An integrative cross-tumors approach identifies FOSL1 as an oncogene dependency in KRAS-driven lung cancer

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The KRAS oncogene represents a clinically-relevant target in human cancers refractory to current therapies. Thus, the identification of molecules mediating oncogenic KRAS effects may help implement novel therapeutic strategies. Here we describe a new approach integrating a cross-tumors gene-expression screen and patient survival information to unveil KRAS dependencies in human tumors. This strategy uncovered the transcription factor FOS-like antigen 1 (FOSL1) as a critical mediator of KRAS-driven lung tumors. FOSL1 was up-regulated in mouse and human mutant KRAS cells and its high expression was a marker of poor survival in patients harboring KRAS mutations. Additionally, FOSL1 loss led to impaired cell viability in mutant KRAS cancer cells in vitro and in vivo and in a genetically-engineered mouse model of Kras mutated lung adenocarcinoma. Mechanistically, this effect involved the transcriptional down-regulation of genes involved in mitosis, a pathway previously postulated to act orthogonally to KRAS signaling, whose high expression was associated with shortened survival of mutant KRAS patients. Lastly, pharmacological inhibition of FOSL1 downstream targets involved in mitosis progression had a preferential deleterious impact on mutant KRAS tumors than wild type. Collectively, our findings identify FOSL1 as a critical factor in KRAS-driven tumors, thereby implicating FOSL1 and downstream targets as potential candidates for therapeutic intervention.

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Intelligent forceps for solitary pulmonary nodule diagnostics*

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Recently, SPNs have become more frequently encountered in pulmonary medicine. Therefore, an efficient and reliable method for detecting SPNs based on their morphological characteristics is needed.

We have validated the efficacy of near infrared (NIR) spectroscopy based catheter connected to biopsy forceps for solitary pulmonary nodule (SPN) diagnostics.

Methods: Between May 2014 and May 2015 we examined 20 male and 18 female patients having a median age of 62 years with positron emission tomography-computed tomography findings of metabolically active SPN between 1, 5 to 3 cm in diameter.

Fluoroscopic guidance was combined with a radial EBUS (without guide-sheath). In the case radial EBUS
conclusively showed catheter position in the centre of SPN (26 cases) NIR spectroscopy probe based forceps were placed at the same place in order to gather tissue information. Mean measurement time was less than one minute after establishing ideal position. Results of spectroscopy measurements from NIR spectroscopy were obtained as differences between spectral characteristics of normal tissue (same side, different lobe) to SPN tissue.

**Results:** The results are expressed as sensitivity of NIR spectroscopy towards EBUS navigated biopsies. Statistical analysis of the results showed very high sensitivity for NIR spectroscopy in confirmation of SPN tissue. From 26 EBUS positive visualisations of SPN there were 26 correct discriminations of SPN tissue, leading to 23 conclusive histological findings.

**Conclusions:** Every confirmatory method brings different information about tissue. EBUS describes volume of the SPN and gives valuable information about the position of catheter in the SPN. NIR spectroscopy brings information about biochemical/ optical characteristics of the tissues. Prototype of intelligent NIR based forceps showed good discriminative ability in the diagnosis of SPN.

*Due to unforeseen circumstance, this poster was not presented.*

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**Characterization of programmed cell death-1 ligand (PD-L1) expression in circulating tumor cells (CTCs) of lung cancer**

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**Introduction:** The programmed cell death-1/programmed cell death-1 ligand (PD-1/PD-L1) pathway plays a key role in tumor immune invasion. Emerging literature suggests that PD-L1 over-expression on solid tumor tissue, including lung cancer, is associated with PD-1/PD-L1 immunotherapy efficacy. Epic Sciences has developed circulating tumor cells (CTCs) based PD-L1 assay, and we sought to evaluate the feasibility of detecting PD-L1 biomarker in lung cancer CTCs.

**Methods:** 74 samples from newly diagnosed lung cancer patients prior to therapy were recruited and blood specimens were collected and shipped to Epic Sciences. All nucleated cells were plated onto glass slides and subjected to immunofluorescence (IF) staining and CTC identification by fluorescent scanners and algorithmic analysis. CK(+) CTCs(CK+ CD45- w/intact DAPI nuclei and morphologically distinct), apoptotic CTCs (CK+, CD45-, non-intact nuclei), CTC Clusters (two or more CTCs together) and CK(-) CTCs (CK-, CD45-, intact and distinct) were identified. Samples were characterized with PD-L1 IF to assess expression.

**Results:** Assays for the PD-L1 protein were developed and specificity confirmed utilizing Colo205 (negative control) and H820 (high PD-L1 expression) cells spiked into donor blood and run through the Epic Assay. Additionally, analysis of Colo205, A549, and SU-DHL-1 cell lines show increased differential expression when cells were exposed to Interferon gamma. PD-L1 protein expression was visualized in CTCs of 15/74 (20%) patients with lung cancer. PD-L1 expression was seen in CK(+) CTCs, CK(-) CTCs, apoptotic CTCs, and CTC Clusters.

**Conclusion:** PD-L1 protein assessment in CTC subpopulations from lung cancer patients at diagnosis is feasible on the Epic CTC platform. The test demonstrates analytical sensitivity and specificity and may aid in the identification of patients suitable for clinical trial studies with novel anti-PD-1 or anti-PD-L1 therapies. The assessment of PD-L1 expression from a circulating marker could enable longitudinal and pharmacodynamic analysis in response to PD-1 axis blockade in patients.