# Clinical validation of Northstar Response, a novel methylated ctDNA therapy response monitoring assay in patients with advanced GI cancer undergoing active treatment.

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**Background:** ctDNA is a non-invasive way to holistically assess the tumor. Several assays developed for therapy response monitoring rely on variant allele frequencies to detect scarce somatic variants in the blood. In contrast, methylated ctDNA measurement has shown promise as a treatment monitoring biomarker without requiring a tumor biopsy, yet accuracy is limited in ability to precisely quantify the amount of methylation present in the ctDNA. The CLIA-approved Northstar Response assay (BillionToOne, Inc.) quantifies on average 90 methylated loci, a 10-fold increase in signal over the average tissue-informed assays. **Methods:** This prospective, observational study assesses the clinical validity of Northstar Response, a quantitative methylated ctDNA therapeutic response monitoring assay that does not require prior tumor tissue. A cohort of 100 advanced cancer patients with a variety of GI malignancies submit serial ctDNA measurements in a blinded fashion. Eligibility includes advanced GI cancer patients starting a new line of systemic therapy (IO, targeted, chemo or combination) who are not pregnant or s/p organ or bone marrow transplantation. Blood samples are collected at baseline, at the time of imaging, and at least two intermediate time points (~30 days and 60 days post tx initiation). The primary endpoint is to measure the concordance and temporal relationship between disease progression measured by ctDNA compared to RECISTv1.1, clinical confirmation, or death. A Cox proportional hazards model will be used to quantify the association between the change in tumor methylation score (TMS; total methylated tumor molecules per 1000 assayed genomic equivalents) and clinical progression. The cohort will be split equally between a training and validation set. **Results:** Enrollment began in June 2023 and continues. To date, 28 patients with a variety of advanced GI cancers (table) have been enrolled with 37 samples processed. All samples have passed established QC metrics with average turn-around-time from receipt of sample to final report of 9.1 days (95% were <15 days). TMS ranged from <120 to >160,000 across individual samples, indicating that the assay can detect a broad range of tumor load in blood. We detected a median of 69 methylated loci per sample (range 4-451). Out of 21 pts, 17 had at least one sample with TMS measured above the noise floor of 120. **Conclusions:** The Northstar Response assay can consistently detect ctDNA across a variety of advanced GI cancers. Enrollment continues with formal comparison to clinical progression planned. Additional data on the complete training set will be available at the meeting. Research Sponsor: BillionToOne, Inc

**Descriptive information of participating patients.**

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| **Median age 65 (32-84); Male (67%); White (86%)** |
| **Cancer types represented** |
| **Esophagogastric** |
| **HCC** |
| **Biliary** |
| **Pancreatic Adenocarcinoma** |
| **Colorectal** |
| **Small Intestine** |
| **GIST** |