Serial changes in whole-genome cell-free DNA (cfDNA) identify disease progression prior to imaging in advanced NSCLC

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Highlights
- We investigated whole-genome analysis on cfDNA from serial blood samples in 35 prospectively enrolled patients receiving treatment for advanced NSCLC.
- Molecular progression by cfDNA was strongly predictive of disease progression at first follow-up and shorter progression-free survival.
- Confirmed predictions of progression were based on blood samples taken a median of 5 weeks before imaging and clinical evaluation.
- Major molecular response was observed in a subset of cases and potentially provides early evidence of treatment efficacy.

Objective
Patients treated for advanced cancer face considerable uncertainty in real time regarding the effectiveness of systemic therapies while incurring a serious burden of cumulative toxicity and out-of-pocket expenses. Today, imaging (CT, PET/CT, MRI), the standard for response assessment, typically requires 2-4 months or longer on therapy before confident conclusions can be made.

Assay Workflow

- Obtain serial blood draws
- Extract cfDNA from plasma and sequence
- Track longitudinal changes to predict progression

With Lexent cfDNA assay

- Progression
- No Progression

Longitudinal Cohort
We prospectively enrolled and serially collected blood from 35 patients with advanced NSCLC, each receiving a new treatment. Blood was collected on a schedule before each cycle of treatment, and imaging was performed per standard practice.

Table 1. Patient characteristics; 2017-2019.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n=35</th>
<th>Median</th>
<th>IQR (25-75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td>Immunotherapy</td>
<td>No treatment</td>
</tr>
<tr>
<td>Treatment start (Days)</td>
<td>126 (42-469)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment end (Days)</td>
<td>195 (119-246)</td>
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<td></td>
</tr>
<tr>
<td>Last follow-up (Days)</td>
<td>62 (29-147)</td>
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Figure 1. Potential use of cfDNA for response monitoring.

Based on the theory that radiographic progression is preceded by changes in tumor biology that are detectable in peripheral blood, what we are calling “molecular progression”, we have developed a novel approach to quantitatively track changes in circulating tumor DNA (ctDNA) to monitor response to treatment. Several distinctive features of cancer can be detected in ctDNA from plasma [1-4], which has led to the development of multiple diagnostic applications.

Figure 2. Sample timing. T1 blood sample was collected before the second cycle of treatment, and T2 was collected before the third cycle.

We have performed per standard practice.

Figure 3. Example workflow showing extraction of cfDNA and sequencing.

Figure 4. Patient characteristics; 2017-2019.

Figure 5. PFS for cfDNA-based molecular progression for (A) all patients (n=35), and (B) patients on immunotherapy (n=22).

Figure 6. PFS for patients with molecular progression (n=14), MMR (n=11), or neither (n=20).

Figure 4. (A,B) Tumor fraction ratio at each time point for patients assessed as PD or nonPD at first follow-up imaging. (B) Timing of molecular progression calls relative to imaging.

Conclusions
- Molecular response assessment by analyzing cfDNA holds promise to identify patients with disease progression faster than traditional methods.
- This technology may enable early switching to other potentially effective therapies, increasing the value proposition of all delivered treatment.
- Blood-based signals of molecular response have potential synergy with imaging to identify patients receiving the greatest therapeutic benefit.
- Further studies are ongoing to develop this assay for use in clinical practice.

Acknowledgements and References
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References