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Evaluation of the Mutant KRAS-Driven NSCLC Tumor Immune Microenvironment Using Patient-Derived Cell Line Xenografts in a Humanized Mouse Preclinical Model for Development of New Immunotherapy Approaches

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Purpose of the Study: Tumor cell and tumor microenvironment (TME) features that influence the response to immune checkpoint blockade in lung cancer are incompletely defined. We wanted to develop preclinical models of lung cancer to explore the relationship of non-small cell lung cancer (NSCLC) molecular characteristics (oncogenotype such as mutant KRAS and LKB1/STK11, and mRNA expression profiles) to the human TME. Experimental Procedures: We exploited the use of 8 (all KRAS mutant) molecularly characterized (whole-exome seq mutation and RNAseq analyses) patient-derived NSCLC lines grown as xenografts in vivo in humanized NSG-SGM3 mice in an effort to determine the effect of different NSCLCs on the immune landscape of lung cancer xenografts. The triple transgenic NSG-SGM3 (NGS) mices express human IL3, GM-CSF, and SCF, which combine the features of the highly immunodeficient NOD scid gamma (NSG) mouse with cytokines that support the stable engraftment of human myeloid lineages and regulatory T-cell populations. Subcutaneous KRAS mutant-driven non-NSCLC xenografts grown in NSG-SGM3 mice "humanized" with CD34+ cord blood cells were subjected to immune landscape analysis through flow cytometry, multiplex immunohistochemistry, and cytokine analysis. The xenografts were also treated with various combinations of checkpoint inhibitors, radiation, and activation of the innate immune pathway with emricasan (pan caspase inhibitor). Summary of New Data: Our results show that the 8 KRAS mutant NSCLCs each were associated with a different spectrum of human TME; 3 of the 8 xenografts had only 0.1% of immune cell infiltrates while other xenografts had 20% immune cell infiltrates; that tumor mutation burden (TMB, absolute mutation calls 233 to 2,076) is not predictive of CD8+ T-cell infiltration in KRAS mutant-driven NSCLC xenografts. In NSCLC xenografts with high CD8+ T-cell infiltrate, the DBD+ cells in the TME were not activated, resulting in limited responses to PD-1/PD-L1 therapy. However, stimulation of the cGAS-STING innate immune pathway with emricasan followed by radiation (15 cGy) resulted in dramatic anti-tumor response. Conclusions: NSCLC xenografts grown in "humanized" mice show great intertumor heterogeneity effects on the TME even within the KRAS mutant subgroup, and it is possible to demonstrate targeted therapy such as emricasan/radiation can lead to changes in improved antitumor responses.

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Phase 1 Trial of In Situ Vaccination with Autologous CCL21-Modified Dendritic Cells (CCL21-DC) Combined with Pembrolizumab for Advanced NSCLC


Effective immunotherapy options are lacking for patients with advanced non-small cell lung cancer (NSCLC) who progress on a programmed cell death-1 ligand 1 (PD-L1) inhibitor and for those who are epidermal growth factor receptor (EGFR) mutation- or anaplastic lymphoma kinase (ALK) rearrangement-positive after progression on tyrosine kinase inhibitor (TKI) therapy. One potential approach to improve immune checkpoint efficacy in these patient populations is to promote tumor-specific T-cell activation via in situ vaccination with chemokine gene-modified functional antigen-presenting cells (APCs), which take advantage of the full repertoire of tumor antigens and convert the tumor into a lymph node-like environment. The chemokine C-C motif chemokine ligand 21 (CCL21) promotes colocalization of naive T cells and dendritic cells (DCs) to promote tumor antigen presentation and facilitate T-cell activation. Our preclinical studies and phase I trial of intratumoral (IT) administration of CCL21 gene-modified DC (CCL21-DC) revealed augmentation of tumor CD8+ T-cell infiltration and systemic antitumor immunity. However, increased PD-L1 expression was observed in some patient tumors, suggesting that tumor-mediated impairment of T-cell function may be forestalling a more robust antitumor response. Similarly, improved anti-PD-(L)1 efficacy may be possible with enhanced T-cell infiltration and augmented APC function following IT CCL21-DC. Therefore, we are conducting a phase I trial combining IT CCL21-DC with pembrolizumab in patients with advanced NSCLC with tumors accessible for IT injection, who are either (1) EGFR/ALK wild-type after progression on a PD-(L)1 inhibitor or (2) EGFR/ALK mutant after progression on TKI therapy. This is a phase I, single-institution, nonrandomized, dose-escalating, multicohort trial followed by dose expansion. A maximum of 24 patients (9-12 escalation + 12 expansion) with stage IV NSCLC will be evaluated. Three IT injections of autologous CCL21-DC (days 0, 21, 42) will be concurrently administered with pembrolizumab, followed by pembrolizumab once every 3 weeks for up to 1 year. Primary objective of dose escalation is safety and determination of maximum tolerated dose (MTD) of IT CCL21-DC when combined with pembrolizumab. Primary objective of dose expansion is objective response rate (ORR) of CCL21-DC at MTD combined with pembrolizumab. This trial, NCT03546361, is currently open for enrollment.

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Identification of Th1 Epitopes in Lung Non-Small Cell Lung Cancer Antigens to Develop a Multiantigen Vaccine

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Non-small cell lung cancer (NSCLC) represents 85% of all lung cancer cases and it is highly smoking related. The goal of this project is to develop a vaccine for lung cancer prevention in current or past smokers by identifying immunogenic proteins in lung cancer that are able to induce a potent inflammatory Th1 response. Lung cancer has one of the highest mutation rates of all types of cancer, but driver mutations that could be targeted for a vaccine for lung cancer prevention are unknown. Gene expression profiling of bronchial biopsy specimens from smokers has shown that changes in gene expression in histologically normal epithelia can discern people with and without lung cancer. Many of these changes are proteins aberrantly upregulated, but not mutated. We have used quantitative mass-spectroscopy analysis to identify proteins overexpressed in NSCLC cell lines compared with normal lung epithelial cells. Five NSCLC cell lines (three squamous cell carcinoma and two adenocarcinoma) and two normal lung epithelial cell lines were included in the analysis. A total of 14,219 peptides, corresponding to 2,875 proteins, were identified. We selected for further analysis those proteins identified with >95% confidence and at least 3 peptides per protein, and overexpressed in three or more NSCLC cell lines. We considered that a protein is overexpressed if [expression in the NSCLC cell line/ expression in the normal cell line] >1.5. A total of 154 antigens met our criteria. Candidate antigens were investigated by siRNA screening to identify those genes with a function in maintaining cell tumor growth.
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: FKBPF3, 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-γ (Th1) and IL10 (Th2). Six to seven epitopes (15-20 mer peptides) per antigen were evaluated by IFN-γ 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 NTCL-induced lung cancer mouse model.

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Dendritic Cell in Situ Vaccination Potentiates Anti-PD-1 Efficacy and Induces Immunoeediting 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), KRASG12DPS3+/− (KP), KRASG12DP53−/−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 (MDSCs), 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+Ly6ChiLy6G0 monocyte/myeloid population. Whole-exome sequencing (WES) of tumors revealed immunoeediting 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 neoeptopes. These studies will enhance our understanding of the molecular mechanisms of tumor vaccination and facilitate the development of rational combination strategies.

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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 assessing 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.

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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 non-small cell lung cancer (NSCLC), yet the majority of patients treated with