Evaluation of a tumor informed MRD assay with contrived breast cancer samples

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Abstract

The non-invasive detection of circulating tumor DNA (ctDNA) from plasma has been shown to have clinical value for detection of minimal residual disease (MRD), emergence of resistance, and predicting treatment response. The sensitive detection of MRD following curative treatment allows for the identification of patients destined to recur. Higher assay sensitivities enable the longest lead times to clinical recurrence. Evaluating several contrived pan-cancer samples has shown that the NeoGenomics tumor-informed personalized assay, RaDaR®, has a high sensitivity to detect ctDNA with an established LoD95 of 0.001% VAF. The tumor-informed analysis utilizes sequencing of the tumor tissue to identify up to 48 somatic variants, which are selected to generate a personalized panel to track the variants in patient plasma. To evaluate this technology in a disease specific manner, we generated a set of contrived plasma samples from breast cancer patients and healthy individuals. The biological materials allowed us to generate a plasma sample with defined range of tumor content between 0.2 and 0.001% variant allele fraction (VAF). The sample dilutions were intended to recapitulate challenging cell-free DNA inputs that can be encountered in a clinical setting (median input: 5.76 ng [range 0.22 to 103.73]). Through our pan-cancer and breast cancer evaluations, we were able to generate both sensitivity metrics along with a strict minimum of detection (LoD) study. The assay successfully detected tumor fractions in samples tested at 1X and 1.5X. RaDaR® was able to detect the majority of MRD+ samples at 0.5X LoD. We present data showing the level of sensitivity based on using both 2,000 and 20,000 genomic equivalents. The RaDaR® assay showed high sensitivity for pan-cancer as well as ER+ breast cancer, which is known to have a low TMB compared to other subtypes. In this small LoD study the NeoGenomics assay was highly sensitive for the detection of ctDNA which is imperative to confidently detect ctDNA in advance of overt clinical recurrence.

Methods

Pan-Cancer Matrix:
- Plasma samples were generated using the Matrix sample methodology.
- Healthy plasma was sequenced and verified to contain no incidental findings, e.g., cancer biomarkers.
- Plasma samples from individuals suffering from cancer were sequenced in parallel and biomarkers identified.
- Several variants were used to generate a mean VAF representing the tumor.
- Using our knowledge of the mean cancer plasma VAF, an intermediate mixture was generated.
- Cancer plasma was diluted into healthy plasma to achieve a mean VAF of 1% cancer variants.
- The intermediate was then sequenced and characterized to verify the VAF.
- Intermediates were serially diluted to generate samples with a range of expected VAF from 0.2% to 0.002%.
- These samples were then provided as plasma samples for testing.

Breast Cancer LOD:
- Three separate breast cancer samples were diluted and used to further test the NeoGenomics RaDaR® assay between 0.03% to 0.001% ctDNA VAF with two input ranges (2,000 and 20,000 human genome equivalents).

All tumor samples were acquired commercially, deidentified, and under the strict guidance of AstraZeneca's Human Biological Sample Guidelines.

Table 1: The NeoGenomics RaDaR® assay performed well detecting variants down to samples with mean VAF 0.02% confidence in all three samples.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Sampleid</th>
<th>VAF/Var GE</th>
<th>Target</th>
<th>NeoGenomics LOD 0.001% VAF</th>
<th>VAF</th>
<th>VAF LOD 0.001%</th>
<th>VAF LOD 0.001%</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Patient 1</td>
<td>0.004</td>
<td>WT</td>
<td>Positive</td>
<td>0.52</td>
<td>0.001%</td>
<td>0.001%</td>
</tr>
<tr>
<td>WT</td>
<td>Patient 2</td>
<td>0.003</td>
<td>WT</td>
<td>Positive</td>
<td>0.52</td>
<td>0.001%</td>
<td>0.001%</td>
</tr>
<tr>
<td>WT</td>
<td>Patient 3</td>
<td>0.002</td>
<td>WT</td>
<td>Positive</td>
<td>0.52</td>
<td>0.001%</td>
<td>0.001%</td>
</tr>
</tbody>
</table>

Table 2: During the dilution series, RaDaR® was consistently able to detect the samples down to our estimated 0.01% VAF and in some cases down to 0.003% VAF indicating high sensitivity. In this analysis between 2,000 and 20,000 amplifiable ctDNA haploids.

Table 3: Samples were diluted to be 0.5X, 1X or 1.5X the limit of detection for each patient based on number of variants in the panel and the level of input. The expected VAF (ppm) based on the dilution to achieve the given LoD level is shown with the measured VAF. The assay performed well at the expected LOD and succeeded in detection of ctDNA in most cases at 0.5X. Left: Breakdown of each sample and dilution. Right: summary of results.

Summary

Pan-Cancer Matrix
- The NeoGenomics RaDaR® Assay was able to identify ctDNA positive samples reliably with high sensitivity.
- NeoGenomics reliably processed samples.
- The RaDaR® assay was very sensitive and reliable.

Breast Matrix
- The NeoGenomics RaDaR® assay can return results on difficult samples.
- Even in a TMB-low disease such as breast cancer, RaDaR® was able to detect cancer with good performance.
- 3 samples were diluted to be 1.5X, 1X and 0.5X of the assay’s described LOD and were reliably detected. Even at 0.5X, most of samples were successfully detected as ctDNA positive.
- The NeoGenomics RaDaR® assay achieved a high level of ctDNA sensitivity and robustness.
- The assay showed high sensitivity with breast cancer - a cancer with low tumor burden.
- Using diluted samples, RaDaR® demonstrated a consistent ability to reliably identify ctDNA and performed well even at 1/3 of the established LOD.
- The tumor-informed approach is very sensitive.