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 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?**

**Roy S. Herbst** *Yale Cancer Center, New Haven, CT*

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**

**Karen L. Kelly** *University of California, Davis, Sacramento, CA*

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 chemotherapy, 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 microenvironment, 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 EGFR driven animal models [9].
phase I clinical trial of erlotinib and nivolumab in patients with EGFR mutated tumors has been completed (10). The regimen was feasible and well tolerated with promising antitumor activity. A randomized phase III trials of an EGFR-TKI versus an EGFR-TKI with an immune checkpoint inhibitor has been launched. Similar preclinical evidence supports the evaluations of ALK-TKIs plus an immune checkpoint inhibitor (8). Phase I testing has begun. Immunotherapy combinations. Optimizing the immune system to attack tumors will require exploiting its diverse components. Employing a dual immune approach with anti-PD-1 plus an anti-CTLA-4 agents has been successful in treating advanced melanoma. In lung cancer phase III trials of PD-1 and CTLA-4 inhibitors have been activated based on encouraging phase I data (12). Meanwhile, early phase trials evaluating immune checkpoint inhibitors with immune checkpoint agonists, cytokines, and vaccines are ongoing to determine the safest and most effective combinations.

Overall we are optimistic that combination regimens that can harness the immune system together with tumor directed therapies will lead to improved clinical benefit. Continued pursuit of optimal combinations will however require increased insight into the complex interactions between the tumor, the immune response and pharmacological interventions.

References


Targeting K-Ras cancers

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Activating mutations in K-Ras occur frequently in lung adenocarcinomas, and are mutually exclusive with other mutations that activate the MAPK pathway, such as EGFR and other receptor tyrosine kinases upstream, loss of neurofibromin, loss of Spred1 and activation of B-Raf. Neurofibromin and Spred1 form a functional complex that inactivates Ras by converting Ras.GTP to Ras.GDP. Neurofibromin has a GAP domain, and Spred1 is essential for directing neurofibromin to the plasma membrane. Binding of neurofibromin to Spred1 is regulated by phosphorylation, so that the level of neurofibromin that can inactivate Ras is a highly regulated process. While mutations in EGFR, K-Ras, neurofibromin, Spred1, B-Raf and related genes are likely initiating driver mutations, knock-down of K-Ras in cell lines often has little effect on cell survival. This is presumed to be because feedback loops become activated upon K-Ras ablation, keeping cells viable. In contrast, ablation of K-Ras in pancreatic cancer cells leads to more profound effects on survival, for reasons that will be discussed.

Cells transformed by KRAS display a stem-like phenotype. In contrast, cells transformed by H-Ras do not. This difference is not related to MAPK or PI3K kinase activation, but is due to KRAS 4B’s unique ability to bind calmodulin, and so to inhibit calmodulin-dependent kinase. Low CaM kinase activity initiates a set of transcriptional programs that confer stem-ness. Binding of K-Ras to calmodulin is prevented by phosphorylation of K-Ras on serine-181, by an unknown isoform of protein kinase C. Treatment of mice with agents that activate PKC disrupts KRAS binding to calmodulin and prevents initiation and growth of tumor cells in vivo. Part of the "stem-ness" program initiated by K-Ras involves secretion of the cytokine LIF, an IL-6 family member with a unique role in maintaining stem-ness. Neutralization of LIF with a monoclonal antibody, or ablation of LIF expression using siRNA or CRISPR, reduces stem-ness.