OA01.04 Tumor Mutation Burden (TMB) by Next Generation Sequencing (NGS) Associates with Survival (OS) in Lung-MAP Immunotherapy Trials S1400I and S1400A


INTRODUCTION:
TMB is an emerging biomarker for efficacy of immune checkpoint inhibitors (ICI). Lung-MAP is a master protocol for biomarker-driven trials in advanced NSCLC. Two sub-studies in previously treated ICI naïve advanced squamous cell lung cancer (sqNSCLC), S1400I, a phase III trial randomizing patients to nivolumab plus ipilimumab (N/I) versus nivolumab (N), and S1400A, a phase II trial evaluating durvalumab (D), provided the opportunity to evaluate TMB and related biomarkers by NGS and to determine associations with clinical outcomes.

METHODS:
NGS was performed on DNA from formalin-fixed paraffin-embedded tumor specimens using the FoundationOne T5 platform. TMB was defined as the total number of nonsynonymous mutations per megabase (Mb) of coding sequence. In S1400I, PD-L1 expression was assessed by the 22C3 antibody. A Cox model was used to evaluate associations between TMB (continuous and dichotomized at 10 Mb/mt), PD-L1 (continuous and dichotomized at 0% versus > 0%), overall survival (OS) and progression-free survival (PFS), summarized by hazard ratios (HRs) and 95% confidence intervals (CI). Associations between TMB and genetic alterations were evaluated by Wald test, with false discovery rate (FDR) ≤ 5% scored as positive. Unsupervised hierarchical clustering was performed using combined data from S1400I+S1400A.

RESULTS:
252 patients on N/I or N and 68 patients on D were included in the analysis. Higher TMB (per 10-unit difference in TMB value) was significantly associated with better OS and PFS (OS HR(CI): 0.80 (0.67–0.94), P = 0.008 and PFS HR(CI): 0.80 (0.69–0.93), P = 0.004). In S1400I, PD-L1 expression levels were not significantly associated with OS or PFS (N=161, P > 0.05), alone or in combination with TMB. In S1400I+S1400A, no genetic variants were significantly associated with OS or PFS. Genes whose alterations were significantly associated with TMB are shown in the volcano plot. Unsupervised hierarchical clustering suggested a variant-defined subgroup conferred better PFS (HR (CI): 0.41 (0.19–0.88), P = 0.022) but not OS; notably, this subgroup showed 3.8-fold higher TMB and more frequent alterations of genes shown in the plot, compared to other subgroups.

CONCLUSION:
Several different methodologies have been employed to measure TMB. TMB by FoundationOne NGS is an analytically and clinically validated assay correlating well with WES and predicted neoantigen load. Here we report that high TMB, but not PD-L1, is associated with improved OS and PFS in patients treated with ICI on S1400I/S1400A. How genetic alterations associated with high TMB may biologically contribute to clinical outcomes from ICI warrants further consideration.
INTRODUCTION:
ROS1 gene rearranged tumours are a rare, yet distinct molecular subgroup of non-small cell lung carcinomas (NSCLC). This study was performed in order to determine the comparative diagnostic accuracy of ROS1 immunohistochemistry (IHC), as opposed to fluorescence in situ hybridization (FISH) for its ability to detect ROS1 gene rearrangement in NSCLC.

METHODS:
A validation study designed to assess the diagnostic accuracy of ROS1 IHC using D4D6 (Cell Signaling Technology (CST), Danvers, MA) antibody clone for detection of ROS1 gene rearrangement as compared to FISH testing using ZytoLight SPEC/ROS1 Dual-Colour Breakapart Probe (ZytoVision, Bremerhaven, Germany) was performed, on an enriched cohort of ROS1 rearranged tumours (including 44 ROS1 rearranged and 166 ROS1 non-rearranged cases). The IHC interpretation was performed by two pathologists independently, who were blinded to the FISH results. Receiver operating characteristics (ROC) curves were used to determine the optimal cut-off value for H-score and proportion of positivity for ROS1 IHC, in order to discriminate between patients with ROS1-rearranged and ROS1- non-rearranged tumours.

RESULTS:
Overall ROS1 IHC positivity was observed in 41/210 (19.52%) and 40/210 cases (19.05%) by two different observers, independently, blinded to FISH results, with the almost perfect interobserver agreement (Kappa value 0.985. (95%CI, 0.95 to 1)). The immunoexpression was predominantly cytoplasmic and heterogenous with median H-score of 165(range 5-300). Amongst, 44 ROS1 gene rearranged cases, 40 cases were positive by ROS1 IHC and amongst, 166 ROS1 gene non-rearranged cases, 165 cases were negative by ROS1 IHC. Hence, overall ROS1 IHC had a sensitivity of 90.9%, a specificity of 99.40%, the positive predictive value of 97.56% and negative predictive value of 97.63% considering FISH as the gold standard. A total of 5 discordant cases including one false positive (ROS IHC+/FISH-) and 4 false negatives (ROS IHC-/FISH +) were recorded. ROC curve analysis revealed the optimal cut-off: H-score of ≥ 2.5 and proportion of positivity ≥ 2.5%, can best predict the ROS1 rearrangement using ROS IHC in this study cohort. In 6 cases, of ROS1 rearranged tumours, low IHC expression (i.e.: only weak intensity staining (1+), positivity in <25% tumour cells and H-score <100) was noted. Although, 3 out of these 6 patients had received the Crizotinib, however, the clinical response was not as promising and developed progressive disease, after a short interval of Crizotinib.

CONCLUSION:
The comparative evaluation in this enriched ROS1 rearranged cohort was unique in term of high specificity (99.40%), and relatively low sensitivity (90.9%) of ROS1 IHC and are somewhat contradictory as compared to previous reports. None of the previously reported cut-off criteria (H score ≥100/150, Staining intensity of ≥2+ in 30% tumour cells) can predict the ROS1 rearrangements with 100% accuracy in our study population and, some cases might be devoid of further molecular testing. Hence, it raises concerns about the utility of D4D6 antibody clone for ROS1 screening. Use of the appropriate cut-off interpretative criteria’s can maximize the sensitivity and specificity of ROS1 IHC for predicting the ROS1 rearrangements. The correlation of the level of ROS1 protein expression with response to the Crizotinib needs to be evaluated further.

ES09 BIOMARKERS IN IMMUNOTHERAPY
IASLC 2020 World Conference on Lung Cancer, Singapore.

ES09.03 Role of PD-L1 and Tumor Mutational Burden in NSCLC Immunotherapy
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New cancer immunotherapy strategies have the potential to overcome tumor-mediated immune suppression. However, such therapies are not only associated with significant costs, but also potentially severe adverse side effects. Because only a minority of patients benefit from this strategy, it is of utmost importance to establish biomarkers to guide therapy decisions. For many tumor entities, assessment of programmed death ligand 1 (PD-L1) expression on tumor and/or immune cells by immunohistochemistry (IHC) is the approved companion diagnostic. As each PD-L1 IHC assay was independently developed for specific anti-programmed death 1 (PD-1)/PD-L1 therapy using a different PD-L1 diagnostic assays (primary antibody clone plus immunostaining platform/protocol), each assay potentially demonstrates distinct staining properties, which could prohibit the interchangeability of their clinical use. Several groups have demonstrated the comparability of the various PD-L1 IHC assays and their potential interchangeability in clinical adoption, starting with the IASLC Blueprint project 1 and 2. The results from the Blueprint phase 1 study demonstrated that three PD-L1 assays (22C3, 28-8, and SP263) showed comparable analytical performance for assessment of PD-L1 expression on TCS, whereas the SP-142 PD-L1 assay appeared to stain fewer TCS compared with the other assays. In contrast, all the assays stained tumor-infiltrating immune cells (ICs), but with poor concordance between assays. These data were confirmed in BP2 validation cohort. PD-L1 is currently mainly used to select patients for immunotherapy monotherapy frontline, in the scenario of a high expression on TC (>50%), initially based on KEYNOTE-024, and less commonly and in selected regions/countries, in case of PD-L1 positivity only, based on the more debated results of KEYNOTE-42 trial. As a basis for immunogenicity, it has been hypothesized that tumors with a high number of coding mutations are more likely to generate tumor-specific neoantigens that will be recognized by the immune system. Tumor mutational burden (TMB) has emerged as a novel biomarker to identify patients more likely to respond to immune checkpoint inhibitor therapy targeting the PD(L)-1 axis or cytotoxic T-lymphocyte associated protein 4 (CTLA-4). Recent data support a predictive potential of TMB for checkpoint inhibitor therapy in various cancer types. TMB can potentially identify — strictly independently from PD-L1 expression status — different patient
cohnets likely to respond and, possibly in conjunction with PD-L1 status, help to predict non-responders and exceptional responders. Aided by recent progress in sequencing technologies, an increasing number of panel-based next-generation sequencing (NGS) assays and services to measure TMB is offered. Panel-based NGS fits very well into the clinical workflow of cancer tissue evaluation because (i) formalin-fixed and paraffin-embedded (FFPE) tissue samples can be used as input biomaterial, (ii) analysis of small biopsies is feasible as only small amounts of DNA are needed, (iii) there is no immediate need for analysis of paired normal tissue or blood samples as germline mutation filtering can be performed in silico, (iv) TMB measurement can be performed together with the analysis of druggable targets in a single assay and (v) the entire workflow including wet-lab analysis, bioinformatics pipeline and variant interpretation can be carried out within a few days. Thus, being the present-day mainstay of clinical mutation analysis in oncology, panel sequencing is expected to be the most widely adopted technology for clinical TMB measurement for the next years. Of major importance, such analysis is progressively moving to the use of circulating tumor DNA (ctDNA). ctDNA can be actively released into circulation or shed after tumor cells outgrow their blood supply, become hypoxic, and undergo apoptosis or necrosis. The goals of circulating tumor DNA monitoring are to capture genetic heterogeneity, identify targetable mutations, and monitor tumor evolution in real time. Studies in multiple tumor types have demonstrated high concordance for hotspot mutations between paired tissue and ctDNA biopsies. However, more recent tumor-oriented studies have stressed technical limitations for tissue vs blood comparisons, notably regarding variability in platforms, technologies, genetic alterations identification, sensitivity and thresholds used. Keeping in mind a strong need for harmonization, blood TMB profiled with ctDNA sequencing remains a promising non-invasive strategy for TMB, aiming at possibly more accurately predict immunotherapy benefit. In June 2020, the US Food and Drug Administration (FDA) granted accelerated approval to pembrolizumab for the treatment of adult and pediatric patients with unresectable or metastatic tumor mutational burden-high (TMB-H) (≥10 mut/Mb) solid tumors, as determined by an FDA-approved test, that have progressed following prior treatment and who have no satisfactory alternative treatment options. This approval was based on efficacy data from 10 refractory solid tumor cohorts enrolled in a multicenter, non-randomized, open-label trial (KEYNOTE-158 (NCT02628067)). Altogether, 102 patients (13%) had TMB-H tumors, defined as TMB ≥10 mut/Mb. The objective response rate was 29% [95% confidence interval (CI): 21% to 39%]. Overall, about half the responses were of greater than 2 years with many ongoing, a durability of response rarely observed in heavily pretreated metastatic cancers with treatment modalities other than immunotherapy. TMB, in concert with PD-L1 expression, has been demonstrated to be a useful biomarker for immune checkpoint blockade selection across some cancer types. However, further prospective validation studies are still required. TMB determination by selected targeted panels has been correlated with WES. Calibration and harmonization will be required for optimal utility and alignment across all platforms currently used internationally. Key challenges will need to be addressed before broader use - or routine practice application - across different tumor types References. 1. Budczies, J., Quantifying potential confounders of panel-based tumor mutational burden (TMB) measurement. Lung Cancer 142, 114-119 (2020). 2. Chan, T.A., Development of tumor mutational burden as an immunotherapy biomarker: utility for the oncology clinic. Ann Oncol 30, 44-56 (2019). 3. Hirsch, F.R., PD-L1 Immunohistochemistry Assays for Lung Cancer: Results from Phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project. J Thorac Oncol 12, 208-222 (2017). 4. Kazdald, D., Spatial and Temporal Heterogeneity of Panel-Based Tumor Mutational Burden in Pulmonary Adenocarcinoma: Separating Biology From Technical Artifacts. J Thorac Oncol 14, 1935–1947 (2019). 5. Subbiah, V., nn Oncol 31, 1115–1118 (2020). 6. Tsao, M.S., J Thorac Oncol 13, 1302–1311 (2018).
detected by FMI. We additionally identified homologous recombination defects in 7/90 patients (7.8%, of which BRCA1/2 pathogenic somatic mutations in 3 patients); PI3K-pathway alterations in 8/90 (8.8%, PIK3CA in 2 patients, AKT1/3 in 2, PTEN in 4); FGFR family alterations in 11/90 (12.2%); ERBB2 alterations in 9/90 (10%); MYC amplification in 7/90 (7.8%) and ARID1A mutations in 6/90 (6.6%). When we focus on the 77/90 patients who were QN (EGFR/ALK/ROS1/KRAS wild-type or unknown) by standard molecular tests, 84.4% had at least 1 alteration detected, 66% had ≥2 concomitant alterations. Seven exon 20 or other EGFR mutations, 1 ALK fusion and 10 Kras mutations were additionally detected (previously unrecognized at standard analysis). Regarding potentially druggable alterations: 8 ERBB2 alterations (3 amplification, 5 mutations), 1 NTRK and 1 RET rearrangements, 1 BRAF and 1 MET exon 14 mutation; 8/77 patients (10.4%) received an alteration-matched therapy according to FMI results. In overall population, median OS was not reached, 1-yr OS rate from diagnosis of metastatic disease was 77.5%. At multivariate analysis, in QN patients, age at diagnosis of metastatic disease (≤55) and MYC amplification were associated with a shorter PFS (HR 2.77, 95% CI 1.23-6.26; p=0.014 and HR 3.12, 95% CI 1.06-9.18; p=0.038; respectively).

CONCLUSION:
This analysis suggests that extended molecular profiling of aNSCLC, particularly in QN patients, may provide meaningful results, potentially increasing treatment opportunities. Nevertheless, impact on patients’ survival needs to be further explored. A larger collection of cases is currently ongoing in the context of a multicenter trial and updated results will be presented at the meeting.

MA13 TUMOR BIOLOGY: FOCUS ON EGFR MUTATION, DNA REPAIR AND TUMOR MICROENVIRONMENT
IASLC 2020 World Conference on Lung Cancer, Singapore.

MA13.09 Heterogeneous Genomic Evolution and Immune Microenvironments in Metastatic Lung Cancer

INTRODUCTION:
The comprehensive insights into the genomic evolution and immune microenvironments of lung cancer metastasis remain unknown. Furthermore, whether non-stochastic patterns of lung cancer metastases to different sites exist is elusive.

METHODS:
We investigated the genomic evolution and immune microenvironments of paired primary-metastatic (P-M) tumors by employing multi-region whole-exome sequencing and immunohistochemistry in 179 samples from 51 lung cancer patients with metastases to the pleura, bone, adrenal gland, brain and additional lymph nodes.

RESULTS:
Our data revealed differences in genomic landscapes, molecular determinants, seeding patterns, and lymphocyte infiltration among different metastatic sites. Metastatic lymph nodes showed the highest P-M genomic divergence, followed by pleura, brain, bone, and adrenal gland. We identified MYC amplification as a selected event driving metastasis and associated with worse overall survival (P = 0.013). Interestingly, EGFR amplification and TP53 mutations were preferably selected in distant metastases whereas RICTOR amplification was selected in regional metastases (pleura and lymph nodes). Based on spatial tumor growth model, we demonstrated commonly late arising of metastatic seeding (61.1%) of lung cancer with quantitative evidence. However, mutation rate and timing of dissemination varied among different metastatic sites. Metastases at regional tissues were more frequently infiltrated with CD8+ tumor-infiltrating lymphocytes (TILs) than those at distant organs, among which bone metastases were merely infiltrated with CD8+ TILs. Furthermore, monoclonal and polyclonal seeding were associated with rapid and attenuated progression (P = 0.013), respectively, which supports the potential value as a prognostic predictor. Immune-heterogeneity and -homogeneity were primarily driven by arm-level and focal copy number events in primary tumors, respectively, indicating distinct mechanisms of tumor immune escape during metastasis.

CONCLUSION:
These findings implied the combinatorial role of multiple factors in shaping patterns of dissemination and advanced the clinical evaluation and intervention of lung cancer metastasis.
MA08.06 Stratifying PD-L1 Expression Level Based on Multimodal Genomic Features for the Prediction of Immunotherapy Benefit in NSCLC

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INTRODUCTION:
Programmed cell death 1 (PD-L1) is the first FDA-approved predictive biomarker for non-small cell lung cancer (NSCLC) patients treated with PD-(L)1 blockade therapy. However, many clinical patients are not subject to PD-L1 testing due to different reasons such as limited sample source and tumor heterogeneity. Therefore, this study aimed to understand the association between multimodal genomic alterations and PD-L1 expression, and further explore whether genomic alterations can be leveraged to stratify patients who may benefit from immunotherapy.

METHODS:
Genomic profiling of tumor biopsies from a total of 883 Chinese NSCLC patients, including 651 adenocarcinoma (ADC) and 157 squamous cell carcinoma (SCC), was performed by targeted next-generation sequencing. Immunohistochemical analysis was conducted to evaluate PD-L1 protein expression using commercial PD-L1 antibodies including Dako 22C3 (N = 750) and 28-8 (N = 133), respectively. A predictive model to discriminate PD-L1 level was constructed and validated to predict patient response to immunotherapy.

RESULTS:
Our study showed distinct correlation patterns between PD-L1 expression and clinical/genomic characteristics of ADC and SCC patients, respectively. PD-L1 high expression (TPS ≥ 50%) for ADC patients was more common in male (p = 0.006) and metastatic samples (p = 0.026). In the SCC patients, lymph node metastasis enriched in PD-L1 positive group (TPS ≥ 1%) compared to samples with other metastatic sites (p = 0.031). POLE mutation was enriched in the PD-L1 positive/high group in SCC, but in the PD-L1 negative group in ADC. Besides, copy number gains of PD-L1 and PD-L2 were significantly associated with PD-L1 positive status (TPS ≥ 1%) only in ADC patients. Furthermore, we constructed a model to predict PD-L1 expression in ADC patients using seven genomic features significantly related with PD-L1 level including EGFR oncogenic mutations, KRAS oncogenic mutations, PD-L1 gain, PD-L2 gain MDM2 gain, chr.1q amplification and chr.20q amplification, which yield an area under the curve (AUC) score of 0.764. Model validation with an independent dataset demonstrated its capacity in predicting PD-L1 expression level and potential benefit from immune checkpoint inhibitors (ICIs) treatment. Since PD-L1 expression and tumor mutational burden (TMB) score were two independent predictive biomarkers for immunotherapy, a combination of our model with TMB showed 52% of patients with both TMB high and predicted PD-L1 high benefited from ICIs treatment, and 29% patients with TMB low but predicted PD-L1 high also showed responses.

CONCLUSION:
This study revealed the distinct correlation between PD-L1 expression levels and clinic molecular features of NSCLC patients from
different histological subgroups, and constructed a classifier for PD-L1 expression and ICIs response prediction for patients who did not receive PD-L1 testing. We also find the predicted PD-L1 expression levels combined with TMB may help to identify patients who are most likely to respond to ICIs treatment and improve the likelihood of benefit of the patients with low TMB.