Sequencing of Plasma cfDNA from Patients with Locally Advanced Bladder Cancer for Surveillance and Therapeutic Efficacy Monitoring


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Abstract number: #5964

Introduction

Studies on different cancer types have shown that circulating tumor DNA (ctDNA) levels can be efficiently used to monitor treatment response and/or detect disease recurrence earlier than clinical and radiological detection.12

Bladder cancer mutations in plasma have been previously used to monitor response during treatment and identify early signs of metastatic disease.3 4

Recently, longitudinal ctDNA detection in patients with non-small cell lung cancer was described and a personalized ctDNA detection assay was developed and made available for research use only (Signatera™ RUO).5

Objectives

The aim of the study was to use patient-specific mutational identified in the primary tumor to detect metastatic relapses, evaluate prognostics, and monitor treatment response in real-time using cfDNA from longitudinally collected plasma samples.

Methods

Clinical Protocol

Patients diagnosed with locally advanced muscle invasive bladder cancer (MIBC) were prospectively recruited between 2013 and 2017.

All patients were treated with neoadjuvant or the first-line chemotherapy before radical cystectomy (CX) and had up to 2 years follow-up (Figure 1). A small subset of patients who volunteered for additional follow up visits were monitored for a longer period.

Plasma samples were longitudinally collected before, during and after therapy and analyzed by mutation-based ctDNA quantification.

Molecular Protocol (Signatera RUO)

The workflow of the molecular protocol is outlined in Figure 2.

Patient-specific somatic mutation were identified whole exome sequencing (WES) of tumor and matched normal tissue.

Personalized multiplex PCR assays and NGS were used to detect patient-specific tumor DNA in plasma using ctDNA from longitudinally collected plasma samples.

For each patient, sequencing of 16 tumor-specific targets was performed and data were analyzed in a blinded fashion for the presence of ctDNA.

Samples were considered ctDNA positive if at least two patient-specific targets were called that met the call calling confidence score threshold.

Clinical results (radiographic imaging and treatment response) were unblinded and were directly compared with the plasma ctDNA results.

Results

A total of 68 patients were included in the study (Table 1).

Table 1. Patient Characteristics and Demographics

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age (years), median (range)</th>
<th>Time from CX to sample (days)</th>
<th>Months relative to CX</th>
<th>ctDNA status</th>
<th>ctDNA VAF (%)</th>
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<tr>
<td>4424</td>
<td>65 (43-83)</td>
<td>100</td>
<td>0</td>
<td>Single assay positive</td>
<td>0.1</td>
</tr>
<tr>
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<td>72 (56-86)</td>
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</table>

Figure 2. Schematic of Molecular Protocol

Figure 3. Summary of Individual ctDNA Analysis

Figure 4. Recurrence-Free Survival and ctDNA Status Before Chemotherapy and After Cystectomy (CX)

Prediction of Outcome

Figure 5. Early Relapse Detection

Response to Therapy

Figure 6. Treatment Responses

Conclusions

ctDNA analyses (eg, via Signatera) can help inform on treatment response and identify disease recurrences with an average lead time of 100 days compared to radiographic imaging.

Survival analyses identified significantly shorter relapse-free survival for patients with ctDNA both at diagnosis and after CX.

Ultimately, ctDNA analysis could be incorporated into routine follow-up for early detection of relapse and consequently earlier initiation of treatment.

The benefit in overall survival gained by ctDNA relapse detection should be assessed in randomized clinical trials.

References

3. PMID: 28412804
4. PMID: 28926162
5. PMID: 26054000
6. PMID: 25200235
7. PMID: 26024205
8. PMID: 26710589
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12. PMID: 28926162
13. PMID: 26054000
14. PMID: 25200235
15. PMID: 26710589
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EUROPEAN SOCIETY FOR MEDICAL ONCOLOGY 2016 Congress, Munich, Germany | October 18–21, 2016