**Background**

NSCLC patients commonly have insufficient tumor sample or high co-morbidities preventing access to tissue for molecular characterization. Molecular subtyping of NSCLC patients has identified molecular drivers of disease progression and enabled an era of precision medicine through the matching of driver mutations with targeted therapies. Currently, testing for EGFR (10-35%) and KRAS (15-25%) mutations and ALK (3-7%) rearrangements in NSCLC patients to determine differential sensitivity to TKIs has been incorporated into this standard of care. Recent demonstration of concordance in EGFR mutational status between cfDNA and tumor tissue in NSCLC patients highlights the utility of a liquid biopsy in a clinical setting. Additional candidate biomarkers, including CTC enumeration, CTC-pan/pan expression, detection of gene fusions (RET and ROS1) and amplifications (MET), may further enrich treatable patient populations for therapy sensitivity. We sought to develop a platform to enable diagnostically testing for clinically actionable NSCLC biomarkers from a single tube of blood utilizing a combination of protein and molecular CTC characterization with mutational analysis of cfDNA.

**Methods**

1. **Solid tissue (spleen)**
   - Trucut biopsy
   - Histology
   - Hematoxylin and eosin (H&E)
   - Immunohistochemistry
   - Detection of biomarker of interest

2. **Liquid tissue (blood)**
   - Blood drawn into 10mL BD vacutainer edTA tube
   - Detected, isolated and stored in BD biorepository

3. **Cell pellet**
   - Cells stained with CD45, DAPI, ALK IF
   - Cell pellet was isolated and resuspended in 200mL Tris-HCl buffer
   - Detection of ALK rearrangement

4. **Exo/ctDNA isolation**
   - Exo/ctDNA isolation (MagNaPure or Qiagen) and quantitative analysis
   - cfDNA amount determined by Qubit and real time PCR analysis

5. **DNA amplification**
   - DNA amplification by ddPCR
   - ddPCR analysis

6. **Bioinformatics**
   - Bioinformatics for ddPCR
   - Bioinformatics for CTC assay

**Table 1. Study Population**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Gene ID</th>
<th>Specific Mutation</th>
<th>Mutation Detection in Plasma</th>
<th>CTC</th>
<th>CTC-IF</th>
<th>Exo/ctDNA</th>
<th>ddPCR</th>
<th>cfDNA</th>
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<tbody>
<tr>
<td>2102</td>
<td>EGFR</td>
<td>AExon 19</td>
<td>Detected in ddPCR only</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td></td>
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<tr>
<td>2632</td>
<td>EGFR</td>
<td>AExon 19</td>
<td>Detected in ddPCR only</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>3147</td>
<td>EGFR</td>
<td>AExon 19</td>
<td>Detected in real time and ddPCR</td>
<td>16</td>
<td>0</td>
<td>0</td>
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<td></td>
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<tr>
<td>3786</td>
<td>EGFR</td>
<td>AExon 19</td>
<td>Detected in real time and ddPCR</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>3228</td>
<td>KRAS</td>
<td>Codon 61</td>
<td>G12C detected in real time or ddPCR (not tested for Codon 61)</td>
<td>16</td>
<td>0</td>
<td>4</td>
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<td>4198</td>
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<td>Detected in ddPCR only</td>
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<td>1</td>
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<tr>
<td>4098</td>
<td>KRAS</td>
<td>G12C</td>
<td>Detected in real time and ddPCR</td>
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<td>0</td>
<td>5</td>
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</tbody>
</table>

**Results**

- The combination of CTC and cfDNA liquid biopsy test confirmed the EGFR, KRAS and ALK mutational status in NSCLC patient samples.
- ddPCR demonstrated higher sensitivity vs. real-time PCR in cfDNA.
- EGFR mutational status was confirmed in 4/4 patients from cfDNA.
- KRAS mutational status was confirmed in 2/3 patients from cfDNA.
- Patients with higher levels of EGFr (cfDNA) also possessed higher frequency of CTC.
- ALK rearrangement confirmed by both IF and FISH from CTC.
- ALK and/or EGFR protein was observed in traditional, apoptotic, small and CK-CTCs.

**Conclusions**

Characterization of biomarkers is feasible utilizing a combination of protein and molecular endpoints starting from CTCs and cfDNA from a single tube of blood. These tests demonstrate sensitivity and specificity in patient selection for standard of care therapies or in the monitoring of residual disease following treatment. The identification of biomarker positive CTC subpopulations identifies unique tumor cell morphology and suggests evidence of epithelial plasticity. The use of both CTC and cfDNA analysis represent complementary approaches for evaluation of a larger, more robust biomarker panel at the protein and genomic level.