Analytical development of the RaDaR™ assay, a highly sensitive and specific assay for the monitoring of minimal residual disease

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RaDaR™ RESIDUAL DISEASE AND RECURRENCE

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INTRODUCTION

- 5-30% of patients with primary non-metastatic cancer relapse and die of metastatic disease, even though no macroscopic disease remains after initial curative-intent treatment.
- Adjuvant therapy is often administered to target minimal residual disease (MRD) without improving outcome for most patients.
- Current standard of care includes routine physical examinations and imaging, but these methods are frequently inconclusive and suffer from significant false-positive and false-negative results.
- Liquid biopsies can identify patients who have MRD without macroscopic disease in a minimally invasive fashion.
- Detecting MRD in advance of current clinical practice provides physicians with a time-window to adjust patient treatment: for example, directing patients with MRD to adjuvant therapy.

OBJECTIVE

- Describe the RaDaR™ assay, a highly sensitive and specific method for detection of MRD and recurrence in plasma cell-free DNA.
- Present a technical validation study on DNA from cancer cell-line material to assess specificity and sensitivity.
- Show application to multiple tumor types.

VALIDATION WORKFLOW

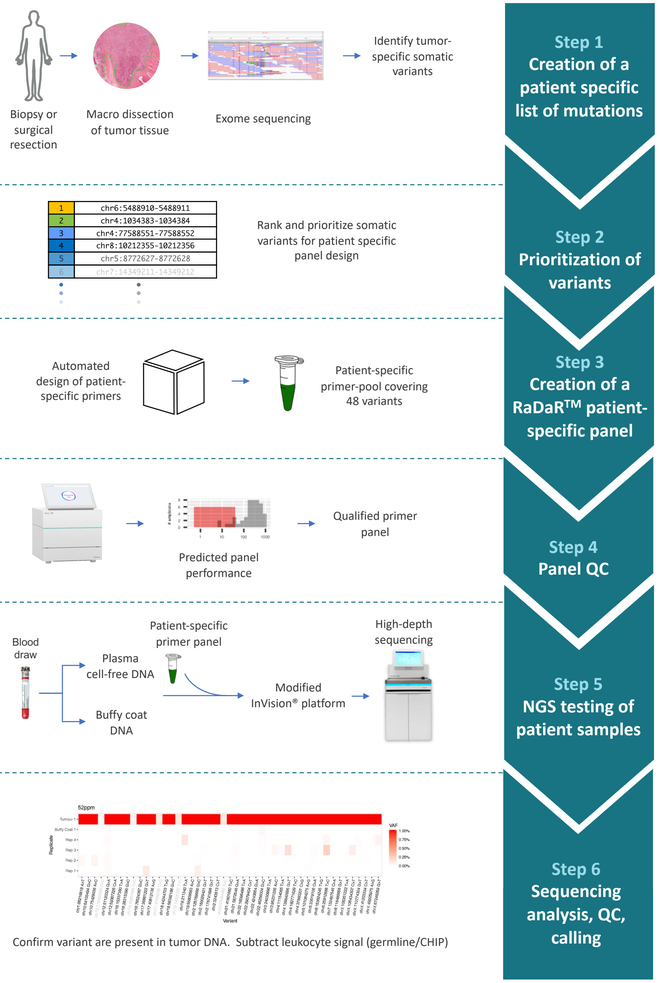
Materials

- Samples: 3 cancer cell lines (> 1,000 positive reactions); reference material (320 negative reactions); 7 FFPE samples (breast, colon, melanoma); 366 lung cancer samples (LUCID study, poster # 735).
- Cell-line dilutions: 5 dilution points (160, 80, 40, 20, 10 and 0 ppm, a variant allele frequency [VAF] range of 0% to 0.016%); 10 samples per dilution; 80 negative reference samples.
- Input: 20,000 and 4,000 copies.
- Primer Panels: customized panels designed against 48 cell-line or tumor specific variants plus 21 SNP amplicons.
- Workflow: multiplex PCR based on the InVision® platform.

Methods

- Sequencing: Illumina NovaSeq flow cell with read depth > 100,000 reads per locus. Processing with proprietary pipeline.
- Performance: RaDaR™ assay assessed using samples from cancer patients and cell-line dilutions.
- Statistical analysis: sample calling proprietary algorithm; sensitivity and specificity analysis; variants sub-subsetting performed by bootstrapping.

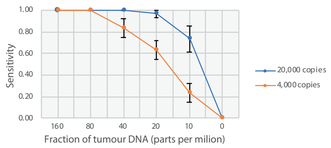
RaDaR™ ASSAY



RESULTS

- Using 48 variants: sensitivity of 97% (20,000 copies) and 63% (4,000 copies) at 20 ppm, with a specificity of 100%.
- Subset of 16 variants: sensitivity of 97% at 40 ppm, 75% at 20 ppm and 38% at 10 ppm, with specificity of 99.7%.
- RaDaR™ assay applied to early-stage NSCLC cohort from the LUCID study (poster # 735) shows a wide range of tumor detection (6-20,000 ppm).
- Assay tested on multiple different cancer samples (FFPE material from breast, colon, melanoma): tumor DNA was detected in plasma at concentrations as low as 78 ppm.

Sensitivity for high (20,000) and low (4,000) input copies



Concentration lookup	
ppm	VAF
160	0.016%
80	0.008%
40	0.004%
20	0.002%
10	0.001%

Figure 1: Average sensitivity for 3 cell lines at different dilutions (0 is reference DNA) for different input copies. Error bars: SEM. At 0 ppm, 0% sensitivity indicates no false positive calls. LoD_{90} ≤ 20 ppm for 20,000 copies; LoD_{90} ≤ 80 ppm for 4,000 copies. Lookup table conversion from ppm to VAF shown for clarity.

Heatmaps of samples from patients with early-stage NSCLC

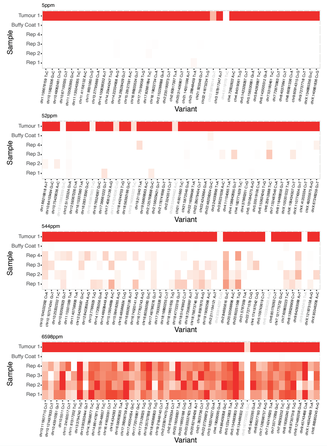


Figure 4: In the heat map examples, each column represents a different variant and each row a different sample type. Variants whose label is shaded in grey were excluded from analysis due to absence in tumor DNA or presence in buffy coat (e.g. CHIP mutations from leukocytes). Plasma replicates are shown as Rep 1 to 4. Top to bottom: patient samples with undetected, low (~50 ppm), medium (~500 ppm) and high (~6,000 ppm) levels of ctDNA. Samples are from patients with early-stage non-small cell lung cancer (LUCID study, see poster #735)

Sensitivity for high (48) and low (16) numbers of variants at high input copies

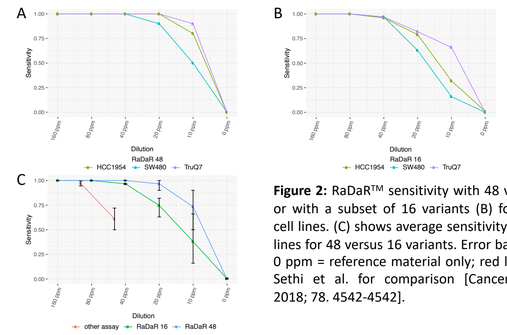


Figure 2: RaDaR™ sensitivity with 48 variants (A) or with a subset of 16 variants (B) for different cell lines. (C) shows average sensitivity across cell lines for 48 versus 16 variants. Error bars = range; 0 ppm = reference material only; red line is from Sethi et al. for comparison [Cancer Research 2018; 78. 4542-4542].

Tumor fraction estimation from detected allele frequency

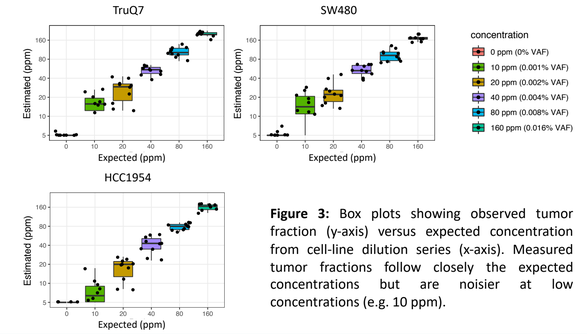


Figure 3: Box plots showing observed tumor fraction (y-axis) versus expected concentration from cell-line dilution series (x-axis). Measured tumor fractions follow closely the expected concentrations but are noisier at low concentrations (e.g. 10 ppm).

CONCLUSION

The RaDaR™ assay provides a highly sensitive and specific automated method to detect low levels of tumor DNA in plasma of cancer patients to test for minimal residual disease and for recurrence monitoring. The assay demonstrates high sensitivity, while maintaining specificity above 99%. The RaDaR assay was used to analyze cancer samples and shows applicability to multiple cancer types. Detection of residual disease in patients treated for early-stage cancer showed its potential for use in a real-world clinical scenario.