Deep sequencing of circulating tumor DNA for personalized lung cancer detection and radiotherapy response monitoring

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Circulating tumor DNA (ctDNA) represents a promising biomarker for sensitive, specific, and dynamic detection of disease burden in cancer patients. Mutations in tumor-derived DNA represent ideal potential biomarkers since they are highly specific to tumor cells and involved in disease pathogenesis. However, even in advanced cancer patients, concentrations of ctDNA are often very low and therefore difficult to detect. We have developed a novel, ultra-sensitive and specific method for detection of circulating tumor DNA called Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq). This method was developed specifically for detection of ctDNA in non-small cell lung cancer patients, although it is broadly applicable to other cancer types. In this presentation I will describe recent improvements to CAPP-Seq that further improve the sensitivity of the assay, with a focus on applications of ctDNA analysis in lung cancer patients treated with radiotherapy.

Circulating tumor cell eXplants (CDX) to advance small cell lung cancer (SCLC) research and drug development

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SCLC accounts for ~15% of all lung cancer cases and ~220,000 deaths annually worldwide. Although response rates to first line platinum-based chemotherapy are high (~80%), progression free survival in most patients is short (3-12 months) due to the development of chemotherapy resistance and progressive disease. In addition, approximately 20% of SCLC patients are classified as chemorefractory with progressive disease within 3 months of their initial treatment. The lack of durable chemotherapy responses contributes to the poor prognosis in SCLC; with a 5 year survival rate of ~5% (5,6). Platinum based chemotherapy regimens have remained the same as standard of care for SCLV for the past 30 years. Targeted therapies have yet to make impact on patient outcomes, although several ongoing clinical trials of small molecular and immunotherapies begin to show promise.

The majority of SCLC patients present with metastatic disease and < 5% patients are suitable for resection with curative intent. Biopsies of SCLC are generally challenging, often providing a low quantity and quality of specimen for research purposes: specimens are usually necrotic and exhibit crush artefact. Serial biopsy in SCLC is rare. Understanding the biology of drug resistant, advanced and progressive disease is hampered by limited clinical specimens to study. In SCLC, CTCs detected by the EpCAM dependent CellSearch platform are prevalent, CTC number is prognostic for overall and progression free survival, the dynamic range is sufficient to use CTC number as a pharmacodynamic biomarker and predictive CTC based biomarkers are feasible. Marker independent CTC enrichment platforms reveal the presence of EpCam negative SCLC CTCs. To address the paucity of SCLC biopsies for research, we recently developed Circulating Tumour Cell patient Derived eXplant models in immune-compromised mice (CDX, Hodgkinson et al, Nature Medicine, 2015).

I will describe our SCLC CDX panel derived from chemosensitive and chemorefractory patients at baseline and CDX matched pairs at baseline and progression. The CDX models tested to date mirror patient responses to standard of care chemotherapy. These models are being exploited to search for new druggable targets, to test targeted treatments and to interrogate treatment resistance mechanisms. As few as 10 SCLC CDX cells can recapitulate the CDX tumour suggesting a high frequency of SCLC ’stem-like’ cells. We also discovered that a subset of SCLC CTCs exhibit vasculogenic mimicry, a ’stem-like’ angiogenic switch whereby tumour cells form their own blood vessel walls. The clinical relevance of this biology is under investigation using CDX and xenograft models. We hypothesize that VM facilitates dissemination, but may also enhance drug delivery and alleviate hypoxia.

In summary, our SCLC CDX models of metastatic lung cancer offer new avenues to study biology, mechanisms of tumor cell dissemination.

Immunotherapy in small cell lung cancer

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Small cell lung cancer (SCLC) is a high-grade neuroendocrine malignancy that accounts for approximately 15% of all lung cancers in the United States. Patients with metastatic disease have a median survival of less than one year, and despite a large number of early phase
trials, standard therapies and outcomes for patients have changed very little over the past 30 years.

Immunotherapy represents a new treatment paradigm that may have clinical activity in SCLC. Multiple lines of evidence suggest that SCLC tumors may be immunogenic. First, a subset of SCLCs are associated with production of auto-antibodies or paraneoplastic syndromes, and production of auto-antibodies predicts a higher likelihood of complete response to first-line chemotherapy and prolonged survival (Graus et al., 1997). Second, higher ratios of circulating T effector cells to T regulatory cells have been observed in patients with earlier stage disease and correlate with prolonged response to therapy (Koyama et al., 2008). Third, although expression of programmed death ligand-1 (PD-L1) is low on SCLC tumor cells, PD-L1 expression is observed on tumor-infiltrating macrophages and is correlated with the presence of tumor-infiltrating lymphocytes (Schultheis et al., 2015).

Clinically, SCLCs are associated with a history of significant tobacco exposure. In patients with non-small cell lung cancer (NSCLC), response rates to inhibitors of the immune checkpoint programmed death 1 (PD-1) receptor are higher in current or former smokers compared to never or minimal smokers. This increased response rate is attributed to a higher mutational burden of these carcinogen-associated tumors, leading to expression of immunogenic neoantigens (Rizvi et al., 2015). Indeed, SCLCs are characterized by a high somatic mutation burden (George et al., 2015). This is likely largely attributable to the carcinogenic effects of tobacco exposure, though genomic instability may also be potentiated by the universal loss of the tumor suppressor gene Tp53.

On the basis of these preclinical observations, several clinical trials are now underway to assess role of immunotherapy in SCLC. Emerging data suggest that immune checkpoint inhibitors may have meaningful clinical activity in this disease. CheckMate 032 (NCT01928394) is a phase 1/2 trial of nivolumab either alone or in combination with ipilimumab in various tumors. Calvo et al. (ESMO, 2015) recently presented preliminary findings from this study, including an overall response rate of 12.7% with nivolumab and 31.1% with nivolumab plus ipilimumab. In a separate phase 1b study, Keynote-028 (NCT02054806), Ott et al. (ASCO, 2015) reported an overall response rate of 35% among PD-L1 positive SCLC patients treated with the PD-1 inhibitor pembrolizumab. Other inhibitors of PD-1, PD-L1 and CTLA-4 are also in clinical development. The durability of responses to immune checkpoint inhibitors is not yet well established, and close monitoring for signs/symptoms of paraneoplastic syndromes and autoimmunity is necessary. Current and future trials utilizing immune checkpoint inhibitors and other modulators of the immune response will further explore clinical activity, biomarkers, toxicities, and the role of combination strategies in SCLC.

Using stem cell biology to design precision medicine for non-small cell lung cancer

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Stem cell biology is integral to understanding the development and treatment responses of lung cancers. Our laboratories used ideas from stem cell biology to explore one of the first genetic mouse models of the lung squamous cell carcinoma, driven by conditional biallelic inactivation of Stk11 (Lkb1) and Pten in the murine lung. Cells that could serially transplant disease (i.e. cancer stem cells) expressed markers of basal cells, the upper airway stem cells, and highly expressed the immune evasion molecule PD-L1. This was in contrast to the lung adenocarcinoma stem cells from the Kras/p53 mouse model that rely upon other pathways and share characteristics of distal lung stem cells. Since anti-PD1 blockade has shown promise in treating lung squamous cell carcinomas, we are now exploring if anti-PD1 targets cancer stem cells, and if so, in what genotypes and contexts. We have also used ideas from stem cell biology to build a rationale for combining epigenetic therapies with common chemotherapies. It is often found that cancer stem cells are resistant to chemotherapy, and combination of chemotherapies with targeted therapies could improve treatment outcomes. Expression of the histone methyltransferase EZH2 in lung tumors is correlated with a poor survival of lung cancer patients, and is therefore an attractive targeted therapy candidate. Because EZH2 is highly co-expressed with the Topoisomerase II (Topoll) gene TOP2A in lung cancers, we examined whether EZH2 inhibition synergized with the common chemotherapy etoposide, which targets Topoll. We found that lung cancers with activating mutations in EGFR, or with inactivating mutations in the BAF complex member BRG1, were both sensitized to etoposide by EZH2 inhibition. We are now exploring the mechanism through which BRG1 loss of function lung cancers, which represent up to 40% of lung adenocarcinomas, are specifically sensitized to etoposide by EZH2 inhibition.