Clinical Validation of the Northstar Response: A Novel Quantitative Methylation ctDNA Monitoring Assay for Advanced GI Cancer Treatment Response

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INTRODUCTION

Improved therapy response monitoring is needed in advanced GI tumors

BACKGROUND

Circulating tumor DNA (ctDNA) is a promising biomarker buttressing clinical decision-making from therapy selection through on-treatment response monitoring to post-therapy surveillance.

Currently, most ctDNA-based therapy response monitoring strategies track variant allele fraction (VAF) of predetermined somatic alterations, which has significant limitations:

- Selected variants may not accurately portray the tumor's genetic composition due to heterogeneity and prevalence of metastases
- Not all tumors have sufficient somatic variants available for reliable tracking
- For tissue-informed assays, not all patients can be feasibly biopsied to inform liquid biopsy monitoring

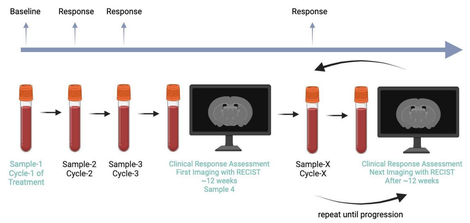
To address these limitations, quantification of methylated loci from ctDNA has emerged as a viable alternative due to a greater abundance of tumor-derived methylated molecules compared to somatic variants, thereby enhancing assay sensitivity.

OBJECTIVE

We utilized Northstar Response, a methylation-based assay tailored to track tumor-specific ctDNA signals to evaluate the association between the change in tumor methylation score (TMS) with patient outcomes.

METHODS

Prospective, observational trial design



- Patients with unresectable or advanced GI cancers were enrolled with ctDNA blood samples taken at baseline, at the time of routine imaging (~ 90 days post-treatment initiation), and at least two intermediate time points (~30 days and 60 days post-treatment initiation).
- Eligibility included adult patients starting a new line of systemic therapy, not pregnant, and no previous transplants
- Statistical analysis includes descriptive, median TMS scores, log-rank, logistic, and hazard regression analysis.

RESULTS

Baseline ctDNA measurements align with known shedding patterns

a

Patient Summary	Whole Cohort
Age	
Median	66
Range	32-91
Sex	
Female	39 (38.6%)
Male	62 (61.4%)
Race and Ethnicity	
White	74 (73.3%)
Black	16 (15.8%)
Hispanic/Latino	6 (5.9%)
Not Specified	5 (5.9%)
Tumor Type	
Biliary Tract	18 (17.8%)
CRC	25 (24.8%)
Esopho-gastric	13 (12.9%)
HCC	29 (28.7%)
Pancreatic	11 (10.9%)
Other*	5 (5%)
Disease Burden	
Primary Tumor Only	10 (9.9%)
Metastatic Lesions Only	54 (53.5%)
Both	37 (36.6%)
Line of Therapy	
1st Line	66 (66%)
2nd Line	25 (25%)
3rd Line	9 (9%)
Type of Systemic Therapy	
Immunotherapy Only	36 (35.6%)
Chemotherapy Only	19 (19%)
Chemo + Targeted Therapy	45 (44.5%)
1st Response Assessment	
Complete Response (CR)	2 (2.6%)
Partial Response (PR)	12 (15.8%)
Progressive Disease (PD)	28 (36.8%)
Stable Disease (SD)	34 (44.7%)

*Includes anal, GIST and undifferentiated tumors;
#First response scan at ~3months on treatment

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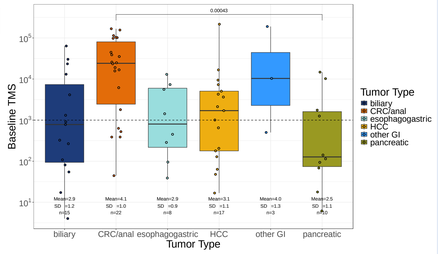

(a) Cohort demographics. In this analysis, we evaluated baseline TMS of 101 cases. Among these, 76 had at least two more blood collections and at least one radiographic or clinical response assessment. The cohort captured was representative of a breadth of GI tumor types with first and second-line therapies and responses

(b) Baseline TMS varies across tumor types, demonstrating statistically significant greater TMS in CRC versus pancreatic cancer, and consistent with expectations regarding known ctDNA shedding rates across common tumor types

RESULTS

Baseline Tumor Methylation Score is prognostic of outcome

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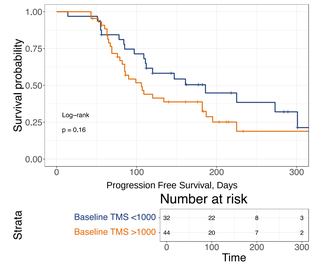

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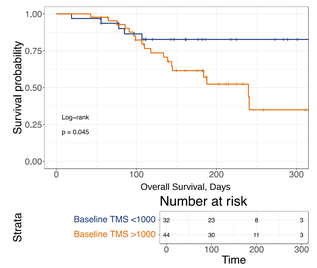


Subjects were separated into two groups: baseline TMS ≥1000 versus <1000.

The cutoff, 1000, is an approximate median for TMS in general and was not modified for this assay to avoid bias.

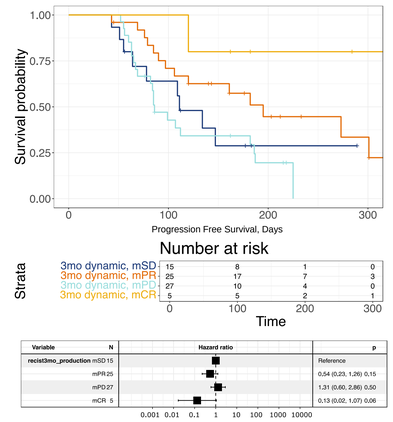
(a) Progression-Free survival by baseline TMS. Patients with higher TMS baseline scores trend towards more rapid progression.

(b) Overall survival by baseline TMS. Lower initial TMS levels are associated with better survival.

RESULTS

Dynamic timepoint comparisons improve prognosis estimates

Note: All analyses were conducted among patients who had evaluable on-treatment blood collections for n=72 patients. Four cases were excluded from the on-treatment cohort due to missing data or quality control (QC) failures during the on-treatment phase.



Progression Free Survival using a dynamic comparison modality.

Instead of comparing on-treatment TMS to baseline, comparisons to the TMS baseline (for partial response) or to the TMS *nadir* (for progressive disease), in a manner analogous to RECIST sum of diameters, result in the potential to stratify patients with greater performance. Using this method, patient prognosis was categorized by molecular response (mCR, mPR, mSD or mPD).

RESULTS

On-treatment changes in Tumor Methylation Score are associated with patient outcomes

*Note: All analyses were conducted among patients who had evaluable on-treatment blood collections for n=72 patients. Four cases were excluded from the on-treatment cohort due to missing data or quality control (QC) failures during the on-treatment phase.

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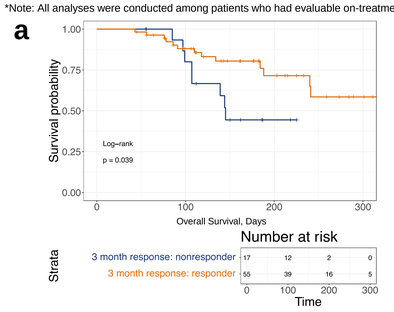

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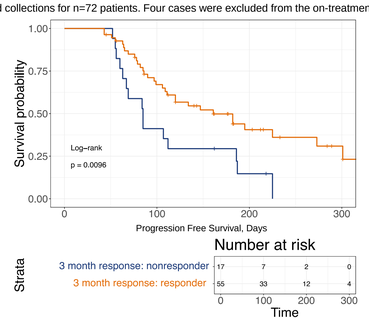

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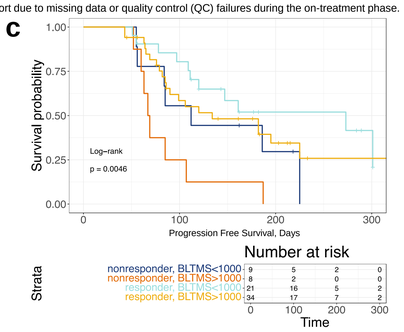

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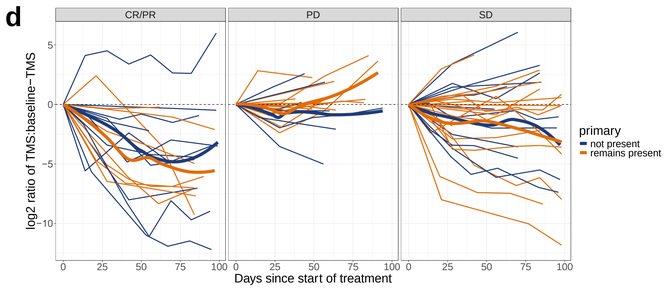

(a) Overall survival and (b) Progression Free Survival by TMS molecular responder vs non-responder at 3 months. Non-responders are defined by increases in TMS versus baseline

(c) Progression free survival across 3-month molecular response and baseline (BL) TMS shows that the combination of these measurements may further refine PFS prognosis (p<0.004)

(d) Trends in TMS score per patient and presence (or absence) of primary tumor at up to 100 days, organized by best overall response (BOR). The trends of increase, neutral, or downward TMS align with radiomic and clinical BOR categories

CONCLUSION

Serial monitoring of methylated ctDNA may provide insight into treatment response

- Baseline TMS differs across the spectrum of GI tumors evaluated, reflecting biological variability in ctDNA shedding within and between tumor types.
- Baseline TMS can be prognostic of outcome, with statistically significant associations versus progression-free survival.
- The data from this analysis justify continued assessments of the Northstar Response, which measures methylated ctDNA, across a variety of locally advanced and metastatic GI malignancies.
- Dynamic comparisons to baseline or the *nadir* (e.g. the lowest TMS value among past measurements) may improve the prognostic capacity of ctDNA based assays.
- Merging baseline TMS alongside 3 month molecular response may increase the prognostic capabilities of ctDNA based assays
- Recruitment and analyses are ongoing through targeted expansion cohorts to support assay sensitivity and specificity determinations and radiomic assessments.

Acknowledgements & Contacts

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