P1.11 METASTATIC NON-SMALL CELL LUNG CANCER – IMMUNOTHERAPY

SUNDAY, SEPTEMBER 7, 2025 - 10:30 - 12:00

P1.11.63 Shifts of Mutational Signatures Reveal Response Variability to Pembrolizumab-Based

Immunotherapy in NSCLC Patients

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Introduction: Immune checkpoint inhibitors, such as pembrolizumab, have revolutionized NSCLC treatment. However, response to

immunotherapy remains highly variable and understanding the genomic adaptations is crucial for optimizing the therapy outcomes. In this

study, we performed targeted next-generation sequencing (NGS) on tumor (T) and longitudinal liquid biopsy (LB) samples to investigate

dynamic mutational changes in NSCLC patients with different responses to pembrolizumab. Analyzing mutational signatures and key driver

mutations over follow-up, we aimed to uncover molecular mechanisms associated with durable response or resistance to therapy.

Methods: Within this study, we collected T and LB1 samples before treatment initiation, followed by additional longitudinal LB samples (LB2,

LB3, etc.) approximately every 2-3 months during clinical follow-up until therapy completion. Targeted NGS libraries (SureSelect CD Glasgow

Cancer Plus Panel, Agilent Technologies, UK) were sequenced on the NovaSeq XPlus system (Illumina, USA) in 2×150 bp read mode. Data

preprocessing, including alignment and variant calling, was performed using AGeNT, BWA-MEM, MUTECT2, and VEP, while downstream

variant analysis and visualization were conducted using the R package Maftools. This study was funded by the Polish National Centre for

Research and Development (LIDER/46/0237/L-12/20/NCBR/2021).

Results: We sequenced 293 samples (77-T, 78-LB1, 55-LB2, 34-LB3, 26-LB4, 23-LB5) from 78 patients (45 males, 33 females; median age:

67±8) treated with pembrolizumab in monotherapy (n=26) or in combination with chemotherapy (n=52) (median PFS: 4 months). Among

them, 23 patients were long responders (PFS >12 months), while 55 were short responders (PFS ≤12 months). Mutational analysis showed

70% concordance between T and LB1 samples, with TP53 and KMT2C/D being the most frequently mutated genes in both tissue and liquid

biopsy samples. KRAS mutations co-occurring with STK11 (three cases) and KEAP1 (one case) mutations were observed exclusively in

short responders. In both T and LB1 samples, short responders showed significant enrichment of the COSMIC\_3 signature (associated with

HR-related DNA-DSB repair defects), whereas long responders had COSMIC\_2 signature (APOBEC), which was significantly associated

with TP53 mutations. Over time, both groups transitioned to COSMIC\_6 (DNA mismatch repair defects) and COSMIC\_30 (unknown etiology)

at 3 and 6 months of treatment, respectively. By 12 months (LB4), short responders reverted to COSMIC\_3, while long responders retained

COSMIC\_6 and COSMIC\_30, which persisted in LB5. These shifts correlated with the early elimination of TP53 mutations in long responders

and their gradual decrease in short responders, as well as the persistence of a KMT2C/DNMT3A-mutated clone, which frequency fluctuated

over time.

Conclusions: The dynamic shifts in mutational signatures during treatment reflect ongoing tumor evolution under pembrolizumab pressure,

revealing distinct genomic adaptation patterns between long and short responders. Long response was associated with a pre-treatment

APOBEC signature and the early elimination of TP53 mutations, potentially supporting the maintenance of adaptive mutational profiles.

Conversely, short responders initially exhibited a mutational shift but eventually reverted to their original HR-related DNA-DSB repair defect

signature, suggesting an inability to preserve adaptive genomic changes under treatment

P3.03 TUMOR BIOLOGY – TRANSLATIONAL BIOLOGY TUESDAY, SEPTEMBER 9, 2025 - 10:00 - 11:30 P3.03.13 HLA II Genetics Shapes Lung Cancer Immunosurveillance E.A. Olumuyide, M. Saffern, D. Chowell, R. Samstein Icahn School of Medicine at Mount Sinai, New York/NY/USA Introduction: The risk of developing cancer is one’s lifetime is about 50% after the sixth decade of life and it is associated with internal factors such as inherited mutations in DNA repair genes, replication errors, and environmental factors. Lung cancer is the most common cancer worldwide and the leading cause of cancer-related deaths worldwide. Recent advances in immunotherapy demonstrate the need to understand the complex choreography of immune genetics and cancer risk, as well as how antigen presentation, particularly MHC molecules, shapes cancer immunosurveillance. MHC molecules are encoded by the polygenic HLA locus, which is the most polymorphic region of the human genome, therefore the possibility of presentation of diverse tumor antigens to the immune system. We hypothesize that germline heterozygosity at the HLA locus allows for binding of a greater repertoire of antigens that can be presented to the immune system, thereby decreasing lung cancer risk. Methods: We used clinical and genetic information from over 390,000 individuals in the UK Biobank to study how HLA zygosity affects lung cancer risks among smokers. To study the mechanism behind this, we used a carcinogen murine model that allows us to assess tumor multiplicity across MHC homozygous and MHC heterozygous genotype groups. To explore how immune surveillance defers between genotypes, we used flow cytometry to explore early immunosurveillance across multiple mice genotypes and using cell lines we developed from the carcinogen model, we explored how tumor cell MHC II shapes tumor surveillance. Results: We show that germline heterozygosity at the HLA class II loci confers decreased risk of cancer among smokers. Furthermore, we showed using murine models that maximal heterozygosity at the HLA locus (H2 in mice) leads to decrease tumor incidence, which we further show is due to increase in T lymphocytes in the tissue microenvironment at early tumor development. We further show using murine models that loss of HLA class II on the cancer cells as well as epithelial cells ameliorate this protective effect against cancer risks. Conclusions: Our findings demonstrate that HLA class II heterozygosity enhances tumor immunosurveillance and reduces lung cancer risk, highlighting the critical role of immune genetics in cancer susceptibility. These results provide a foundation for developing immunopreventive strategies targeting MHC II neoantigens. Keywords: Lung Cancer, immunosurveillance, Immunoprevention

P1.11.59 Effect of IFNG Expression Levels on Real-World Survival Outcomes in Patients With Metastatic Non-Small Cell Lung Cancer Receiving Immunotherapy M. Altoe1, S. Papillon-Cavanagh2, M. Poi2, S. Yip1, T. Oakland1, M.R. Rossi1 1ConcertAI LLC, Cambridge/MA/USA ,2Caris Life Sciences, Phoenix/AZ/USA Introduction: Despite advances in immunotherapy for metastatic non-small cell lung cancer (NSCLC), a significant proportion of patients do not respond to immune checkpoint inhibitors (ICIs). Identifying effective prognostic biomarkers that are independent of squamous and nonsquamous histology is critical for optimizing treatment strategies in NSCLC. Interferon gamma (IFNG) has emerged as a potential marker for predicting immunotherapy outcomes due to its role in immune modulation. Methods: We retrospectively evaluated metastatic NSCLC patients treated with first-line ICIs or ICI plus chemotherapy with an ECOG performance status of 0 or 1 at the time of initial diagnosis. Patients were identified from the ConcertAI linked with Caris Life Sciences data, a U.S.-based, de-identified, patient-level dataset that integrates EHR-based clinical data with exomic, transcriptomic, and whole slide imaging data. Patients were grouped based on high and low IFNG expression, defined as the top and bottom quartiles of expression (transcripts per million, TPM). Survival analyses, including Kaplan-Meier estimates and Cox proportional hazard models, were performed, stratified by histological subtypes of adenocarcinoma (N = 414) and squamous cell carcinoma (N = 147), and by IFNG expression levels. Results: We evaluated 561 patients who met the selection criteria. High IFNG expression was associated with significantly longer median overall survival (OS), regardless of histological subtype. The median OS for high IFNG expression was 26.7 months in adenocarcinoma (N = 111) and 27.6 months in squamous cell carcinoma (N=38). In contrast, low IFNG expression corresponded to a median OS of 15.1 months in adenocarcinoma (N = 99) and 12.9 months in squamous cell carcinoma (N = 42). Cox regression analysis confirmed that low IFNG expression was significantly associated with higher mortality risk (Hazard Ratio [HR] = 1.8, 95% CI = 1.35-2.4, P < .001). Histological subtype did not independently predict OS (p > 0.05). Conclusions: High IFNG expression is associated with improved survival outcomes in metastatic NSCLC patients treated with ICIs or ICIs plus chemotherapy as first-line therapy independent of histological subtype. These findings suggest the potential utility of IFNG as a prognostic biomarker to guide therapy decisions in higher-risk NSCLC and tailor treatment strategies in this patient population.

P1.11 METASTATIC NON-SMALL CELL LUNG CANCER – IMMUNOTHERAPY SUNDAY, SEPTEMBER 7, 2025 - 10:30 - 12:00 P1.11.54 Lack of Durable Response to ICI Therapy in Advanced NSCLC: The Role of Circulating Lazy T Cells A. Palladini1,2, P.L.S. Michel2, I.C. Chillico2, N.L. Alessio1,2, H.R. Recalde1, C. Meletti Cavallari1, M. Frascino1, S. Angelicola3, S. Borgetto2, F. Rifaldi1,2, I. Lanzetta1,2, A. Tortorella1,2, F. Mascaro1,2, M. Festari2, S. Croci4, C. Bortolotto1,2, L. Brizzi1,2, F. Gelsomino3, P. Pedrazzoli1,2, F. Agustoni1,2 1University of Pavia, Pavia/IT ,2IRCCS Policlinico San Matteo Foundation, Pavia/IT ,3IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna/IT ,4Azienda USL, IRCCS Reggio Emilia, Reggio Emilia/IT Introduction: Treatment of advanced Non-Small Cell Lung cancer (NSCLC) with immune checkpoint inhibitors (ICIs) leads to long-lasting responses in only 20% of patients. Conversely, 14-37% of patients experience rapid disease progression following ICI therapy. Efforts to investigate the causes of fast tumor progression by dissecting the tumor microenvironment at baseline did not yield any clear clinical biomarkers. Further, real-life longitudinal studies focusing on circulating immune components remain limited, and no specific indexes have been established for clinical monitoring. Previously, we demonstrated that low IFN-γ doses modulate NSCLC plasticity associated with ICI-mediated hyperprogression. However, few is known about the regulation of IFN-γ production by T cells. The goal of this study was to investigate the ability of circulating T cells from advanced NSCLC patients to be stimulated and to produce IFN-γ, comparing long-responder with fast-progressor ICI-treated patients. Methods: Advanced NSCLC patients undergoing ICIs were enrolled and categorized into patients who responded after 1 year of treatment (long responders-LR) and patients who progressed within the first 3 months (fast progressors-FP). Consecutive blood samples of LR were monitored over time to minimize inter-sample variability and record changes associated to progression. Classical, intermediate, and non classical monocyte subsets were identified using CD14-PE and CD16-FITC. HLA-DR-APC staining was used to assess antigen presentation capacity. PBMCs isolated from FP and LR patients were cultured with ImmunocultTM medium and hu-IL-2 and stimulated with anti-CD3/ CD28/CD2 antibodies. Then, intracellular IFN-gamma level was measured. Plasma soluble factors were measured through human Luminex Discovery Assay. Results: The expression of HLA-DR resulted lower in classical monocytes compared to intermediate and non-classical counterparts. Notably, FP patients had a more than 50% reduction in HLA-DR expression on intermediate monocytes compared to LR patients. This decrease was also observed in LR patients who later experienced sudden disease progression. Pentraxin PTX3, which can inhibit HLA-DR expression, enhance TGF-β production, and bind CD44, was found to be significantly increased in FP compared to LR patients.LR-derived CD4+ T and CD8+ T cells showed a 2-fold increase in IFN-γ production after three days of stimulation with anti-CD3/CD28/CD2 antibodies, compared to FP T cells. Prolonged exposure to immune stimulators further increased IFN-γ secretion by FP T cells but did not in LR ones. Treatment with nivolumab reduced FP CD4-mediated IFN-γ production, while LR T cells showed increased IFN-γ release under nivolumab treatment. Surprisingly, circulating T regulatory cells resulted slightly higher in LR compared to FP patients. This could indicate a lower intratumor Treg localization in the former. Tregs mainly modulate the immune response through IL-10 and TGF-β1 cytokines. We found a 3-fold increase of TGF-β1 in FP respect to LR, while IL-10 was low or undetectable in both groups. Conclusions: Advanced NSCLC patients receiving ICI therapy showed longer-lasting therapeutic responses in presence of circulating intermediate monocytes with higher HLA-DR expression. Conversely, elevated PTX3—capable of inhibiting antigen presentation and promoting cancer cell plasticity—was found in FP patients. Despite a slower activation profile, T lymphocytes from FP patients retained the capacity to produce IFN-γ. Keywords: Interferon gamma

PT2.11 METASTATIC NON-SMALL CELL LUNG CANCER – IMMUNOTHERAPY MONDAY, SEPTEMBER 8, 2025 - 14:15 - 15:00 PT2.11.04 Divergent Mechanisms of Hyper-Progressive Disease in Non-Small Cell Lung Cancer Treated With Immune Checkpoint Inhibitors S. Shim1,2, D.Y. Jeong1, Y.J. Oh2, H. Kim1, J. Kim1, S. Park1, H.A. Jung1, J-M. Sun1, J.S. Ahn1, M-J. Ahn1, H.Y. Lee1,2, S-H. Lee1,2 1Samsung Medical Center, Seoul/KR ,2Sungkyunkwan University, Seoul/KR Introduction: Hyper-progressive disease (HPD) has emerged as an atypical response pattern to immune checkpoint inhibitors (ICIs), characterized by accelerated tumor progression and profoundly unfavorable clinical outcomes. Although its underlying mechanisms remain unclear, emerging evidence implicates both tumor-intrinsic properties and immune-mediated processes in its development. Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC), the two major histological subtypes of non-small cell lung cancer (NSCLC), exhibit fundamentally distinct tumor biology and immune microenvironments. This study aims to delineate the clinical and molecular characteristics of HPD across tumor subtypes to improve patient stratification and optimize the application of ICIs. Methods: We retrospectively analyzed 630 patients with advanced NSCLC who received ICI monotherapy. Among them, 318 patients who exhibited progressive disease (PD) according to RECIST 1.1 were further classified into HPD and non-HPD based on volumetric assessment. Patients with PD who did not meet the volumetric criteria for HPD were defined as non-HPD. Subsequent analyses were conducted to evaluate clinical and molecular landscapes between these two groups. Transcriptomic profiling of pre-treatment tumor specimens was performed to investigate subtype-specific features of HPD, including gene set enrichment, cytokine signaling inference, and immune cell deconvolution. Systemic immune alterations were assessed using multiparametric flow cytometry on paired peripheral blood samples collected before and during treatment. Results: HPD occurred in 10.2% of patients (N = 64; LUAD 9.8%, LUSC 11.1%) and was associated with significantly inferior overall survival compared to non-HPD (median OS, 4.6 vs 8.7 months; hazard ratio [HR] = 2.05; 95% CI, 1.55-2.71; P < 0.0001). In LUAD HPD, transcriptomic analysis revealed a marked upregulation of granulocyte-macrophage colony-stimulating factor (GM-CSF) signaling (P = 0.01), accompanied by increased M2-like macrophages (P < 0.001) and macrophage/dendritic cell trafficking signatures (P = 0.01). Peripheral immune profiling further demonstrated an expansion of circulating myeloid populations following ICI treatment. Collectively, these findings suggest that HPD in LUAD may be driven by a pronounced shift toward an immunosuppressive phenotype. In contrast, LUSC did not exhibit HPD specific transcriptional or systemic immune changes. A pervasive immunosuppressive tumor microenvironment (TME) was observed in LUSC non-HPD, characterized by enrichment of M2-like macrophages (P = 0.01), angiogenic signatures (P = 0.002), and epithelial mesenchymal transition (EMT) pathways (P = 0.003). However, these features were not significantly observed in LUSC HPD, suggesting that its development in this subtype may involve alternative mechanisms, potentially driven by tumor-intrinsic programs. The presence of immunosuppressive traits in LUSC may obscure the distinction of HPD within this subtype, whereas aberrant immune perturbations may serve as key determinants of HPD development in LUAD. Conclusions: HPD in advanced NSCLC may arise through divergent biological mechanisms shaped by histological subtypes. In LUAD, HPD may appear to be associated with immunosuppressive remodeling, while in LUSC, it is more likely governed by tumor-intrinsic pathways independent of immune modulation. These findings highlight the clinical relevance of histology-specific molecular stratification for identifying patients at heightened risk of HPD and underscore the importance of tailoring immunotherapeutic strategies to each tumor subtype. Keywords: Hyper-progressive disease, Non-small cell lung cancer, Immune checkpoint inhibitor

EP.07 EARLY-STAGE NON-SMALL CELL LUNG CANCER TUESDAY, SEPTEMBER 9, 2025 EP.07.38 Immune Checkpoint Inhibitors May Not Fully Mitigate the Adverse Prognostic Impact of PD L1 Expression in Early Lung Cance K. Onodera, H. Notsuda, S. Kumata, T. Watanabe, Y. Watanabe, T. Suzuki, T. Hirama, H. Oishi, Y. Okada Institute of Development, Aging and Cancer, Tohoku University, Sendai/JP Introduction: PD-L1 expression is thought to correlate with the grade of malignancy in lung cancer. On the other hand, immune checkpoint inhibitors have emerged as an option for recurrence after lung resection and are expected to be highly effective in patients of lung cancers with PD-L1 expression. In this study, we investigated the impact of treatment with immune checkpoint inhibitors after recurrence on postoperative prognosis in early stage lung cancer patients. Methods: We analyzed 141 patients with stage pI non-small cell lung cancer who underwent complete resection at Tohoku University Hospital from January 2017 to December 2018. We retrospectively evaluated pathological factors and the treatment after recurrence and examined recurrence-free and overall survival rates according to PD-L1 expression. Results: PD-L1 expression was classified as high (≧50%) in 19 patients, low (1-49%) in 50 patients, and negative in 72 patients; with increasing PD-L1 expression, the rates of pleural invasion, vascular invasion, and lymphatic invasion increased. Recurrence patterns included local recurrence in 8 patients, distant recurrence in 5 patients, and both local and distant recurrence in 4. Patients with negative PD-L1 expression had better OS and RFS than those with positive expression. Patients who bared lung cancer with PD-L1 expression and without driver mutations were predominantly treated with immune checkpoint inhibitors after relapse, but two of the five patients were untreatable after recurrence. Conclusions: PD-L1 expression in early-stage lung cancer appears to be associated with the grade of oncological malignancy. Treatment with immune checkpoint inhibitors may not sufficiently mitigate the adverse prognostic implications of PD-L1 expression despite its high efficacy for lung cancers with PD-L1 expression. Keywords: PD-L1, early-stage lung cancer.

EP.06 PATHOLOGY AND BIOMARKERS EP.06.44 Tertiary Lymphoid Structures in the Tumor Micro Immune Environment of EGFR Positive Lung Cancer Are Associated Prognosis H. Yamaguchi Fukushima Medical Univercity, Fukushima City/JP Introduction: We have been studying the tumor micro immune environment(TME) in lung cancer such as tertiary lymphoid structure(TLS), and there are still many unknowns about TME in EGFR mutation-positive lung cancer. We investigated TME in EGFR mutation-positive lung cancer patients. Methods: We included 213 patients with non-small cell lung cancer who underwent surgery at our institution between January 2007 and December 2015, who were positive for EGFR mutations and were further considered for TLS; TLS was defined as a population of lymphocytes containing PNAd-positive epithelial vessels. Results: The median age was 67 years (37-90 years), 34% were male, 66% were female, and 62 patients had a history of smoking. The histological type was adenocarcinoma in 209 cases: Stage I in 172 cases and II-IV in 41 cases; L858R in 132 cases and Exon19 del in 81 cases; TLS High in 75 (57%) of L858R-positive cases and 31 (37%) of Exon19 del-positive cases (P=0.011). DFS was significantly higher in TLS High in all cases (P=0.028). There was no clear difference in Stage I, but in Stage II and above, the TLS High group tended to do better, especially in L858R. In univariate and multivariate analysis of DFS, p-Stage and TLS were independent predictors of prognosis. Conclusions: There are reports that TLS is associated with DFS in EGFR-positive lung cancer, although L858R-positive lung cancer is more likely to have TLS, and there are scattered reports that Exon19del has better PFS and TKI efficacy. On the other hand, the ATTLAS trial reported that ICI tended to be more effective than L858R. This study suggests that differences in EGFR mutations may be related to the tumor microenvironment, especially TLS expression, and may affect the efficacy of TKIs and ICIs. Keywords: Lung cancer, EGFR, TLS