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. Evaluations using several contrived pan-cancer samples have 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 small limit 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 albeit 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:

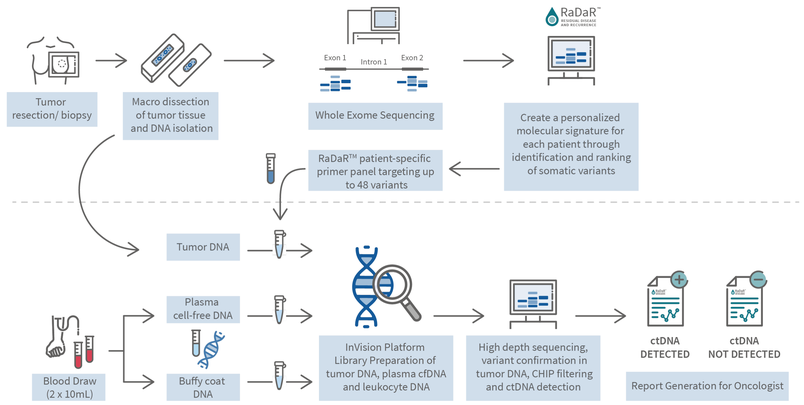
- Plasma dilutions were constructed using our Matrix sample methodology.
- Healthy plasma was sequenced and verified to contain no incidental findings, e.g. cancer biomarkers.
- Plasma samples from individuals suffering from cancer were sequenced in parallel and biomarkers identified.
- Several variants 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 VAFs 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 Samples guidelines.

RaDaR® Assay Methodology



Pan-Cancer Matrix

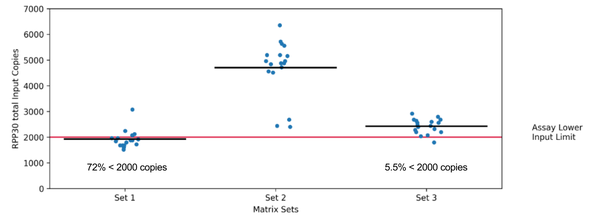


Figure 2: Genome equivalent copies of extracted DNA. Matrix samples are often on the low end of the clinical sample range due to the limitations of available plasma. This makes the samples both challenging and relatable to the difficulties of sample acquisition during clinical studies. Cell-free DNA was quantified using a ddPCR assay targeting the RPP30 gene.

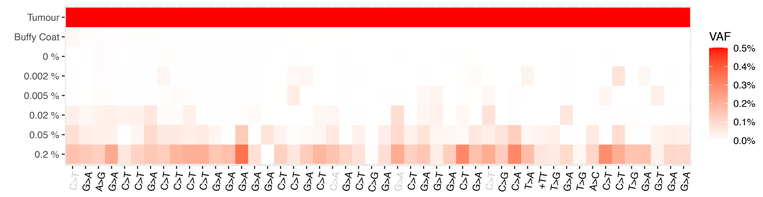


Figure 3: Initial variants chosen during the NeoGenomics RaDaR® methodology were compared to sequencing of the tumor, buffy coat, and the sample plasma. Variants were removed from consideration if present in the normal control (Buffy Coat). The figure illustrates variant detection for one set of matrix samples (squamous lung) from the normal control to the highest tested VAF.

expected VAF	Colorectal	Melanoma in Lung	Squamous Lung
0.2	Positive	Positive	Positive
0.2	Positive	Positive	Positive
0.2	Positive	Positive	Positive
0.05	Positive	Positive	Positive
0.05	Positive	Positive	Positive
0.05	Positive	Positive	Positive
0.02	Positive	Positive	Positive
0.02	Positive	Positive	Positive
0.02	Positive	Positive	Positive
0.005	Negative	Positive	Positive
0.005	Negative	Negative	Positive
0.005	Negative	Negative	Positive
0.002	Negative	Negative	Positive
0.002	Negative	Negative	Negative
0.002	Negative	Negative	Positive
WT	Negative	Negative	Negative
WT	Negative	Negative	Negative
WT	Negative	Negative	Negative

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

Breast Cancer LOD

ctDNA Positive
ctDNA Negative

Dilution series (%)	NeoGenomics (RaDaR) Patient id_13	Patient id_14	Patient id_15
0.03	0.058 % VAF 5000 Input GE 2.90 Var GE	0.034 % VAF 3800 Input GE 1.29 Var GE	0.054 % VAF 3640 Input GE 1.97 Var GE
0.03	0.025 % VAF 4840 Input GE 1.21 Var GE	0.026 % VAF 4880 Input GE 1.27 Var GE	0.042 % VAF 3760 Input GE 1.58 Var GE
0.01	0.017 % VAF 2880 Input GE 0.49 Var GE	0.013 % VAF 3520 Input GE 0.46 Var GE	0.019 % VAF 3600 Input GE 0.68 Var GE
0.01	0.039 % VAF 3600 Input GE 1.40 Var GE	0.006 % VAF 3240 Input GE 0.19 Var GE	0.028 % VAF 2800 Input GE 0.78 Var GE
0.003	2520 Input GE	2840 Input GE	0.006 % VAF 2440 Input GE 0.15 Var GE
0.003	2920 Input GE	0.007 % VAF 3560 Input GE 0.25 Var GE	0.013 % VAF 3080 Input GE 0.40 Var GE
0.001	3280 Input GE	3640 Input GE	2480 Input GE
0.001	3200 Input GE	2000 Input GE	2720 Input GE
0	2720 Input GE	2360 Input GE	2400 Input GE
0	2200 Input GE	2600 Input GE	2000 Input GE

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 5,000 amplifiable cfDNA haploid Genome equivalents (Input GE) were added to library preparation as measured by dPCR. The assay was therefore frequently detecting ctDNA when there was less than 1 variant containing genome equivalent (Var GE) present. Samples where ctDNA was detected are identified in green.

Patient ID	Input	Level	Expected ppm (VAF)	Measured ppm (VAF)	Replicates Detected	Replicates tested	Success Rate	Passing Variants	Average detected variants
lod_01	2000	0.5x LoD	111	130	3	5	60%	15	3.3
	2000	1.0x LoD	222	317	5	5	100%	15	4.8
	2000	1.5x LoD	333	382	5	5	100%	15	7.2
lod_01	20000	0.5x LoD	17.5	10	4	5	80%	15	2.8
	20000	1.0x LoD	35	13	5	5	100%	15	2.8
	20000	1.5x LoD	52.5	35	5	5	100%	15	4.4
lod_02	2000	0.5x LoD	77	55	4	5	80%	26	3.8
	2000	1.0x LoD	154	76	5	5	100%	26	4.8
	2000	1.5x LoD	231	168	5	5	100%	26	7.6
lod_03	2000	0.5x LoD	82	92	3	5	60%	24	4
	2000	1.0x LoD	164	67	5	5	100%	24	4.2
	2000	1.5x LoD	246	122	5	5	100%	24	6.2
lod_03	20000	0.5x LoD	12.5	5	3	5	60%	24	2
	20000	1.0x LoD	25	11	5	5	100%	24	3.2
	20000	1.5x LoD	37.5	14	4	5	80%	24	3.8
lod_04	2000	0.5x LoD	62	45	2	5	40%	34	2
	2000	1.0x LoD	124	100	5	5	100%	34	5.4
	2000	1.5x LoD	186	138	5	5	100%	34	8.8

2,000 Input Copies			
Level	Detected	Tested	Success
0.5X LoD	12	20	60%
1X LoD	20	20	100%
1.5X LoD	20	20	100%

20,000 Input Copies			
Level	Detected	Tested	Success
0.5X LoD	7	10	70%
1X LoD	10	10	100%
1.5X LoD	9	10	90%

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 give 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 and 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 ½ of the established LOD.
- The tumor-informed approach is very sensitive.

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