational burden detected by tissue next-generation sequencing (NGS) correlates with checkpoint inhibitor response. However, tissue biopsy may be costly and invasive. We sought to investigate the association between hypermutated blood-derived circulating tumor DNA (ctDNA) and checkpoint inhibitor response.

**Experimental Design:** We assessed 69 patients with diverse malignancies who received checkpoint inhibitor-based immunotherapy and blood-derived ctDNA NGS testing (54–70 genes). Rates of stable disease (SD) ≥6 months, partial and complete response (PR, CR), progression-free survival (PFS), and overall survival (OS) were assessed based on total and VUS alterations.

**Results:** Statistically significant improvement in PFS was associated with high versus low alteration number in variants of unknown significance (VUS, >3 alterations versus VUS ≤3 alterations), SD ≥6 months/PR/CR 45% versus 15%, respectively; \( P = 0.014 \). Similar results were seen with high versus low total alteration number (characterized plus VUS, ≥6 vs. <6). Statistically significant OS improvement was also associated with high VUS alteration status. Two-month landmark analysis showed that responders versus nonresponders with VUS >3 had a median PFS of 23 versus 2.3 months (\( P = 0.0004 \)).

**Conclusions:** Given the association of alteration number on liquid biopsy and checkpoint inhibitor-based immunotherapy outcomes, further investigation of hypermutated ctDNA as a predictive biomarker is warranted. *Clin Cancer Res; 23*(19); 5729–36. ©2017 AACR.
Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab

David R. Gandara\textsuperscript{1,7*}, Sarah M. Paul\textsuperscript{2,7}, Marcin Kowanetz\textsuperscript{2,7}, Erica Schleifman\textsuperscript{2,7}, Wei Zou\textsuperscript{2,7}, Yan Li\textsuperscript{2}, Achim Rittmeyer\textsuperscript{3}, Louis Fehrenbacher\textsuperscript{4}, Geoff Otto\textsuperscript{5}, Christine Malboeuf\textsuperscript{5}, Daniel S. Lieber\textsuperscript{5}, Doron Lipson\textsuperscript{5}, Jacob Silterra\textsuperscript{5}, Lukas Amler\textsuperscript{2}, Todd Riehl\textsuperscript{2}, Craig A. Cummings\textsuperscript{2}, Priti S. Hegde\textsuperscript{2}, Alan Sandler\textsuperscript{2}, Marcus Ballinger\textsuperscript{2}, David Fabrizio\textsuperscript{5}, Tony Mok\textsuperscript{6*} and David S. Shames\textsuperscript{2*}

Although programmed death-ligand 1–programmed death 1 (PD-L1–PD-1) inhibitors are broadly efficacious, improved outcomes have been observed in patients with high PD-L1 expression or high tumor mutational burden (TMB). PD-L1 testing is required for checkpoint inhibitor monotherapy in front-line non-small-cell lung cancer (NSCLC). However, obtaining adequate tumor tissue for molecular testing in patients with advanced disease can be challenging. Thus, an unmet medical need exists for diagnostic approaches that do not require tissue to identify patients who may benefit from immunotherapy. Here, we describe a novel, technically robust, blood-based assay to measure TMB in plasma (bTMB) that is distinct from tissue-based approaches. Using a retrospective analysis of two large randomized trials as test and validation studies, we show that bTMB reproducibly identifies patients who derive clinically significant improvements in progression-free survival from atezolizumab (an anti-PD-L1) in second-line and higher NSCLC. Collectively, our data show that high bTMB is a clinically actionable biomarker for atezolizumab in NSCLC.
Tumor mutational burden in blood (bTMB) is associated with Atezolizumab efficacy in 2nd-Line+ NSCLC (POPLAR & OAK Trials)

Increasing Atezolizumab benefit with higher bTMB cut-points in OAK

**Progression-Free Survival – OAK**

- Enrichment of PFS benefit was observed in the bTMB ≥16 subgroup.
- OS was consistent between the bTMB ≥16 subgroup and the BEP
- The bTMB ≥16 subgroup represents 27% of the study population

**Overall Survival – OAK**

Adapted from Gandara et al: *NatMed*
LIMITED Overlap between $bTMB \geq 16$ and PD-L1 expression$^a$ (OAK BEP)

- Non-significant overlap between the $bTMB \geq 16$ and TC3 or IC3 subgroups (Fisher exact test, $P = 0.62$)
  - 19.2% of tumors with $bTMB \geq 16$ were also TC3 or IC3
  - 29.1% of tumors with TC3 or IC3 also had $bTMB \geq 16$

- Efficacy was greatest in those patients with specimens positive for both PD-L1 TC3 or IC3 plus $bTMB \geq 16$

<table>
<thead>
<tr>
<th></th>
<th>PFS HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$bTMB \geq 16$</td>
<td>0.64 (0.46, 0.91)</td>
</tr>
<tr>
<td>TC3 or IC3</td>
<td>0.62 (0.41, 0.93)</td>
</tr>
<tr>
<td>$bTMB \geq 16 +$ TC3 or IC3</td>
<td>0.38 (0.17, 0.85)</td>
</tr>
</tbody>
</table>

$^a$ PD-L1 expression was evaluated by immunohistochemistry (IHC) using the VENTANA SP142 assay; TC3 or IC3, ≥50% of TC or ≥10% of IC express PD-L1.

BEP, biomarker-evaluable population; IC, tumor-infiltrating immune cell; TC, tumor cell.
Background: Identifying biomarkers to select patients who respond to immune checkpoint blockade in non-small cell lung cancer (NSCLC) remains a challenge. Cell-free circulating tumor DNA (ctDNA) has emerged as a non-invasive, quantitative method of monitoring genomic alterations in the peripheral blood. We evaluated the clinical utility of ctDNA mutant allele frequency (MAF) and tumor burden based on imaging as biomarkers for response to immune checkpoint blockade in NSCLC. Method: From a cohort of 136 patients with ctDNA samples, 20 patients were retrospectively identified with ctDNA testing before initiation of anti-PD-1/PD-L1 treatment or within 90 days of therapy initiation. ctDNA testing was performed by Guardant360 (Guardant Health, Redwood City, CA). MAF of the dominant clone was identified quantitatively for each patient. In addition, baseline tumor burden was estimated using RECIST version 1.1. MAF and tumor burden were correlated with progression free survival (PFS) and overall survival (OS). Logistic regression of response rate (RR) and clinical benefit rate (CBR) was also performed. Result: Higher median ctDNA MAF was correlated with significantly shorter PFS and OS (hazard ratio (HR) 3.4, p=0.03 and HR 10.4, p=0.03, respectively) (Fig 1). There was no significant association between tumor burden estimation and PFS and OS. However, tumor burden was significantly correlated with MAF (r=0.58, p=0.007). MAF and tumor burden estimation did not correlate with RR or CBR in this small sample. Conclusion: ctDNA MAF appears to be a promising, non-invasive, prognostic biomarker for response to immune checkpoint blockade in NSCLC with higher MAF associated with shorter PFS and OS. ctDNA MAF may also serve as a surrogate for tumor burden. Prospective studies with serial ctDNA sampling are necessary to further validate these findings.
Inclusion Criteria
- Measurable disease per RECIST v1.1
- ECOG PS of 0 or 1
- Immunotherapy naive
- PD-L1 unselected
- Provision of blood

Exclusion Criteria
- Sensitizing EGFR mutations or ALK rearrangements
- Active brain metastases requiring treatment

Primary analysis
- All enrolled patients with at least 6 months of follow-up
- Prespecified bTMB biomarker cutoff of 16

Co-Primary Endpoints
- Efficacy endpoint: INV-assessed ORR per RECIST v1.1
- Biomarker endpoint: INV-assessed PFS per RECIST v1.1

Secondary Objectives
- Safety and assessment of efficacy by INV-assessed DOR, OS

ALK, anaplastic lymphoma kinase.
\(^b\) Total enrolled, N = 153; however, 1 patient was never treated and was not included in the intent-to-treat population.
\(^c\) Tissue biopsy was optional.
B-F1RST: Patient population

- ITT analysis population enrolled from 20 US regional and community practice sites

- The biomarker-evaluable population (BEP) included patients with a baseline evaluable blood sample with adequate tumour content (i.e., maximum somatic allele frequency [MSAF] ≥ 1%) to test on the FMI bTMB assay

- The bTMB cutoff score of 16 was prespecified to evaluate efficacy (bTMB high, ≥ 16; bTMB low, < 16)

- Excludes 1 patient who was never treated.
- Assay quality-control failures.
- The MSAF < 1% population was considered as non-biomarker evaluable (non-BEP).

FMI, Foundation Medicine.

Enrolled (N = 153) → ITT\(^a\) (n = 152) → BEP MSAF ≥ 1% (n = 119) → bTMB low, < 16 (n = 91) → bTMB high, ≥ 16 (n = 28)

bTMB not evaluable\(^b\) (n = 4) → MSAF < 1\(^c\) (n = 29)

\(^a\)Excludes 1 patient who was never treated. 
\(^b\)Assay quality-control failures. 
\(^c\)The MSAF < 1% population was considered as non-biomarker evaluable (non-BEP).
B-F1RST: Atezolizumab overall response rate\textsuperscript{a} per RECIST v1.1

Minimum follow-up: 6 months

**bTMB Subgroups**

- **≥ 10 Cutoff**
  - ITT \textsuperscript{b} (N = 152): 14.5%
  - BEP (n = 119): 10.1%
  - High (n = 49): 16.3%
  - Low (n = 70): 5.7%
  - **P = 0.0595**

- **≥ 16 Cutoff**
  - High (n = 28): 28.6%
  - Low (n = 91): 4.4%
  - **P = 0.0002**

- **≥ 20 Cutoff**
  - High (n = 19): 36.8%
  - Low (n = 100): 5.0%
  - **P < 0.0001**

\(\text{PR} \quad \text{CR}\)

**Data cutoff:** May 21, 2018.

**BEP,** biomarker- evaluable population.

\textsuperscript{a} Confirmed.

\textsuperscript{b} ORR in non-BEP population (MSAF < 1%) was 34.5% (n = 29).

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B-F1RST: PFS with atezolizumab in bTMB high (≥ 16) vs low (< 16) subgroups

- **Data cutoff:** May 21, 2018.

<table>
<thead>
<tr>
<th></th>
<th>bTMB High (n = 28)</th>
<th>bTMB Low (n = 91)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median PFS</td>
<td>4.6 mo</td>
<td>3.7 mo</td>
</tr>
<tr>
<td>90% CI</td>
<td>1.6, 11.0</td>
<td>2.6, 4.3</td>
</tr>
<tr>
<td>HR</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>90% CI</td>
<td>0.42, 1.02</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

- 6-month PFS: 41.6% vs 32.8%
- 9-month PFS: 37.4% vs 9.7%
- Approximately 70% of events for PFS
B-F1RST: OS with atezolizumab in bTMB high (≥ 16) vs low (< 16) subgroups

6-month OS
85.3% vs 72.3%

9-month OS
68.1% vs 66.3%

<table>
<thead>
<tr>
<th></th>
<th>bTMB High (n = 28)</th>
<th>bTMB Low (n = 91)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median OS</td>
<td>NE</td>
<td>13.1 mo</td>
</tr>
<tr>
<td>90% CI</td>
<td>8.8, NE</td>
<td>10.5, NE</td>
</tr>
<tr>
<td>HR</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>90% CI</td>
<td>0.41, 1.43</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.48</td>
<td></td>
</tr>
</tbody>
</table>

≈ 30% of events for OS
MYSTIC study design

- **Phase 3, global, randomised, open-label, multicentre study**

- Stage IV NSCLC
- All-comers population (i.e. irrespective of PD-L1 status)
- No sensitising *EGFR* mutation or *ALK* rearrangement
- ECOG PS 0/1
- Immunotherapy- and CT-naïve

N=1118 randomised

Durvalumab (n=374)
20 mg/kg q4w until disease progression

Durvalumab + tremelimumab (n=372)
D 20 mg/kg q4w until disease progression + T 1 mg/kg q4w for up to 4 doses

Platinum-based chemotherapy (n=372)
- Paclitaxel + carboplatin OR
- Gemcitabine + cisplatin/carboplatin (squamous) OR
- Pemetrexed + cisplatin/carboplatin (non-squamous)*
  for up to 6 cycles

Primary endpoints (PD-L1 TC ≥25%*):
- PFS ‡ (D+T vs CT)
- OS (D vs CT)
- OS (D+T vs CT)

Key secondary endpoints:
- PFS ‡ (D vs CT; PD-L1 TC ≥25%*)
- OS (D+T vs CT; PD-L1 TC ≥1%*)
- ORR ‡
- DoR
- Safety and tolerability

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*Ventana PD-L1 (SP263) assay using newly acquired or archival (<3 months) tumour biopsy;
†Followed by pemetrexed maintenance therapy if eligible; ‡Blinded independent central review per RECIST v1.1
CT, chemotherapy; D, durvalumab; DoR, duration of response; ECOG, Eastern Cooperative Oncology Group;
ORR, objective response rate; PFS, progression-free survival; PS, performance status; q4w, every 4 weeks; T, tremelimumab
Mystic trial: OS

**OS: D vs CT (PD-L1 TC ≥25%; PRIMARY ENDPOINT)**

| Event, n (%) | 108 (66.3) | 128 (79.0) |
| mOS, months (95% CI) | 16.3 (12.2–20.8) | 12.9 (10.5–15.0) |
| HR (97.5% CI) | 0.76 (0.564–1.019) | 0.036 |

**OS: D+T vs CT (PD-L1 TC ≥25%; PRIMARY ENDPOINT)**

| Event, n (%) | 113 (69.3) | 128 (79.0) |
| mOS, months (95% CI) | 11.9 (9.0–17.7) | 12.9 (10.5–15.0) |
| HR (97.5% CI) | 0.85 (0.611–1.173) | 0.202 |

---

**Received post-discontinuation anticancer therapy, n (%)**

<table>
<thead>
<tr>
<th>Durvalumab (n=163)</th>
<th>Chemotherapy (n=162)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>CT</td>
</tr>
<tr>
<td>73 (44.8)</td>
<td>95 (58.6)</td>
</tr>
<tr>
<td>Subsequent immunotherapy</td>
<td>10 (6.1)</td>
</tr>
</tbody>
</table>

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**Received post-discontinuation anticancer therapy, n (%)**

<table>
<thead>
<tr>
<th>Durvalumab + tremelimumab (n=163)</th>
<th>Chemotherapy (n=162)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D+T</td>
<td>CT</td>
</tr>
<tr>
<td>61 (37.4)</td>
<td>95 (58.6)</td>
</tr>
<tr>
<td>Subsequent immunotherapy</td>
<td>5 (3.1)</td>
</tr>
</tbody>
</table>

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DCO: 4 Oct 2018; mOS, median overall survival.
MYSTIC TRIAL: BLOOD TMB ANALYSIS

- tTMB ≥10 mut/Mb cutoff used to define high TMB in CheckMate 227 for the primary PFS endpoint
- This correlated with a bTMB 16 mut/Mb cutoff in MYSTIC (overall tTMB vs bTMB correlation: rho=0.6)

### TMB evaluable dataset

<table>
<thead>
<tr>
<th></th>
<th>Durvalumab</th>
<th>Durvalumab + tremelimumab</th>
<th>Chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT, n (%)</td>
<td>374 (100)</td>
<td>372 (100)</td>
<td>372 (100)</td>
</tr>
<tr>
<td>tTMB, n (%)</td>
<td>145 (38.8)</td>
<td>164 (44.1)</td>
<td>151 (40.6)</td>
</tr>
<tr>
<td>bTMB, n (%)</td>
<td>286 (76.5)</td>
<td>268 (72.0)</td>
<td>255 (68.5)</td>
</tr>
</tbody>
</table>

Large bTMB dataset: 809 samples (72.4% of patients)
**OS: bTMB subgroups (exploratory analysis)**

### bTMB <16 mut/Mb population

- **Durvalumab** (n=175)
  - mOS, months (95% CI): 12.2 (9.0–15.5)
  - HR vs CT*
  - 0.92 (0.715–1.174)

- **Durvalumab + tremelimumab** (n=162)
  - mOS, months (95% CI): 8.5 (6.6–9.7)
  - HR vs CT*
  - 1.23 (0.964–1.575)

- **Chemotherapy** (n=153)
  - mOS, months (95% CI): 11.6 (9.1–13.1)

### bTMB ≥16 mut/Mb population

- **Durvalumab** (n=111)
  - mOS, months (95% CI): 11.0 (7.8–16.1)
  - HR vs CT*
  - 0.80 (0.588–1.077)

- **Durvalumab + tremelimumab** (n=106)
  - mOS, months (95% CI): 16.5 (10.3–22.9)
  - HR vs CT*
  - 0.62 (0.451–0.855)

- **Chemotherapy** (n=102)
  - mOS, months (95% CI): 10.5 (8.8–12.4)

---

**No. at risk**

<table>
<thead>
<tr>
<th></th>
<th>Durvalumab (n=175)</th>
<th>Durvalumab + tremelimumab (n=162)</th>
<th>Chemotherapy (n=153)</th>
<th>Durvalumab (n=111)</th>
<th>Durvalumab + tremelimumab (n=106)</th>
<th>Chemotherapy (n=102)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D</strong></td>
<td>175</td>
<td>162</td>
<td>153</td>
<td>111</td>
<td>106</td>
<td>102</td>
</tr>
<tr>
<td><strong>D+T</strong></td>
<td>138</td>
<td>128</td>
<td>132</td>
<td>128</td>
<td>106</td>
<td>93</td>
</tr>
<tr>
<td><strong>CT</strong></td>
<td>112</td>
<td>101</td>
<td>111</td>
<td>101</td>
<td>83</td>
<td>75</td>
</tr>
<tr>
<td><strong>mOS, months (95% CI)</strong></td>
<td>12.2 (9.0–15.5)</td>
<td>8.5 (6.6–9.7)</td>
<td>11.6 (9.1–13.1)</td>
<td>11.0 (7.8–16.1)</td>
<td>16.5 (10.3–22.9)</td>
<td>10.5 (8.8–12.4)</td>
</tr>
<tr>
<td><strong>HR vs CT</strong></td>
<td>0.92 (0.715–1.174)</td>
<td>1.23 (0.964–1.575)</td>
<td>–</td>
<td>0.80 (0.588–1.077)</td>
<td>0.62 (0.451–0.855)</td>
<td>–</td>
</tr>
</tbody>
</table>
IMpower133: Phase 1/3, randomized, placebo-controlled trial evaluated atezolizumab + carboplatin + etoposide in 1L ES-SCLC: OS

Atezolizumab + CP/ET (N = 201) vs Placebo + CP/ET (N = 202)

**Clinical data cutoff date:** April 24, 2018, 11 months after the last patient was enrolled.

- **OS events, n (%):**
  - Atezolizumab: 104 (51.7%)
  - Placebo: 134 (66.3%)

- **Median OS, months (95% CI):**
  - Atezolizumab: 12.3 (10.8, 15.9)
  - Placebo: 10.3 (9.3, 11.3)

- **HR (95% CI): 0.70 (0.54, 0.91)**
  - **p = 0.0069**

- **Median follow-up, months**
  - Atezolizumab: 13.9
  - Placebo: 13.9

---

Atezolizumab + CP/ET
Placebo + CP/ET
Censored

---

*Clinical data cutoff date: April 24, 2018, 11 months after the last patient was enrolled. CI, confidence interval; HR, hazard ratio; CP/ET, carboplatin + etoposide.*
OS in key subgroups

<table>
<thead>
<tr>
<th>Population</th>
<th>Atezolizumab + CP/ET</th>
<th>Placebo + CP/ET</th>
<th>OS hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n = 261)</td>
<td>12.3</td>
<td>10.9</td>
<td>0.74 (0.54, 1.02)</td>
</tr>
<tr>
<td>Female (n = 142)</td>
<td>12.5</td>
<td>9.5</td>
<td>0.65 (0.42, 1.00)</td>
</tr>
<tr>
<td>&lt; 65 years (n = 217)</td>
<td>12.1</td>
<td>11.5</td>
<td>0.92 (0.64, 1.32)</td>
</tr>
<tr>
<td>≥ 65 years (n = 186)</td>
<td>12.5</td>
<td>9.6</td>
<td>0.53 (0.36, 0.77)</td>
</tr>
<tr>
<td>ECOG PS 0 (n = 140)</td>
<td>16.6</td>
<td>12.4</td>
<td>0.79 (0.49, 1.27)</td>
</tr>
<tr>
<td>ECOG PS 1 (n = 263)</td>
<td>11.4</td>
<td>9.3</td>
<td>0.68 (0.50, 0.93)</td>
</tr>
<tr>
<td>Brain metastases (n = 35)</td>
<td>8.5</td>
<td>9.7</td>
<td>1.07 (0.47, 2.43)</td>
</tr>
<tr>
<td>No brain metastases (n = 368)</td>
<td>12.6</td>
<td>10.4</td>
<td>0.68 (0.52, 0.89)</td>
</tr>
<tr>
<td>Liver metastases (n = 149)</td>
<td>9.3</td>
<td>7.8</td>
<td>0.81 (0.55, 1.20)</td>
</tr>
<tr>
<td>No liver metastases (n = 254)</td>
<td>16.8</td>
<td>11.2</td>
<td>0.64 (0.45, 0.90)</td>
</tr>
<tr>
<td>bTMB &lt; 10 mut/mb (n = 139)</td>
<td>11.8</td>
<td>9.2</td>
<td>0.70 (0.45, 1.07)</td>
</tr>
<tr>
<td>bTMB ≥ 10 mut/mb (n = 212)</td>
<td>14.6</td>
<td>11.2</td>
<td>0.68 (0.47, 0.97)</td>
</tr>
<tr>
<td>bTMB &lt; 16 mut/mb (n = 271)</td>
<td>12.5</td>
<td>9.9</td>
<td>0.71 (0.52, 0.98)</td>
</tr>
<tr>
<td>bTMB ≥ 16 mut/mb (n = 80)</td>
<td>17.8</td>
<td>11.9</td>
<td>0.63 (0.35, 1.15)</td>
</tr>
<tr>
<td>ITT (N = 403)</td>
<td>12.3</td>
<td>10.3</td>
<td>0.70 (0.54, 0.91)</td>
</tr>
</tbody>
</table>


a Hazard ratios are unstratified for patient subgroups and stratified for the ITT.
Table 2 TMB testing: tissue vs. liquid biopsy

<table>
<thead>
<tr>
<th>Tissue sample</th>
<th>Liquid biopsy sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pros</td>
<td></td>
</tr>
<tr>
<td>(I) Relatively well-established procedure; (II) possibility to interrogate a large number of genes for different types of genetic alterations (SNV, CNA, Fusions); (III) possibility to normalize for tumor cell content</td>
<td>(I) Low invasive procedure; (II) possibility to obtain updated information on mutational status; (III) possibility to follow molecular evolution of the disease; (IV) possibility to better reflect disease heterogeneity</td>
</tr>
<tr>
<td>Cons</td>
<td></td>
</tr>
<tr>
<td>(I) Obtained through invasive procedures; (II) mutational count might change during the progression of the disease; (III) potential artifacts introduced by fixation</td>
<td>(I) Low cfDNA concentration in patients with low tumor burden; (II) low VAF might affect the results of the test</td>
</tr>
</tbody>
</table>

TMB, tumor mutation burden; SNV, single nucleotide variants; CNA, copy number alteration; VAF, variant allelic frequency; cfDNA, cell free DNA.
**BFAST (Blood first assay screening trial): Phase II/III in advanced treatment-naïve NSCLC**

**Cohort A**
- ALK Positive
- Alectinib 600 mg orally BID
- n=78

**Cohort B**
- RET Positive
- Alectinib 900, 1200, 760 mg orally BID
- n=62-62

**Cohort C**
- bTMB
- Above the pre-specified cutpoints of ≥ 10 and ≥ 10

**Blood-based NGS cDNA assays**

**Patients with confirmed stage IIIIB/IV advanced or metastatic NSCLC (any histology)**
- N=3500 screened

**Key Inclusion Criteria**
- Measurable disease per RECIST v1.1
- ECOG PS 0 or 1
- Treatment naïve
- Adequate organ function
- Provision of blood samples

**Key Exclusion Criteria**
- Active, untreated brain metastases
- History of other malignancies within 5 years prior to screening
- Significant cardiovascular disease

**Platinum-based chemotherapy**

**Atezolizumab**
- 1200 mg IV q3w

**R 1:1 n=440**

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ALK, anaplastic lymphoma kinase; BID, twice daily; bTMB, blood tumor mutational burden; cDNA, circulating tumor DNA; ECOG PS, Eastern Cooperative Oncology Group performance status; IV, intravenously; NGS, next-generation sequencing; q3w, every 3 weeks; R, randomized; RECIST, Response Evaluation Criteria In Solid Tumors; RET, rearranged during transfection kinase.
Growing body of evidence that TMB in blood is predictive of immunotherapy efficacy in NSCLC

TMB and PD-L1 do not significantly overlap

Preliminary data also suggest a predictive role of TMB in SCLC

Relevant challenges
- Methodology standardization
- Definition of high/low TMB
- Clinical validation
Gracias!!!

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efelip@vhio.net