Clinical study design

- A study was performed to assess ctDNA mutations present in plasma from 20 non-small cell lung cancer (NSCLC) patients (Table 1).
- Plasma was taken from 18 treatment-naive patients, and from 2 patients post-therapy.
- For 10 patients a second blood sample was collected 21 days after starting chemotherapy to assess change in their mutational profile.

ctDNA has numerous potential clinical applications (Figure 2) including:

- Minimally invasive detection of cancer mutations
- Molecular stratification of patients for treatment
- Monitoring response to treatment
- Identifying the emergence of resistance to therapy

Table 1: Summary of patient characteristics

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Subtype</th>
<th>Number of patients</th>
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<tbody>
<tr>
<td>Age</td>
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<tr>
<td>Gender</td>
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<td>Race</td>
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<td>Histology</td>
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<td>Smoking history</td>
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<tr>
<td>Performance status</td>
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<tr>
<td>EGFR mutation</td>
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</tbody>
</table>

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Figure 3. (A) An overview of the TAm-Seq ctDNA assay which includes (1) designing primer pairs targeting hotspot DNA regions; (2) amplifying hotspot regions in plasma DNA using our optimized TAm-Seq method; (3) sequencing amplicons from both the tumor biopsy and plasma DNA using enhanced TAm-Seq; (4) bioinformatic data analysis of the sequencing reads to identify mutations in the plasma DNA.

Table 2 shows the mutations identified in plasma and tissue samples, demonstrating that mutations can be reliably detected in ctDNA using TAm-Seq.

- Only 40% of tumour biopsies from this study provided sufficient sample for analysis, reinforcing the importance of an assay which is not reliant on tumour biopsy tissue.
- ctDNA analysed by TAm-Seq showed a high concordance with tumour biopsy (where available).

- No new mutations were detected. For some patients identical mutations were detected at Day 21, whereas for other patients no mutations were detected.
- 100% of the patients that showed partial response in the CT scan either had a lower frequency of mutations, or not detectable (ND) levels of mutations at Day 21.

Table 2. Mutations identified in plasma by TAm-Seq from 18 treatment-naive and 2 post-treatment NSCLC patients, compared to mutations identified in tissue biopsies. Mutations are either in the light blue region (above 1% in plasma), pink region (between 0.001% - 1% in plasma), or grey region (ND). The stars represent a tumour specific mutation.

Table 3. Table showing correlation of ctDNA data with clinical response data, measured by TAm-Seq.

- ctDNA changes in response to treatment

In this study, ctDNA has been used to monitor response to treatment, and showed good correlation to clinical response data. 50% of mutations detected in patient plasma by enhanced TAm-Seq were observed at a frequency lower than 1%, reinforcing the importance of using a high sensitivity assay for ctDNA analysis.

This data warrants additional larger studies, which are underway to further evaluate and validate use of ctDNA analysis and TAm-Seq in patient stratification and monitoring.

5. Conclusions

- ctDNA can be used as a 'liquid biopsy' for molecular profiling of mutations in NSCLC patients in the absence of an invasive biopsy.
- ctDNA analysed by enhanced TAm-Seq showed a high concordance (90%) with tumour biopsies.
- TAm-Seq is a flexible and sensitive assay which enables quantification of tumour-specific mutations in plasma.

6. Conclusions

- DNA released from tumour cells into the bloodstream is known as circulating tumour DNA (ctDNA). It can be distinguished from other cell-free DNA of non-cancerous origin by the presence of cancer-specific mutations (Figure 1).
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- Mutations were identified using our robust bioinformatic analyses, and compared with NGS and Sanger sequencing data from tissue biopsy.
- We developed enhanced TAm-Seq that allows for reduced background levels and increased sensitivity for identification of mutations in DNA from plasma samples (Smalley et al., EACR Cancer Genomics 2015). We used a TAm-Seq amplicon panel covering hotspots and entire coding regions in 35 genes (Figure 3). In selected genes, entire coding regions are covered by tiles with a series of short overlapping amplicons.

4. Molecular profiles detected in ctDNA

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Unlike single hotspot assays, TAm-Seq allows for simultaneous analysis of multiple genomic regions, including the presence of potentially actionable mutations.

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