Ultra-sensitive, tumor-informed ctDNA profiling in pembrolizumab-treated gastroesophageal cancer patients reveals longitudinal ctDNA kinetics

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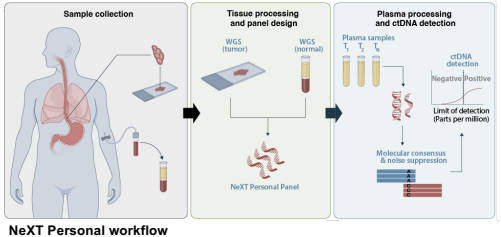
BACKGROUND

Metastatic esophagogastric cancer (mEGC) is a lethal disease with poor long-term survival. Recent studies have established anti-PD-1 therapy in combination with chemotherapy as the standard of care for first-line therapy for mEGC. KeyLargo (NCT03342937 [1]) was a single-arm phase II study of pembrolizumab in combination with oxaliplatin and capecitabine in the first-line treatment of patients with HER2 negative mEGC. While high response rates were noted, not all patients received benefits, emphasizing the need for better biomarkers. Paired tumor biopsies and plasma were longitudinally collected, processed, and stored for optimal biomarker testing. In this retrospective study, we employed a novel, tumor-informed circulating tumor DNA (ctDNA) approach for longitudinal disease monitoring and dynamic tumor evolution.

OBJECTIVES

Investigate the application of ultra-sensitive ctDNA assay and explore ctDNA parts per million (PPM) as a prognostic biomarker for the clinical benefit of anti-PD-1 immunotherapy in combination with oxaliplatin and capecitabine in the first-line treatment of patients with HER2-negative mEGC.

METHODS



NeXT Personal workflow

A total of 159 plasma samples from 25 patients have been retrospectively evaluated at baseline and during treatment. An ultra-sensitive, tumor-informed ctDNA approach was used to assess molecular/minimal residual disease (MRD) and evaluate tumor evolution. NeXT Personal uses whole-genome sequencing of both tumor and normal samples to generate a personalized liquid biopsy panel for each patient consisting of up to 1,800 selected somatic variants, enabling ultra-sensitive detection down to 1-3 PPM [2]. The resulting individual bespoke panels used to detect MRD across each patient ranged from 506 to 1,875 target MRD variants. The median MRD panel size was 1,827 variants. NeXT Personal results were analyzed along with imaging assessments and clinical outcomes for each patient.

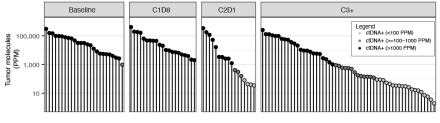
RESULTS

Thirty-six patients were enrolled between January 2018 and January 2020. Twenty-five patients were analyzed for the presence and quantification of ctDNA in plasma. Reasons for exclusion included the absence of FFPE blocks (n=4), poor DNA yields (n=5), and low tumor content (n=2). Out of these pts, the best overall response consisted of 2 complete response (CR), 14 partial response (PR), 2 stable disease (SD), and 5 progressive disease (PD) patients. Data was missing for two patients, specified as withdrew/non-evaluable (NE).

Demographics	Patients, n (%)	Patients in this study, n (%)
Age, median (range)	**60 (34-78)**	**61 (34-76)**
Male	**30 (83)**	**21 (84)**
Female	**6 (17)**	**4 (16)**
Primary Site		
Esophagus	**9 (26)**	**5 (22)**
GEJ	**18 (53)**	**15 (65)**
Gastric	**7 (21)**	**3 (13)**
Withdrew/NE	**2**	**2**

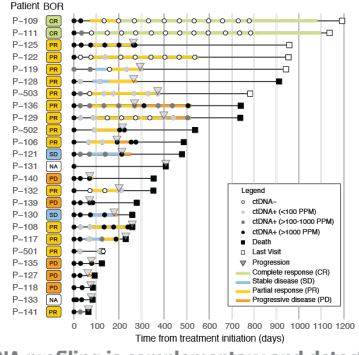
Tracking patient response requires ultra-sensitive profiling

NeXT Personal detects a large spectrum of ctDNA-positive events, allowing for ultra-high sensitivity for low tumor fractions (<0.01%). The dynamic range of ctDNA-positive samples varied from 406,067 down to 1.5 ctDNA molecules PPM (Figure 2).



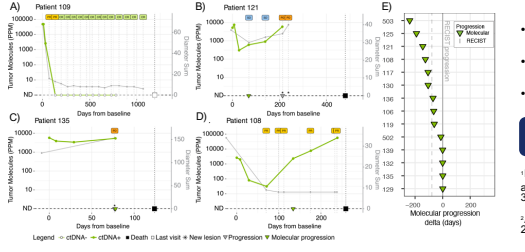
All patients demonstrated ctDNA positivity at baseline and C1D8. Most patients exhibited ctDNA positivity across the majority of their longitudinal plasma samples (119/159) with a median limit of detection of 2.03 with quartiles spanning 1.66 to 2.74 PPM. A significant number of ctDNA-positive detections were observed at low PPM levels, with 20% (24/119) of ctDNA-positive (ctDNA+) time points displaying ultra-low ctDNA levels below 0.01% in plasma (or 100 PPM).

Longitudinal ctDNA changes correlate with patient survival

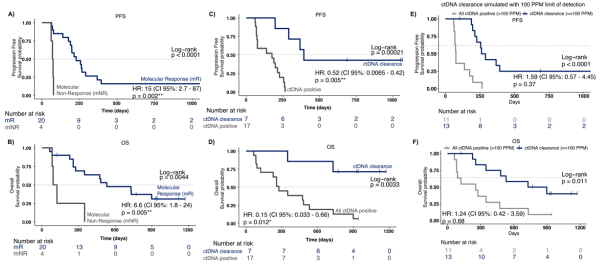


ctDNA profiling is complementary and detects disease progression earlier than imaging

We identified a strong correlation between change in ctDNA levels and change in overall tumor burden, measured as the sum of diameters of target lesions according to RECIST criteria ($\rho = 0.58$, $p = 7.3e\text{-}9$). Representative examples across multiple BOR categories show great concordance between ctDNA (green) and tumor size (gray). Additionally, we observe cases suggesting ctDNA profiling is strongly complementary, being able to detect patient response dynamics which were undetected by imaging (Patient 108). For all patients, molecular progression was detected before or at clinical progression with a median lead time of 65 days (Panel E).



Molecular clearance and molecular response were highly prognostic for improved clinical outcomes



Lack of early molecular response (ctDNA decrease) was associated with worse overall survival (OS) and progression-free survival (PFS). Molecular clearance of ctDNA was associated with improved OS and PFS. To simulate what the ctDNA results would have been with an assay with a 100 PPM limit of detection, we re-analyzed our data, changing all of our positive detections at <=100 PPM to "not detected". With this 100 PPM simulated LOD, clearance of ctDNA was no longer associated with improved OS ($p = 0.68$) and PFS ($p = 0.37$). Additionally, nearly 17% (3/18) of patients classified as molecular progressors (specifically patients 503, 119, and 132) would no longer have observable molecular progression. In patient 106, who had molecular progression detected 65 days ahead of imaging, molecular progression would instead be detected at the same time as imaging, reducing the lead time to zero days.

CONCLUSIONS

- Even in late-stage disease, an ultra-sensitive ctDNA platform is critically important for accurately tracking and predicting response to therapy.
- ctDNA profiling is complementary to imaging, allowing for more granular assessment of patient response to therapy.
- Ultra-sensitive ctDNA profiling correlates with the patients' best response and tumor size dynamics.
- Tumor molecule PPM dynamics are prognostic of patient response to immunotherapy.

REFERENCES

[1] KEYlargo: A phase II study of first-line pembrolizumab, capecitabine, and oxaliplatin in HER2-negative gastroesophageal adenocarcinoma. (https://ascopubs.org/doi/abs/10.1200/JCO.2021.39.3_suppl.228) Uronis et al. Journal of Clinical Oncology 2021 39:3_suppl, 228-228

[2] Analytical validation of NeXT Personal(R), an ultra-sensitive personalized circulating tumor DNA assay. Northcott J. et al. Oncotarget 2024;15:200-18 doi:10.18632/oncotarget.28565.