Characterization of ALK Fusions in Circulating Tumor Cells (CTCs) of NSCLC

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Background

As many as 40% of non-small cell lung cancer (NSCLC) adenocarcinoma patients have insufficient tumor samples or have high co-morbidities preventing access to tissue biopsies for FISH or IHC to identify an ALK fusion and subsequent eligibility for Crizotinib therapy. This unmet medical need could be mitigated by the development of a fluid biopsy that can characterize patient blood for the presence of ALK-driven NSCLC. We developed a geno/protein assay for the ALK fusion and molecularly characterized CTCs and CTC subpopulations in newly diagnosed NSCLC patients. In addition to traditional CTCs with epithelial morphology, CTC subpopulations that include apicoblastic, small, and cytokeratin (CK)-negative cells have been identified as CTCs in miRCPR (ASCQ GU 2014) via assessment of PTEN/ERG alterations. The Epic Platform enables the detection of NSCLC CTCs with unique morphology and epithelial plasticity. We set out to measure the frequency of CTCs and identify CTC subpopulations (traditional, small, or CK-) in NSCLC, and to further develop a geno/protein assay to assess ALK status of NSCLC patients with confirmed ALK+ or ALK- lung biopsies by FISH analysis.

Methods

Ten newly diagnosed NSCLC patients were recruited prior to therapy, and blood specimens were shipped to Epic Sciences. 2/10 had known ALK rearrangement through FISH analysis performed on tissue biopsies. All nucleated cells were plated onto glass slides and subjected to immunofluorescence (IF) staining and CTC identification by fluorescent scanners and algorithmic analysis. CTCs, defined as traditional (CK+, CD45, and intact DAPI nuclei) and apicoblastic (CK+, CD45, non-intact nuclei) and CK-(CK-, CD45-, distinct and intact nuclei) were identified. Samples were characterized with ALK IF to assess protein expression. Patients with known ALK rearrangements in tissue also had their CTCs assessed by Epic’s ALK assay.

Representative images of CTCs with various biomarker profiles detected in NSCLC patient blood.

NSCLC CTC Subpopulations Detected on Epic Platform

Clinical Feasibility

ALK+ CTCs were found in blood samples from 1 of 2 patients with confirmed ALK rearrangement (assessed via biopsy), which contained both CK+-ALK+ and CK-/ALK+ cells.

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ALK Protein & Gene Assay

Assays for ALK protein were developed and specificity was confirmed utilizing H2228 (ALK+) and A549 (ALK-) cells spiked into healthy donor blood.

Conclusions

Epic’s ALK fusion protein assay and ALK FISH analysis demonstrate both sensitivity and specificity, potentially enabling the identification of patients eligible for Crizotinib. The Epic Platform enables the detection of traditional, small and EMT (CK- and/or EpCAM) CTCs in NSCLC, allowing for the detection of ALK in CTC subpopulations with unique cell morphology and epithelial plasticity (i.e., lacking specific epithelial markers). Further clinical testing is ongoing to determine the clinical value of Epic’s ALK assay and the relevance of these CTC subpopulations to determining Crizotinib therapy and NSCLC progression. We are also establishing methods for detailed genomic analysis of ALK+ CTCs with various morphologies inclusive of both FISH and single-cell genomics.

These studies will further validate the clinical utility of a specific ALK protein assay using the Epic CTC platform to identify patients eligible for Crizotinib, especially in those that may be ineligible for biopsy due to co-morbidity or tumor inaccessibility.