**IA13**

**Targeting Myeloid Cells that Define the Tumor Immune Microenvironment in NSCLC**


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The composition of the tumor immune microenvironment dictates responsiveness to cancer immunotherapies, though discrete biomarkers to predict responsiveness are yet to be defined. Much of the focus of the field of biomarker research has been on the spatial distribution and activation status of CD8 T cells when defining whether a tumor is “inflamed” and potentially responsive to immunotherapy. However, the majority of the leukocyte composition of most tumors, including non-small cell lung cancer (NSCLC), is of myeloid, not lymphoid, origin, and these cells appear to play a role in dictating therapeutic efficacy. Through single-cell analysis at the proteomic and transcriptomic level we can define the resident myeloid populations within the tumor, to determine the role these subsets play in developing an immunosuppressive tumor microenvironment, and identify potential therapeutic targets. Through mass cytometry our group has demonstrated selective depletion of resident alveolar macrophages, with enrichment of a distinct tumor-associated macrophage (TAM) population within the tumor. Single-cell RNA sequencing confirms the disparate transcriptome of these TAMs contrasted with resident lung macrophages, and direct concordance between a defined monocye-derived TAM and T regulatory cell enrichment, as well as T effector cell dysfunction. These inhibitory myeloid subsets present a potential therapeutic target to further potentiate current immunotherapy approaches. We have identified multiple chemokine and cytokine pathways that may be integral to recruitment and maintenance of this immunosuppressive milieu, and validated dependence on these pathways in preclinical studies. To investigate the role of two of these myeloid-recruitment pathways in vivo in humans, we have designed a neoadjuvant “window-of-opportunity” trial that will evaluate the synergy of PD-1 blockade with disruption of the CCR2/5 or interleukin-8 mediated myeloid recruitment and retention within early-stage NSCLC lesions.

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**IA14**

**Preclinical and Translational Approaches to Capturing Mechanisms of Immunotherapy Response and Resistance in NSCLC**

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Strategies incorporating immune checkpoint inhibition have achieved unprecedented successes and been rapidly incorporated into standard-of-care regimens for patients with locally advanced or metastatic non-small cell lung cancer. Unfortunately, high rates of primary or acquired therapeutic resistance limit their broader efficacy for patients or durability. Using preclinical models, we have studied response and resistance to both single-agent and combination checkpoint blockade strategies. Consistently we have observed that the initial therapeutic response is accompanied by an overall reprogramming of the cellular immune microenvironment, followed by the development of resistance. Upregulation of molecules such as CD38 on tumor cells and cells of the myeloid compartment orchestrate changes in the metabolic environment as well as the cellular landscape, each of which define important and targetable components of resistance. We have similarly built patient cohorts and interventional trials for patients who are treated with surgical resection to leverage the neoadjuvant treatment space for tissue-based examination of response and resistance. This approach allows us to monitor for clinical response or resistance to treatment, while obtaining appropriate tissues for deep, multiparameter profiling of the tumor, normal tissues, and circulating factors. These efforts include the ICON Project (Immungeneomic Profiling of Non-small cell lung cancer), which has profiled 150 patient tumors without neoadjuvant treatment or with neoadjuvant chemotherapy only and longitudinally followed patients for outcomes. Additionally, in the phase II NEOSTAR trial patients received neoadjuvant nivolumab (n=23) or nivolumab plus ipilimumab (n=21) before undergoing surgical resection, with tumors from both of the arms undergoing multiparameter profiling. These individual efforts and comparison of the tumor data between them allow us to understand the baseline immunogenomic profiles and heterogeneity of NSCLC, as well as the effects of standard chemotherapy or immunotherapy. Using these datasets, we can define subsets of patients likely to respond to therapy, while identifying types of responses, biomarkers, and potential mechanisms that define resistance that can be targeted by combination or sequential therapies.

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**IA15**

**Decoding Critical Targets of LKB1/STK11 in NSCLC**

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Inactivating mutations in the LKB1 (STK11) tumor suppressor are the third most frequent genetic alteration in non-small cell lung cancer (NSCLC). LKB1 encodes a serine/threonine kinase that directly phosphorylates and activates 14 members of the AMP-activated protein kinase family. The function of many of the AMPK-related kinases (AMPKRs) remains obscure, and which are most critical to the tumor-suppressive function of LKB1 remains unknown. Recently we have combined CRISPR and genetic analysis of the AMPK family in NSCLC cell lines and mouse models, revealing multiple surprises. First, despite an unwavering role in inhibiting mTOR progrowth signaling, loss of AMPK at initiation in Kras GEMMs results in a block in tumor progression, which we could connect to a loss of lysosome and metabolic adaptive capability. Moreover, we found a surprising critical role for the SIK subfamily. Conditional genetic loss of SIK1 revealed increased tumor growth in mouse models of Kras-dependent lung cancer, which was further enhanced by loss of the related kinase SIK3. As most known direct substrates of SIK1 and SIK3 control transcription, gene-expression analysis was performed, revealing specific transcriptional programs that contribute to LKB1-dependent tumorigenesis. Additional pathways by which one might therapeutically target these tumors based on the signaling and metabolic pathways dysregulated from LKB1-deficiency will be discussed.

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**IA16**

**A New Generation of Anti-Myc Mini-Proteins as Potential Therapy for NSCLC**

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MYC is one of the most wanted targets for therapeutic intervention in cancer, having a key role in driving and maintaining most, if not all, human tumors, including lung cancer. Despite this indisputable therapeutic opportunity, MYC has long been perceived as “undruggable” for its intrinsically disordered nature and fear of catastrophic side effects...
in normal tissues. Indeed, to date, there is still no MYC inhibitor in the clinic. We previously designed a dominant negative form of MYC called Omomyc and used its conditional transgenic expression to inhibit MYC function both in vitro and in vivo, demonstrating a potent therapeutic impact in various mouse models of cancer, including non-small cell lung cancer (NSCLC), causing only mild, well-tolerated, and reversible side effects. Importantly, we recently showed that the purified Omomyc mini-protein displays unexpected cell-penetrating properties and can be used by direct tissue delivery or systemic administration to target NSCLC harboring different oncogenic mutation profiles, indicating its potential for clinical development for the treatment of NSCLC patients. Clinical trials testing this use are due to begin in 2020.

IA17
Druggable Vulnerabilities in Therapy-Resistant Lung Cancers
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Oncogene-targeted therapies often drive therapeutic responses in patients with advanced lung cancers, but frequently these responses eventually give way to acquired resistance. Blocking acquired resistance is a substantial and open-ended challenge whose difficulty is underscored by the fact that resistance within individual patients is often polyclonal in nature, driven by diverse and co-occurring mechanisms. Here, I will discuss two broad approaches we are employing in the search for therapeutic strategies that delay or circumvent resistance evolution. In the first area, we have identified vulnerabilities present in tumors at minimal residual disease states. For example, we have identified a molecular pathway through which targeted therapies such as EGFR, ALK, and BRAF inhibitors trigger double-strand DNA breaks in the cancer cells that comprise the minimal residual disease state. These cells rely upon an ATM-dependent DNA repair process for their survival and are thus hypersensitive to ATM inhibition. As such, combining oncogene-targeted therapies with an ATM inhibitor leads to more penetrant and durable responses to these agents in vivo. In the second area, we have identified vulnerabilities that arise specifically in tumor cells that develop acquired resistance to oncogene-targeted therapies. Importantly, we have identified scenarios in which these “collateral sensitivities” are conserved across heterogeneous resistant clones with distinct resistance mechanisms, implying that targeting these mechanisms may simultaneously eradicate diverse clones. I will describe examples of mechanism-based collateral sensitivities we have uncovered in lung cancers, melanomas, and leukemias, then demonstrate that by targeting these mechanisms in the upfront setting, it is possible to construct combination therapies that select against resistance.

IA18
A New World for Lung Cancer Vaccines: Beyond Picking a Single Antigen for Everyone
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Immunotherapy was hypothesized as an effective approach for the treatment of lung cancer for decades. Until the last decade, this enthusiasm was met with the cold reality of clinical trials, showing no benefit in engaging the immune system to fight lung cancer. This string of disappointing clinical trials included several high-profile trials of vaccines targeting a single, specific antigen. While enthusiasm for this approach of vaccines targeting a specific antigen has been reinvigorated in the era of PD-1 and PD-L1 inhibitors (generally in combination with these agents), new approaches to vaccines are also emerging. These new approaches often use patient-specific factors, such as autologous antigen presenting cells or vaccines directed at antigens specific to the patient being treated.

IA19
Evaluating the Role of B Cells and Tertiary Lymphoid Structures in Lung Cancer Development and Progression
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Lung cancer is the leading cause of cancer death in both the United States and the world. Even with the best current treatments, the 5-year survival is only 15%. Immunotherapy has been impressively successful in multiple solid tumors, including non-small cell lung cancer (NSCLC), which, until recently, was always considered to be immune quiescent. Blockade of the inhibitory PD1:PDL1 pathway on CD8+ and CD4+ tumor-infiltrating lymphocytes (TILs) has revolutionized standard of care for NSCLC patients. Anti-PD1 can specifically target tumor cells without harming normal lung epithelial cells, which ultimately allows for fewer adverse events compared to standard chemotherapy or radiation. However, these approaches do not work in 80% of NSCLC patients; thus, a better understanding of the immune response prior to the development of cancer (heavy smokers and patients with chronic obstructive pulmonary disease [COPD]) compared to active disease (adenocarcinoma [LIDAJ] and squamous cell carcinoma [LUSC]) is necessary to develop new therapeutic approaches to enhance the antitumor immune response but also to assemble additional noninvasive, accurate screening methods for patients. The current immunotherapies for NSCLC patients do not consider or target B cells despite their predominance in the tumor microenvironment (TME) and key role in the adaptive immune response. Further, in NSCLC patients, current evidence suggests an antitumor role for B cells as they can generate tumor-specific antibodies, present antigens to CD4+ TILs, and are detected within tertiary lymphoid structures (TLS), which also correlate with better prognosis. TLS predominantly contain B cells, CD4+ T conventional cells, and CD14+ myeloid cells; however, unlike normal lymphoid tissues, i.e., lymph node or tonsil, TLS in cancer patients do not always have well-defined germinal centers (GCs). GCs are paramount for proper B-cell development and function. Thus, in order to successfully implement B-cell targeting into future immunotherapies, we must increase our understanding of B-cell function in TLS within premalignancy and overt cancer. We hypothesize that B cells help generate potent, long-term immune responses against lung tumor cells by educating CD4+ T cells in TLS and producing tumor-specific antibodies. Toward this hypothesis, we have evaluated B cells and TLS in premalignancy and overt cancer via single-cell RNA sequencing, advanced spectral cytometry (Cytek Aurora), and multispectral imaging (Vestra and Nanostring GeoMx platforms). These analyses have revealed key differences in B-cell infiltration and TLS formation as lung cancer develops and progresses. We have utilized our results to create an objective signature for TLS identification. Specifically, we have observed an increase in GC-like TLS as patients develop cancer. We have also begun to evaluate the ex vivo function of B cells in patient tumors via antigen presentation and antibody production assays. We have evidence for a differential function for B cells within the TME that correlates with activation status. Since B cells and TLS are great prognostic indicators in NSCLC patients, an improved objective measure of the different tiers of these structures and how they correlate with disease progression could offer new and viable (a) biomarkers to predict lung cancer progression, (b) targets for early immunotherapeutic intervention in COPD patients that might trigger better antitumor immunity as patients develop lung cancer, and (c) immunotherapeutic targets in patients with already established NSCLC.