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Comparison of a personalized sequencing assay and digital PCR for circulating tumor DNA based Molecular Residual Disease detection in early-stage triple negative breast cancer in the cTRAK-TN trial

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Background

Detection of circulating tumor DNA (ctDNA) in patients (pts) who have completed treatment for early-stage breast cancer is associated with a high risk of future relapse. Identifying those at high risk of subsequent relapse may allow tailoring of further therapy to delay or prevent recurrence.

Multiple different assays are in development for the assessment of ctDNA, yet few studies have compared different assays to assess relative performance. Assays with improved analytical sensitivity could increase detection of low levels of ctDNA and lengthen the lead time between ctDNA detection and relapse.

We compared ctDNA detection of Molecular Residual Disease (MRD) via Invivata's Residual Disease and Recurrence (RaDaR®) personalized sequencing assay to digital PCR (dPCR) in pts from the c-TRAK-TN clinical trial.

Methods

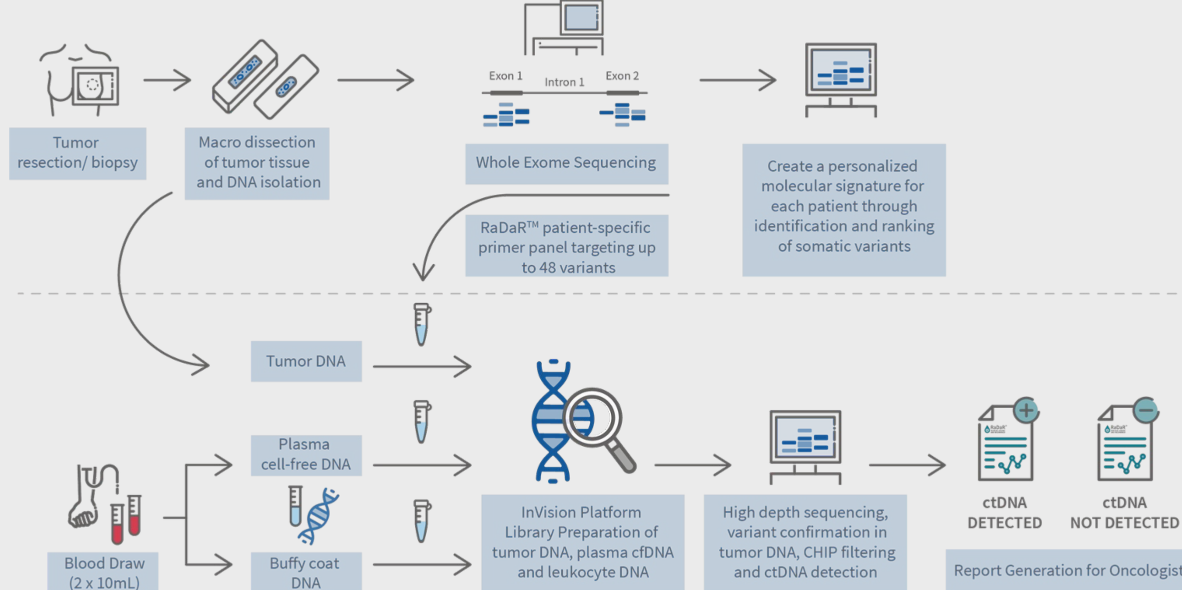
The *cTRAK-TN clinical trial* recruited 161 pts into prospective ctDNA surveillance with dPCR. Pts had serial post-treatment surveillance plasma samples collected every 3 months for up to 2 years. ctDNA positive pts were randomized double blind to 1) CT staging plus pembrolizumab therapy for pts without relapse or 2) observation.

Retrospectively, whole exome sequencing (WES) was performed on tumor DNA from FFPE samples to design personalized RaDaR® multiplex PCR-based NGS assays. Plasma DNA extracted from a minimum of 2mL banked plasma was sequenced with personalized RaDaR® assays. ctDNA detection was identified with a proprietary algorithm.

Prospectively assessed dPCR assays tracked 1-2 mutations, as previously described[1].

The primary endpoint was rate of ctDNA detection by 12 months from start of surveillance in both assays. Secondary endpoints were agreement in ctDNA detection between RaDaR® and dPCR assays, and lead-time between ctDNA detection and disease recurrence.

RaDaR® Workflow



Results

Overall, 238 tissue samples from 147 pts were subjected to WES, and RaDaR® assays were designed for 141 pts with sufficient plasma for testing. RaDaR® assays tracked a median of 47 variants (range 33-56) per pt. A total of 899 plasma timepoints were analyzed (median 7 (1-11) timepoints per pt) by both RaDaR® and dPCR.

During a median follow up of 22 (0-30.8) months, 39.7% (56/141) of pts had ctDNA detected with the RaDaR® assay. ctDNA was detected at a median 0.0812% (0.00015-36.6%) estimated variant allele fraction (eVAF). With dPCR, 35.5% (50/141) pts tested had ctDNA detected.

839/899 plasma timepoints tested had concordant test results, giving an overall test agreement between RaDaR® and dPCR assays of 93.3% (95%CI; 91.4% – 94.8%).

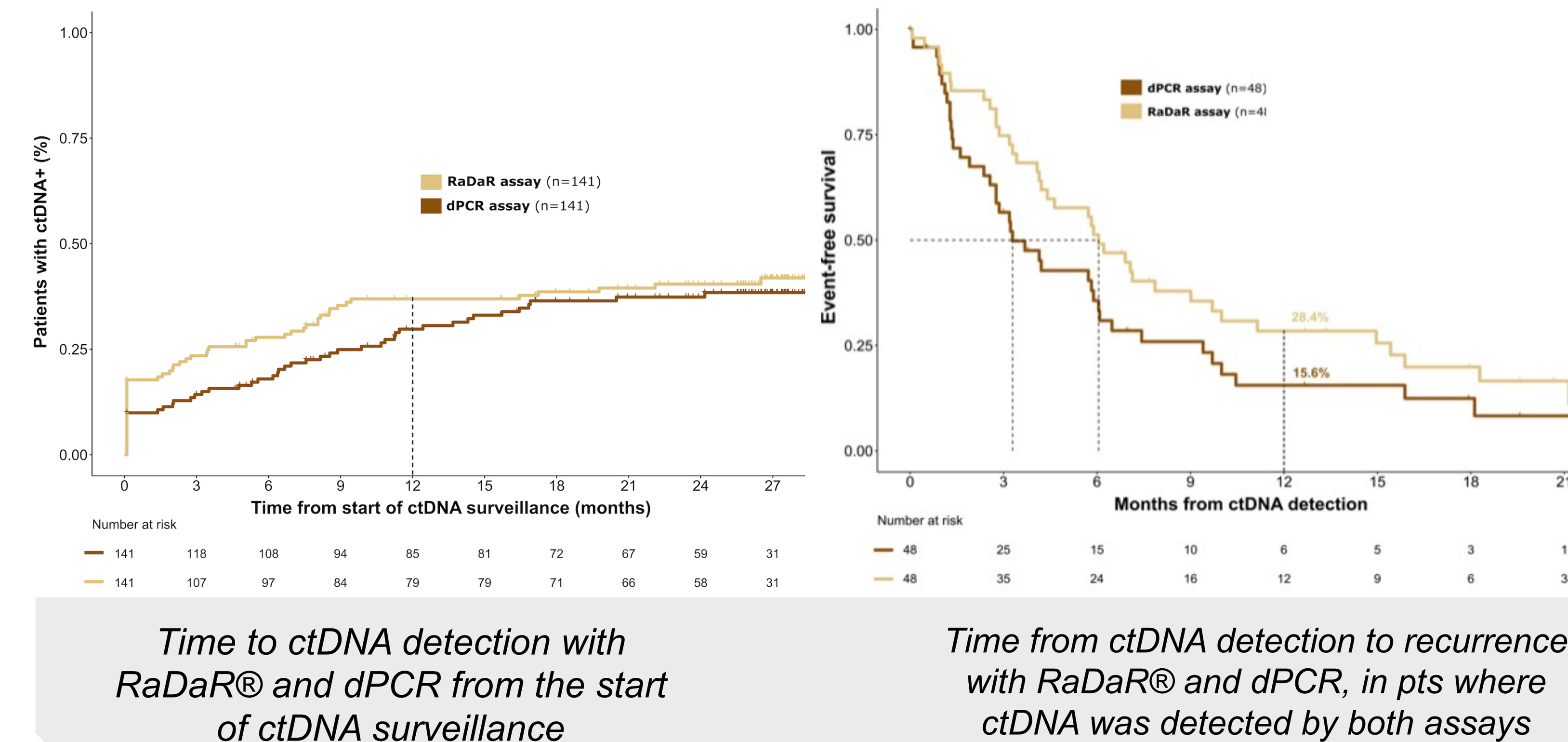
From a pt perspective, 131/141 pts had concordant test results, as 58.9% of pts were ctDNA negative for both assays, and 34.0% ctDNA were positive for both assays (although potentially at different timepoints).

Discordant test results were found in 10/141 (7.1%) of pts. 8 pts had ctDNA detected by RaDaR® but not by dPCR, and 2 pts had ctDNA detected by dPCR but not by RaDaR®. 6 of these pts relapsed within the follow up period, 5 who had ctDNA detected by RaDaR® only, and 1 who had ctDNA detected by dPCR only.

Among pts where ctDNA was detected by both assays (n=48), 47.9% were first detected by RaDaR®, 0% by dPCR, and 52.1% were first detected at the same time-point (test of proportions, $p<0.001$).

The median lead time from ctDNA detection to relapse was 6.1 months (95% CI 4.2 – 10.0) with RaDaR® and 3.3 months (95% CI 2.8 – 6.1) with dPCR.

Time to Event Analysis



Time to ctDNA detection with RaDaR® and dPCR from the start of ctDNA surveillance

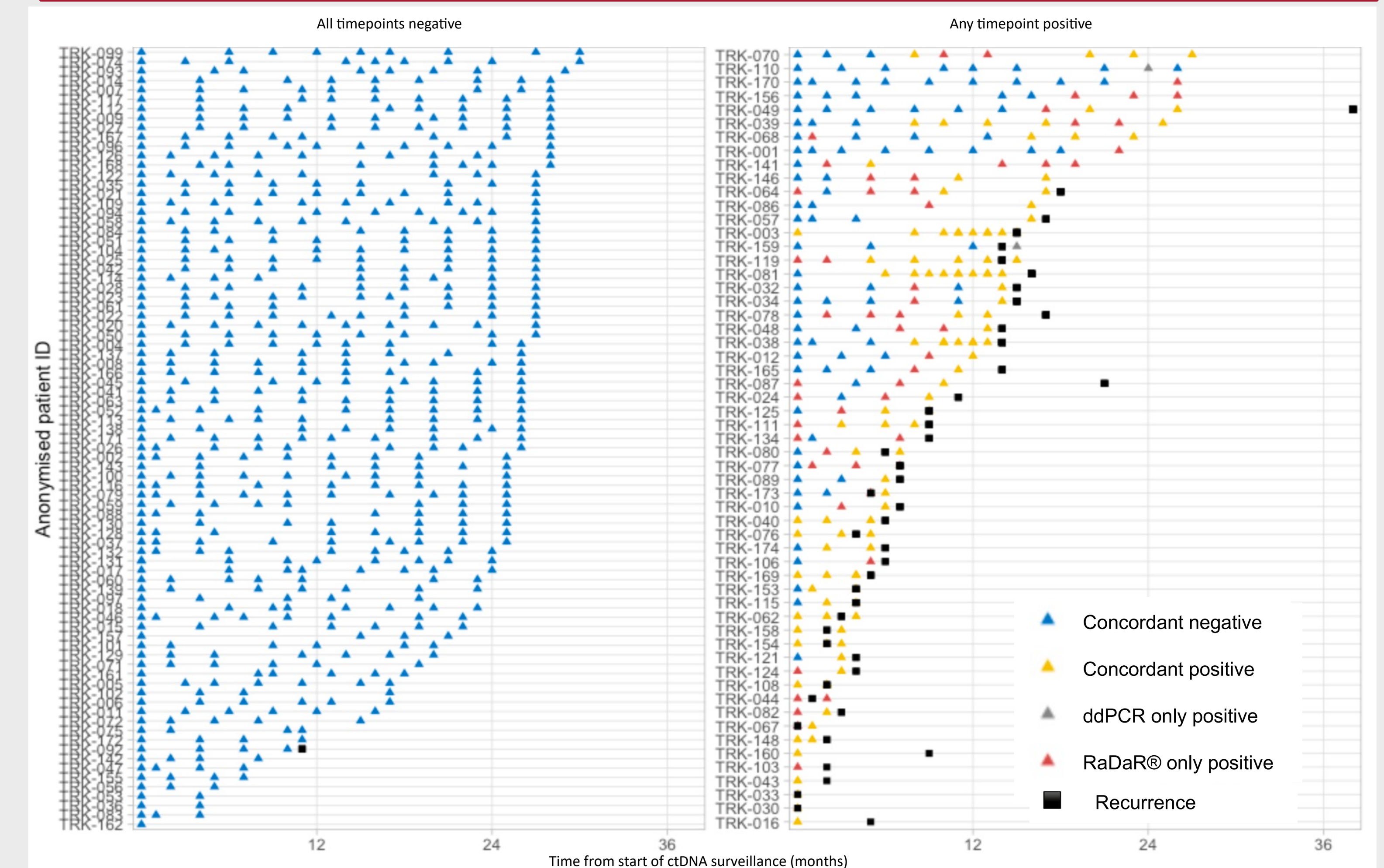
Time from ctDNA detection to recurrence with RaDaR® and dPCR, in pts where ctDNA was detected by both assays

Results summary

	dPCR	RaDaR®
ctDNA detection at 12 months	29.8% (21.5% - 37.1%)	36.9% (28.3% - 45.5%)
HR for Relapse ctDNA via RaDaR® compared to dPCR*	0.69 (0.44 – 1.08)	
Median Lead time from ctDNA detection to relapse*	3.3 months	6.1 months
Percentage of pts first detected by assay*	0%	47.9%

** Where ctDNA was detected by both assays (n=48)*

Assay concordance by patient



Plasma timepoints tested by both RaDaR ® and dPCR assays

Conclusion

The RaDaR® personalized multi-mutation sequencing assay detected MRD with a longer median lead time prior to relapse than dPCR mutation tracking assays.
These findings have implications for the choice of ctDNA assay in clinical trials designed to treat pts at the point of MRD detection.

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1. Turner N, Swift C, Jenkins B, et al. Primary results of the c-TRAK TN trial: A clinical trial utilising ctDNA mutation tracking to detect minimal residual disease and trigger intervention in pts with moderate and high risk early stage triple negative breast cancer. SABCS 2021; Abstract GS3-06.

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