If a gene is required for tumor cell proliferation, knocking down the gene by siRNA should decrease cell survival and proliferation. We looked at both viability and apoptosis by caspase 3/7 activation after siRNA knockdown. We selected those antigens for which: [(mean of viability in NSCLC cell line) / (mean of viability in the normal lung cell line)] < 0.75 with a p-value of 0.1. We identified 14 candidates that are overexpressed in lung cancer and necessary for tumor cell survival. We have prioritized those proteins that have been previously described to play a role in lung cancer invasion, proliferation, metastasis, or survival. We selected 5 candidates to move forward: FKBP3, PARP1, RAN, S100A6, and SART3. An effective anticancer immune response needs to elicit a strong inflammatory Th1 response and avoid a Th2 response that promotes tumor tolerance. We used web-based modeling to predict epitopes that preferentially elicit a Th1 response, and assessed the presence of Th1 and Th2 responses via IFN-g (Th1) and IL10 (Th2). Six to seven epitopes (15-20 mer peptides) per antigen were evaluated by IFN-g and IL10 ELISPOT. Th1 epitopes identified in NSCLC antigens are the base for a preventive vaccine for NSCLC. The efficacy of the multiantigen Th1 vaccine to prevent lung cancer is currently under evaluation in the NTCCI-induced lung cancer mouse model.

A35
Dendritic Cell in Situ Vaccination Potentiates Anti-PD-1 Efficacy and Induces Immunediting in a Murine Model of NSCLC

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Studies reveal that responses to checkpoint blockade in non-small cell lung cancer (NSCLC) are associated with high tumor mutational burden (TMB), preexisting CD8+ T-cell infiltration, and high baseline PD-L1 expression within the tumor microenvironment (TME). In contrast, co-occurring KRAS/LKB1 mutation is associated with primary resistance to PD-1 blockade and decreased overall survival. In preclinical studies as well as a phase I clinical trial, we have discovered that intratumoral (IT) vaccination with gene-modified dendritic cells expressing CCL21 (CCL21-DC) promotes tumor effector T-lymphocyte infiltration, PD-L1 upregulation, and systemic tumor-specific immune responses. We hypothesized that in situ vaccination with CCL21-DC could restore tumor antigen presentation and promote T-cell priming and activation, thereby sensitizing nonresponsive NSCLC tumors to checkpoint blockade. Although genetically engineered murine models (GEMMs) of NSCLC bear driver mutations of the disease, recent studies reveal that these GEMMs possess low mutational burden. We established novel GEMMs of NSCLC [KrasG12D (K), KrasG12D/P53+/- (KP), KrasG12D/P53+/-Lkb1+/- (KPL)] bearing common driver mutations and varying mutational loads by in vitro exposure of tumor cell lines to the carcinogen N-methyl-N-nitrosourea (MNNU). Our preclinical KPL model with high TMB recapitulates the immunologic phenotype of human disease, and contains a predominance of myeloid-derived suppressor cells (MDSC), low-tumor-infiltrating lymphocytes (TILs), and low PD-L1 expression within the TME. As anticipated, the KPL tumors are resistant to anti-PD-1 therapy, even with increased mutational load. We evaluated IT CCL21-DC combined with anti-PD-1 therapy in immunocompetent mice bearing KPL tumors with high TMB, and observed that IT CCL21-DC vaccination induces infiltration of autologous T lymphocytes and conventional type 1 DCs (cDC1s) into the TME and sensitizes the tumors to anti-PD-1 therapy. Combination therapy also reprogrammed the myeloid compartment, resulting in a significant reduction of MDSCs and a concurrent increase in CD11b+Ly6G+Ly6C+ monocyte/myeloid population. Whole-exome sequencing (WES) of tumors revealed immunediting and selective depletion of tumor subclones post IT CCL21-DC and anti-PD1 combination therapy. Future studies will evaluate the evolution of the T-cell receptor (TCR) repertoire in response to the combination treatment and define functional responses to neoepitopes. These studies will enhance our understanding of the molecular mechanisms of tumor vaccination and facilitate the development of rational combination strategies.

A36
Patient-Specific Humanized PDX Model for Overcoming Tumor Resistance to Immune Checkpoint Inhibitors in NSCLC Patients

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Background: Lung cancer is the most common cause of cancer-related mortality worldwide. Over the past few years, immune checkpoint inhibitors (ICI) have been shown to provide unprecedented clinical success in non-small cell lung cancer (NSCLC). However, ICI have some drawbacks, including initial and acquired resistance, which was observed after a complete response during and after previous ICI treatment. This relapse phenomenon was suggested to be associated with the state of the immune system and the tumor-immune response microenvironment interaction. The critical observation of cancer resistance or progression under ICI treatment suggests that a better and deeper understanding of the dynamic responses between the antitumor immune system and the tumor interaction, as it accrues in the patient setting, is therefore of utmost importance. Methods: Using a patient-specific humanized patient-derived xenograft (PDX) (huMicX) model, we will study the coevolution between tumor and the immune system with and without ICI intervention. Comprehensive OMICS analysis on the proteomic, transcriptomic, and genomic levels will be performed on samples collected from human patients and the huMicX model. Results: Sample biobank of whole blood and tumor tissues, and consensus protocols for peripheral HSC CD34+ isolation, are being established from NSCLC patients. Tumor tissue samples have been used to generate a PDX in mice model. Data from PDX models have demonstrated the feasibility of testing the activity of autologous transplanted lymphocytes against the patient’s tumor in vivo with a clinical benefit in the same patient overcoming ICI resistance. Conclusion: The huMicX model is designed to provide vital knowledge of the patient-specific tumor and immune system microenvironment, and the dynamic assessment of the mechanisms of ICI tumor resistance. This preclinical model is expected to present both treatment intervention and prognostic or predictable biomarkers, which will be exploited subsequently in actual clinical settings.

A37
N-803 Plus Nivolumab for Advanced or Metastatic Non-Small Cell Lung Cancer: Update on Phase II Experience of Combination PD1 Blockade with an IL-15 Superagonist

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Immunotherapy has radically altered the treatment landscape of nonsmall cell lung cancer (NSCLC), yet the majority of patients treated with
A38

Gemcitabine Improves Suppressive Immune Microenvironment Induced by Long-Term Treatment with EGFR-TKIs: Implications for Combination Chemotherapy and Immunotherapy

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**Background:** For patients harboring epidermal growth factor receptor (EGFR)-sensitive mutations, the use of EGFR tyrosine kinase inhibitors (EGFR-TKIs) has brought admirable survival. However, patients with EGFR mutation cannot benefit from anti-PD-1/PD-L1 alone as second-line therapy, from the analysis of results of immunotherapy clinical trials. In fact, immunotherapy with programmed cell death 1 ligand and anti-programmed cell death 1 (PD-1/PD-L1) checkpoint inhibitors is less effective in patients who previously received targeted therapy. For poor response to immune checkpoint inhibitors, one mechanism is suppressive immune microenvironment. However, the results of clinical trials, chemotherapy combined with pembrolizumab/nivolumab, ipilimumab and atezolizumab, have mostly improved overall survival (OS) and progression-free survival (PFS) of non-small cell lung cancer (NSCLC) patients. The aims of this study were to determine whether gemcitabine or pemetrexed improves suppressive immune microenvironment induced by long-term treatment with EGFR-TKIs. **Methods:** We adopted long-term use of EGFR-TKI models to investigate the responses of immune microenvironment to gemcitabine and pemetrexed. We analyzed the serum levels of IL-1ß, IL-6, and IL-10 after chemotherapy. **Results:** In our investigation, a significantly higher percentage of myeloid-derived suppressor cells (MDSCs) was detected in long-term erlotinib-treated mice. Compared with the pemetrexed for the long-term use of EGFR-TKI models, the level of MDSCs was consistently reduced, CD8+ T cells, CD4+ T cells, and dendritic cells were elevated. Analysis of inflammatory factors in serum showed that gemcitabine decreased the levels of L-1ß, IL-6, and IL-10. **Conclusion:** These data suggested that gemcitabine could reverse MDSC-mediated immune suppression and modulate the tumor microenvironment, thereby improving the efficacy of immune-based therapies. The results indicated a combination therapy using chemotherapy and immunotherapy for patients with EGFR mutation or who acquired resistance to EGFR-TKIs. It was also suggested that the combination use of MDSC-scavenging drugs may enhance the efficacy of anti-PD-1 immunotherapy.

A39

Reactive Cutaneous Capillary Endothelial Proliferation Caused by Camrelizumab (SHR-1210) Through Activation of HIF-1α/VEGF Signaling Pathway

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**Background:** Monoclonal anti-programmed cell death 1 (PD-1) antibodies are effective cancer therapeutics, but camrelizumab (SHR-1210) caused reactive cutaneous capillary endothelial proliferation (RCCP) in patients. This symptom was not detected in the clinical trials of other PD-1/PD-L1 antibodies approved by the Food and Drug Administration (FDA). Therefore, it is of great significance to verify the phenomenon of camrelizumab (SHR-1210) to promote the proliferation of human blood vessels and to explore its possible mechanisms for the effective control of its side effects and the continuation of clinical trials. **Methods:** We chose the cells mainly involved in the blood vessels, umbilical vein endothelial cells (HUVEC), as experimental object. Cholecystokinin c-terminal octapeptide (CKK-8) assay was used to detect the proliferation of HUVEC cells. Transwell cell migration and invasion assay were used to detect the cell migration ability; apoptosis detection by terminal-deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay was used to detect the apoptosis; ELISA was used to detect the expression of VEGF and bFGF in the cell supernatants; Western blot test was used to detect HIF-1α, p44/42 (ERK1/2), p-p44/42 (p-ERK1/2), p38, p-p38, JNK, p-JNK, Akt, and p-Akt. **Results:** The CKK-8 test suggested that 150 µg/ml and 200 µg/ml camrelizumab (SHR-1210) compared to the control group showed a significant increase in cell proliferation. We chose 150 µg/ml as the working concentration for follow-up experiments. Transwell cell migration and invasion assay suggested that the number of cell migration increased significantly in the camrelizumab (SHR-1210) treated group. The results of apoptosis detection by terminal-deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay showed that there was no significant difference in the number of apoptotic cells and apoptosis index (AI) between the camrelizumab (SHR-1210) treated group and the control group. ELISA results showed that the concentration of VEGF in the supernatant of the camrelizumab (SHR-1210) treated group was significantly higher than that of the control group, but there was no significant difference in the concentration of bFGF. Western blot results indicated the expression of HIF-1α was significantly increased in the camrelizumab (SHR-1210) treated group, and the expression of p-p44/42 (p-ERK1/2) and p-p38 was significantly increased, while p-JNK and p-Akt were not significantly increased. **Conclusion:** Camrelizumab (SHR-1210) can promote proliferation and migration of HUVEC cells without inhibiting apoptosis. It can promote the expression of VEGF in HUVEC cells and promote the proliferation and migration of HUVEC cells through VEGF without promoting the expression of bFGF. By activating the HIF-1α/VEGF pathway and its upstream signal pathways ERK and p38MAPK,