A second topic I will discuss involves adaptive changes in immune cells in the tumor microenvironment which occur over time in response to PD1:PDL1 therapy. These changes, which include upregulation of alternate immune checkpoints, lead to eventual failure of PD1:PDL1 blockade. I will show that TIM3 is a targetable biomarker in this context.

Tobacco smoke toxicant and carcinogen biomarkers and lung cancer susceptibility in smokers

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Lung cancer is the leading cause of cancer death in the United States, with more than 158,000 deaths expected in 2015, approximately 90% of which will have been caused by cigarette smoking. Worldwide in 2012, there were 1,589,800 deaths from lung cancer. Cigarette smoking accounts for 80% of the worldwide lung cancer burden in males and at least 50% in females. Our approach to lung cancer prevention is based on an understanding of the carcinogens in tobacco smoke and their mechanisms of action. This leads to carcinogen and toxicant biomarkers that have the potential to identify susceptible individuals at a young age so they can be targeted for intensive smoking cessation therapy and surveillance. We have developed a panel of urinary tobacco smoke carcinogen and toxicant metabolites that can be used to quantify exposure in cigarette smokers. The panel consists of nicotine metabolites comprising nearly 90% of the nicotine dose (nicotine, cotinine, 3’-hydroxycotinine, total nicotine equivalents, total NNAL, and phenanthrene tetraol, but not the mercapturic acid metabolites of the volatile toxicants and carcinogens, is independently related to lung cancer risk among smokers in this study. These results demonstrate that these urinary metabolites are risk biomarkers as well as exposure biomarkers.

We have also used these biomarkers to investigate potential explanations for differing risks of lung cancer among ethnic groups. The Multiethnic Cohort Study demonstrated that, for the same number of cigarettes smoked, African Americans and Native Hawaiians have a higher risk for lung cancer than Whites while Latinos and Japanese Americans have a lower risk. We analyzed urine samples from 300-700 subjects per group for total nicotine equivalents, total NNAL, phenanthrene tetraol, 3’-hydroxyphenanthrene, and the mercapturic acids of acrolein, crotonaldehyde, and benzene. The results demonstrated that African Americans, although smoking fewer cigarettes per day than any of the other groups except Latinos, had significantly higher levels of total nicotine equivalents, total NNAL, phenanthrene tetraol, 3’-hydroxyphenanthrene, and the benzene metabolite S-phenylmercapturic acid compared to Whites while Japanese Americans had significantly lower levels of total nicotine equivalents, total NNAL, and 3-hydroxyphenanthrene than Whites. The relatively low levels of total nicotine equivalents in the urine of the Japanese American smokers was related to low activity polymorphisms in CYP2A6, the major enzyme responsible for nicotine metabolism. These results partially explain the differing susceptibilities to lung cancer among these ethnic groups, but further research is necessary to fully understand the relatively high risk of Native Hawaiians (which may be related to their acrolein levels) or the low risk of Latinos.
While we have shown that these urinary metabolites are risk biomarkers for lung cancer in these studies, they are not fully predictive. This is because these biomarkers are mainly measuring exposure but do not take into account the critical metabolic activation step leading to the formation of DNA adducts which cause the multiple mutations observed in lung cancer. Quantitation of DNA adducts is challenging because of their low levels, typically less than 1 adduct per $10^7$ normal bases, and the small amounts of DNA available for studies in living humans. To address this question, we are developing high resolution mass spectrometric methods for quantifying oral cell DNA adducts as a surrogate for DNA adduct formation in the lung. In one recent study, we found remarkably high levels of DNA adducts of tobacco-specific compounds in the oral mucosa cells of smokers compared to non-smokers. We are also able to quantify formaldehyde-DNA adducts in oral mucosa cells. As mass spectrometric methods for analysis of carcinogen-DNA adducts become increasingly more sensitive and specific, it appears likely that quantitation of a panel of tobacco carcinogen DNA adducts in smokers, leading to identification of susceptible individuals, may be feasible.

Targeting PD1 and PDL1 in lung cancer treatment: Where are we now?
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The therapy for advanced non-small cell lung cancer has improved dramatically in recent years. We have moved from an era of Cisplatin-based chemotherapy to the use of targeted therapy, immunotherapy, and their combinations. Of equal importance is the evolution of more personalized tissue-based therapy used to guide choice. This lecture will chronicle the 10+ year history of the development of biopsy-based treatments for lung cancer, evolving from the initial BATTLE trials to the Umbrella and Master Protocols of today. The dawn of immunotherapy will be discussed along with efforts designed to administer it in a more targeted way with the development of novel biomarker and combinations.

Beyond monotherapy: Integrating immunotherapy into current treatment regimens
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Immunotherapy strategies targeting immune checkpoints including PD-1, PDL-1, and CTLA-4, have garnered substantial enthusiasm after demonstrating clinical activity in a broad spectrum of tumor types. In metastatic non-small cell lung cancer (NSCLC) two randomized phase III trials have convincingly shown an overall survival benefit in patients receiving nivolumab in the second line setting. The first trial conducted in patients with squamous cell histology randomized 272 patients to receive nivolumab or docetaxel (1). The HR for OS was 0.59 (95% CI 0.44-0.79; p<0.001). The median survival times were 9.2 months for nivolumab and 6.0 months for docetaxel. In the second trial performed in 582 patients with nonsquamous cell histology nivolumab was again shown to be superior to docetaxel with an OS HR 0.73 (95% CI 0.59-0.88; p=0.002) and a median survival time of 12.2 months for nivolumab and 9.4 months for docetaxel (2). Two additional randomized trials comparing docetaxel to pembrolizumab (anti PD-L inhibitor) or atezolizumab (anti PDL-1 inhibitor) have completed accrual and are likely to favor the immunotherapy arm. The efficacy and mild, non-overlapping toxicity profile of immune checkpoint inhibitors make them an ideal partner to combine with systemic agents such as cytotoxic chemotheraphy, molecularly targeted agents and other immune therapies in an effort to further prolong survival for patients with advanced lung cancer.

Combinations with cytotoxic chemotherapy. The rationale for evaluating chemotherapy with immune checkpoint inhibitors include: 1) tumor cell death from conventional therapies releases neo-antigens into the microenvironment leading to immune activation, the recruitment of cytotoxic T cells and additional tumor cell death 2) several cytotoxic chemotherapy agents have been shown to promote “immunogenic cell death” and 3) systemic therapies can influence the immune micro-environment, i.e. gemcitabine can suppress negative immune regulators (3-5). In preclinical experiments, chemotherapy plus PD-1, PDL-1 or CTLA-4 inhibitors have demonstrated enhanced antitumor activity leading to their evaluation in patients. Several phase I clinical trials combining immune checkpoint inhibitors with a variety of platinum based doublet have shown the combination regimens are safe and tolerable (6-8). Objective response rates and progression free survival were favorable. As a result, numerous randomized phase III trials comparing platinum doublets with or without an immune checkpoint inhibitor are underway.

Combinations with molecularly targeted agents. The rationale for pursuing these dual regimens is also based on the release of neoantigens that occurs upon cell death with effective targeted therapy (3,4). In addition targeted therapies have been shown to remodel the immune microenvironment. For example EGFR TKIs can decrease PD-L1 expression and lead to tumor regression in EGRF driven animal models (9). One