P1.04-08
Co-Residence of Patient-Derived Immune Cells in Patient-Derived Xenografts from Lung Cancer Patients

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Background: Patient-derived xenograft (PDX) models have been shown to recapitulate many characteristics of human tumors and have been increasingly used for anticancer drug development, molecular characterization of cancer biology, and development of precision therapies. However, because PDXs are grown in immunodeficient mouse strains, they are regarded as inappropriate for preclinical evaluation of anticancer immunotherapy. Here we evaluated whether patient-derived immune cells co-exist in PDXs derived from lung cancer patients. **Method:** First-generation PDX (F1) was established by subcutaneously implanting human tumor tissue into non-obese diabetic-severe combined immunodeficiency (NOD-SCID) mice with a null mutation of the gene encoding for interleukin-2 receptor g (NSG). When the resulting tumors in these mice grew to about 1.5 cm in diameter, we passaged the tumors in NSG or nude mice for subsequent generations. A small piece of these PDX tissues (about 2-3 mm^3) were minced into fragments and cultured in media containing human interleukin-2 (IL-2) (2000 -6000 units/ml) for up to 6 weeks. The proliferated lymphocytes for analyzed by fluorescence-activated cell sorting (FACS) with antibodies specific for human immune cell surface markers. The provenance of cultured cells was determined by DNA fingerprinting assay together with patients' DNA samples from primary tumors and/or peripheral blood mononuclear cells (PBMC). **Result:** The mean time of PDX growth in NSG mice before harvesting for culturing tumor-infiltrating lymphocytes (TILs) was 120 days (ranging from 63-292 days). TILs were successfully cultured from 8 of 25 PDXs samples (about 32%), with one from F2 PDXs and 7 from F1 PDXs. TILs from five of those PDXs were predominantly human $CD3^+CD8^+$ T cells (72% -99%), while the remaining three were predominantly human $CD19^+$ B cells (77% - 95%). DNA fingerprint analysis showed that genotypes of TILs were identical to patients' primary tumors and/or PBMC, demonstrating that the TILs were from the same patients as the PDXs. Further analysis showed that $CD8^+$ T cells from PDXs were $CD45RO^+$, with either $CD62L^+$ or $CD62L^-$. **Conclusion:** Patient-derived immune cells co-exist with PDXs in some lung cancer PDX models. Most of those immune cells were $CD3^+CD8^+$ and could be memory T cells. These results suggest that some PDXs might be used for evaluating functions of tumor resident immune cells and/or for evaluating anticancer immunotherapies. **Keywords:** Lung cancer, patient-derived xenografts (PDX), Tumor infiltrating lymphocytes (TIL), immunotherapy

P1.04-09
Immunomodulatory Effects of Afatinib and Pembrolizumab in EGFR-Mutant NSCLC with Progression on Prior EGFR-TKI

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Background: EGFR-mutant NSCLC is less responsive to single agent PD-1 blockade than smoking associated NSCLC. Preclinical models suggest EGFR-TKI can render a more immunocompetent tumor microenvironment. This study examined the immunomodulatory effects of combination second generation EGFR-TKI and PD-1 antibody. **Method:** In this phase 1 dose de-escalation study, patients were treated with afatinib 40 mg oral daily and pembrolizumab 200 mg IV q21day. Key Eligibility: advanced EGFR-mutant NSCLC with progression (PD) on prior EGFR-TKI, age$\geq$18, ECOG PS$\leq$1, acceptable organ function, no significant autoimmune diseases, measurable disease and controlled brain metastases. Tissue biopsy performed baseline and week 5-6 on treatment for PD-L1 IHC (22C3) and quantitative immunofluorescence for immune cell subsets and next-generation sequencing. Blood at baseline and at serial on-treatment timepoints were collected for ctRNA of PD-L1, EGFR, HER2 and MET; changes in circulating immune cell subsets, T-Cell repertoire and cytokine levels were evaluated by flow cytometry and Luminex. **Result:** No DLTs were observed in the first 6 patients and the 10 patient expansion cohort proceeded at afatinib 40 mg daily and pembrolizumab 200 mg IV q21day. Eleven patients enrolled to date. Key molecular and pathologic characteristics: adenocarcinoma 9, neuroendocrine 1, squamous 1. EGFR-TKI resistance mechanism: EGFR-T790M 4, EGFR-T790M/C797S 1, HER2 amp 1, MET 2, Her2 amp+neuroendocrine differentiation 1, unknown 2. Five patients had prior second line osimertinib. Three (27%) patients had immune related AEs (G2 adrenal insufficiency, G2 nephritis, G3 colitis). Nine patients were evaluable for response: (1 PR, 7 SD ((6/7) with tumor reduction $<$30%)). The responding patient had squamous histology tumor, prior PD on erlotinib, and PFS of 11 months with PD-L1 (22C3) TPS 40% and PD-L1 and PD-L2 amplification. Treatment with afatinib and pembrolizumab induced systemic immune changes including trend for increased soluble IDO, MIG, TIM3, IP-10, LAG3, PD-L1 and PD-L2 and decreased IFN-gamma. ctRNA for EGFR and PD-L1 were detected in 7/7 and 6/7 patients, respectively, with dynamic changes in allele frequency of EGFR and PD-L1 observed. Baseline PD-L1 (22C3) TPS ranged from 0% to 75% expression. Four patients had repeat biopsy and in paired samples analyzed PD-L1 (22C3) expression decreased in the stroma. **Conclusion:** Immunomodulatory effects of afatinib with pembrolizumab were noted in the tumor microenvironment and peripheral blood. Only modest clinical activity has been observed thus far in patients with PD on prior EGFR-TKI. Genomic and immune-profiling is feasible in EGFR-mutant NSCLC and may identify EGFR-mutant NSCLC patients who may respond or lack benefit to PD-1 blockade. **Keywords:** immune microenvironment, EGFR-mutant NSCLC, Immunotherapy

P1.04-10
The Importance of Suppressor and Cytotoxic T Lymphocyte Subsets and Cytotoxic Mechanisms in Non-Small Cell Lung Cancer

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Background: Lung cancer represents the second most frequent cause of death worldwide, and most common subtype of lung cancer is non-small cell lung cancer (NSCLC). Recent trials showed that deficiencies in

anti-tumor immunity play a major role in carcinogenesis of NSCLC. $CD8^+CD28^-$ cytotoxic T (Tc) cells and $CD4^+CD25^+FoxP3^+$ regulatory T (Treg) cells known as suppresor CD4+ T cells, have been shown to exist in tumor tissues and inhibits the anti-tumoral immune responses. Natural killer (NK) cells are cytokine producing innate lymphoid cells having cytotoxic capacity to kill tumor cells. In this study the prevalence of Treg cells and CD28 expressing Tc cells and cytotoxic functions were analyzed. **Method:** The study groups comprised patients with newly-diagnosed non-small cell lung cancer (NSCLC) (n=46) with T1-3N0M0 NSCLC, none of whom had received preoperative chemotherapy and/or radiotherapy, and healthy subjects (n=46). The prevalence of $CD3^-CD16^+CD56^+$ NK cells, $CD4^+CD25^+Foxp3^+$ regulatory (Treg) and $CD8^+CD28^-$ T cells were analyzed by flow cytometry. Cytotoxic capacity of NK and $CD8^+$ T cells were analyzed by CD107a degranulation assay. **Result:** NK and $CD8^+CD28^-$ suppressor T cells were increased however percentage of activator $CD8^+CD28^+$T lymphocytes were significantly decreased in patients with NSCLC compared to healthy subjects (p=0.002, p=0.005, p=0.000, p=0.001, p=0.001, p=0.02 and p=0.02, respectively). Although the cytotoxic activity of NK cells did not differ between the groups, CD107a expression was found increased in total $CD8^+$ T cells in unstimulated and K562 stimulation (p=0.001). Although the number of Treg cells are decreased in the NSCLC group but this is not statistically significant. No statistically signficant difference was found in terms of lymphocyte subsets and NK cells between the patients with early(T1) and late stages(T2-3). **Conclusion:** Our findings showed that suppressor $CD8^+CD28^-$ T cell subset as well as NK cells were increased in patients with operable NSCLC. Increased $CD8^+CD28^-$ T cells might cause supression of antitumour immunity and their prevalence might be useful to assess immunotherapy outcomes in patients. Although the number of NK cells increased significantly, the activity of NK cells did not show difference. Functional evaluation of cells has been found to be more important than cell populations. **Keywords:** non-small cell lung cancer, anti-tumor immunity, Treg cells

P1.04-11
Exploring the Germ-Line Contribution to Exceptional Response to PD-1/PD-L1 Inhibition in Patients with NSCLC by Whole Genome Sequencing

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Background: Responses to immune checkpoint inhibitors (CPI) may vary between individuals because of somatic mutation differences in the tumour and/or germ-line differences in immunological tolerance. To explore the latter, this ongoing study evaluates patients with metastatic non-small cell lung cancer (NSCLC) treated with single agent PD-1 or PD-L1 inhibitors recruited from a treatment pool of 420 patients (total) / 137 (active since 1 August 2017). **Method:** Rare and common germ-line DNA variants are analysed in exceptional responders and non-responders by whole genome sequencing (WGS) (Illumina HiSeqX Ten). Exceptional responders are defined as patients with complete or partial response of more than 12 months or stable disease of more than 24 months (per RECIST), and a concurrent immune-related adverse event of any grade. Non-responders are defined as patients with best response of progressive disease, having received at least 4 cycles or 2 months of treatment. In these individuals, the burden of rare and common variants in immune tolerance genes is analysed and compared to the Medical Genome Reference Bank (MGRB), comprising WGS of 1144 well-elderly individuals. Comparisons are made with Fisher Exact test. Genetic risk scores for auto-immune conditions are calculated for these cohorts, MGRB and NSCLC patients included in The Cancer Genome Atlas. Scores are calculated using curated risk alleles and OR weightings derived from the NHGRI-EBI GWAS catalogue. **Result:** Recurrent rare variants (Exome Aggregation Consortium (ExAC) frequency $< 1\%$) were found within responders sequenced to date (n=20), including variant A, a frameshift mutation in a protein kinase not present in ExAC, with allelic frequency (AF) of 1.27% in MGRB and 17.5% of our cohort ($p<0.0001$). Multiple common variants (ExAC $\geq 1\%$) were more frequent within the cohort compared with population standard. Among these, three functional variants within gene B, encoding a protein involved in modulating immune-responsiveness, (variant B.1, B.2 and B.3, ExAC AF: 1.3%, 0.99% and 2.3%), were found seven times (total) across six individuals (one compound heterozygous B.2/B.3). The exceptional responders cohort was enriched for subjects with higher genetic risk for Disease A, psoriasis and psoriatic arthritis compared with control groups. **Conclusion:** Preliminary findings suggest individuals harbouring functional variants in genes promoting immune tolerance may be more responsive to PD-1/PD-L1 inhibitors. This may be due to higher basal immune activation, requiring greater reliance on inhibitory checkpoints to maintain homeostasis. Ordinarily, this would be clinically undetectable, however the addition of a pharmacological CPI may more effectively break immune tolerance in this primed environment. **Keywords:** Immunogenomics, Immunotherapy, whole genome sequencing

P1.04-12
Mass Spectrometry-Based Serum Proteomic Signature as a Potential Biomarker for Survival in NSCLC Patients with Immunotherapy

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Background: The Veristrat test is a serum biomarker using a mass spectrometry (MS)-based proteomic signature derived from machine learning. It is used as a prognostic marker for patients with NSCLC undergoing platinum-based chemotherapy. However, its role in patients undergoing immunotherapy has not been investigated. **Method:** 47 patients with advanced stage NSCLC and no activating EGFR mutation underwent VeriStrat testing from 2016 to 2017. Patients were grouped into Veristrat 'Good' risk group (VS-G) or Veristrat 'Indeterminate' and Veristrat 'Poor' risk group (VS-IP). Kaplan-Meier survival analyses with log rank test were performed to compare the progression-free survival (PFS) and overall survival (OS) between the two groups. PFS of NSCLC patients treated with immunotherapy was derived from the time of the immunotherapy to disease progression or death. **Result:** 47 patients had a mean age of 65.6 (range: 30 to 91). 26 patients were female (55%). 26 patients had diagnosis of adenocarcinoma (55%), while 18 patients had squamous cell carcinoma (38%). 32