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Longitudinal evaluation of ctDNA Molecular Response for monitoring clinical benefit and investigating treatment related impacts in metastatic colorectal cancer patients treated with different drug regimens

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INTRODUCTION

- **The patient-centric liquid biopsy approach to detect changes in circulating tumor DNA (ctDNA) can provide an early indication of treatment response to therapies and is an emerging tool to aid clinicians in treatment decision making.**
- **We investigated this approach in the mCRC palliative treatment setting by exploring the potential clinical utility of the validated Guardant Health Molecular Response (MR) algorithm.**

METHODS

70 baseline and longitudinal plasma samples (27-276 days post treatment) were collected from 14 patients with mCRC that had been treated using chemotherapy (CTx) alone or combined with one or more targeted therapies. All patients were clinically stable or had clinical benefit over the course of plasma collection. 6 patients had radiological response by RECISTv1.1 and 10 had tumor tissue-based RAS/RAF mutation status (Table 1).

Table 1: Patient Clinical, Biomarker and Treatment Information

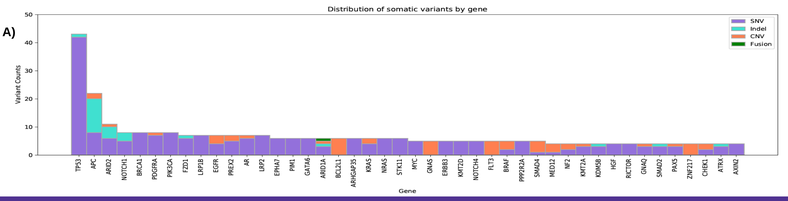
Donor ID	Organ	Clin/Path	Stage	TNM	Tumor Biomarker Status	Treatment
ES_000	Rectum	Adenocarcinoma	IV	pT3 pN2 cM1	NK	CTx + anti-EGFR
ES_002	Rectum	Adenocarcinoma	IV	pT3 pN1 pM1	NK	CTx + anti-VEGF + anti-EGFR
ES_003	Rectum	Adenocarcinoma	IV	pT3 pN2 cM1	BRAF/ KRAS/ NRAS wildtype	CTx + anti-PD-L1 + anti-EGFR
ES_004	Rectum	Adenocarcinoma	IV	rTX NX cM1	BRAF/ KRAS/ NRAS wildtype	CTx + anti-PD-L1 + anti-EGFR
ES_005	Rectum	Adenocarcinoma	III	cT3 cN2b cM0	NK	CTx + anti-EGFR
ES_007	Rectum	Adenocarcinoma	IV	uT3 uN1 cM1	KRAS mut	CTx + anti-EGFR
ES_013	Rectosigmoidal Junction	Adenocarcinoma	IV	cT0 cN0 cM1	KRAS/ NRAS wildtype	CTx + anti-EGFR
ES_018	Rectum	Adenocarcinoma	IV	cT3 cN2 cM1	KRAS/ NRAS wildtype	CTx
ES_027	Colon	Adenocarcinoma	IV	rcT0 cN0 cM1	BRAF/ KRAS/ NRAS wildtype	CTx + anti-EGFR
ES_028	Colon	Adenocarcinoma	IV	rT0 cN0 pM1	BRAF/ KRAS/ NRAS wildtype	CTx + anti-EGFR
ES_029	Colon	Adenocarcinoma	IV	pT4 pN2 pM1b	BRAF/ KRAS/ NRAS wildtype	CTx + anti-PD-L1 + anti-EGFR
ES_030	Colon	Adenocarcinoma	IV	pT3 pN1 cM1	BRAF/ KRAS/ NRAS wildtype	CTx + anti-PD-L1 + anti-EGFR
ES_033	Colon	Adenocarcinoma	IV	rTX cNX cM1	BRAF/ KRAS/ NRAS wildtype	CTx + anti-VEGF
ES_039	Colon	Adenocarcinoma	IV	rTX pNX pM1	NK	CTx

Circulating free DNA (cfDNA) was extracted and tested using the GuardantOMNI™ (500 gene, 2.145Mb) liquid biopsy panel. Small-nucleotide variants (SNVs), insertions/deletions (Indels), copy number variations (CNVs), fusions, microsatellite instability high (MSI-High) status, and tumor mutation burden (TMB) was reported. Somatic classification and status of SNVs and Indels was performed by a beta-binomial model that incorporates genomic context and variant allele frequency (VAF). Selection of qualifying alterations and generation of MR scores were pursued using the Guardant Health MR algorithm[1]

RESULTS

Figure 1. Plasma Variant Summary.

- Aggregate count of somatic alterations detected in most prevalent mutated genes detected in plasma across the patient cohort (Fig 1A)
- All detected somatic ctDNA alterations, including fusions, CNVs, Indels, and SNVs per sample (Fig 1B). Germline and somatic putative clonal hematopoiesis of indeterminate potential (CHIP) variants were excluded.



RESULTS

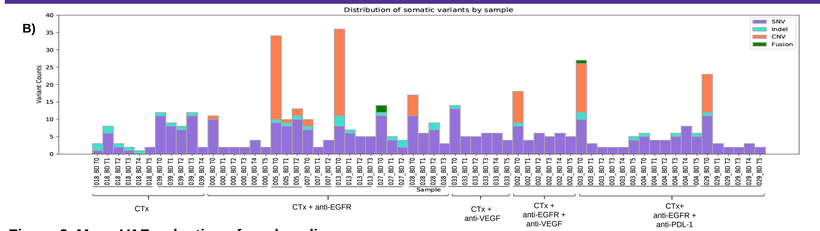


Figure 2. Mean VAF reductions from baseline are similar between early and late treatment timepoints.

- 13/14 patients were evaluable for MR by having somatic alterations at each timepoint that exceeded the molecule threshold required for reliable MR score calculation.
- Mean VAF of all plasma samples in the >10 week on-treatment timepoints trended in the same direction as the early 4-10 week on-treatment plasma samples when compared to baseline.

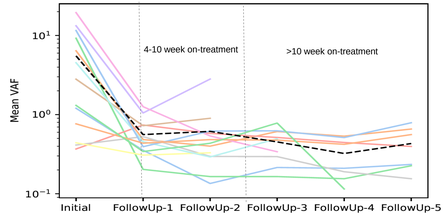


Figure 3. The majority of patients exhibited consistent reduction in mVAF compared to baseline in response to CTx + targeted therapy.

- Consistent mVAF reductions were noted for 10/11 (91%) patients treated with CTx + targeted therapy across longitudinal timepoints in response to treatment (Fig 3A-E).
- However, mVAF notably fluctuated in response to CTx only across treatment timepoints (Fig 3F and 3G)
- Two patients (ES00_18 and ES_004) demonstrated increases in mVAF above pre-treatment baseline levels.

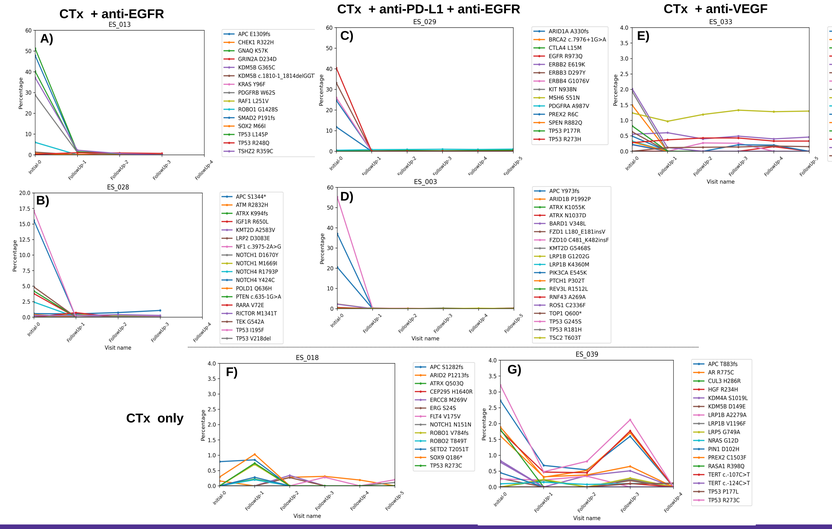


Figure 4. An MR outlier was shown to harbor mutations that could potentially alter the response to targeted therapy.

- Based on FFPE tumor analysis, ES-004 was determined to be *RAS/RAF* wild-type and treated with anti-EGFR and anti-PD-L1 (Fig 4A).
- MR scores for this patient across all timepoints reflected an increase in mVAF compared to baseline (Fig 4B).
- GuardantOMNI™ detected a low level *KRAS* alteration (*KRAS* G13D) in the baseline plasma sample of this patient, indicating this patient's disease harbored a *KRAS* alteration that was not detected by tissue-based testing[2].
- In addition, this patient developed both *KRAS* Q61H and *BRAF* V600E mutations when on treatment.

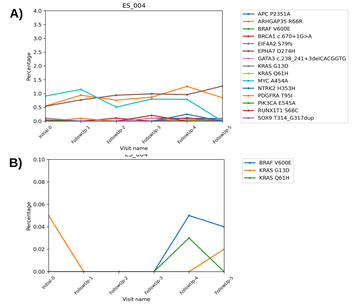


Table 2. The majority of patients with partial response by RECIST1.1 showed a decrease in MR score.

- All 5 patients with RECIST 1.1 information had partial responses (PR) and 4 showed a decrease in the MR score.
- The one patient whose MR score did not align with radiographic assessment (ES_004) was determined to be positive for *KRAS* G13D by GuardantOMNI at baseline. This patient is under further investigation.

DONOR ID	TREATMENT	MR Score(s) 4-10 week OT	MR Score(s) >10 weeks (range 12-39 weeks)	MR Scores Consistency Across Timepoints	BOR by RECIST 1.1
ES_018	Chemo	174, 0	0, 0	N	
ES_039	Chemo	15, 20	55, 0	N	
ES_000	Chemo + anti-EGFR	6, 2	4, 5	Y	
ES_005	Chemo + anti-EGFR	7, 23	NA	Y	
ES_007	Chemo + anti-EGFR	5	14	Y	
ES_013	Chemo + anti-EGFR	4, 1	0	Y	PR
ES_027	Chemo + anti-EGFR	28, 32	NA	Y	
ES_028	Chemo + anti-EGFR	5, 3	3	Y	PR
ES_003	Chemo + anti-EGFR + anti-PD-L1	1, 0	0, 0	Y	PR
ES_004	Chemo + anti-EGFR + anti-PD-L1	143, 110	133, 150	Y	PR
ES_029	Chemo + anti-EGFR + anti-PD-L1	1, 1	1, 1	Y	PR
ES_002	Chemo + anti-EGFR + anti-VEGF	3	4, 5, 5	Y	
ES_033	Chemo + anti-VEGF	21, 24	28, 24	Y	

CONCLUSIONS

We demonstrate that ctDNA analysis of plasma samples taken at early timepoints (4-10 weeks) after treatment initiation with chemotherapy alone or combined with one or more targeted therapies (anti-PD-L1 and/ or anti-EGFR) are sufficient to support MR assessments to supplement patient response determined by radiographic imaging and RECIST1.1.

With the caveat that we have analyzed small sample numbers, the data suggest that the molecular response profiles might differ between CTx only and CTx + targeted therapy in the treatment of metastatic colorectal cancer in that molecular responses are stronger and more consistent with CTx + targeted therapies. Larger cohorts need to be investigated to draw definitive conclusions.

- **We also highlight the advantage of the patient-centric liquid biopsy approach to detect potential resistance mutations prior to therapy initiation that may not be detectable in the diagnostic tissue sample[2]. This concept is especially relevant to patients available for post-progression therapies where the diagnostic tissue may not reflect the current genomic make-up of the disease.**

REFERENCES

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2) Feng *et al. Cancer Res 2020* 80 (16_Supplement): 1992 https://doi.org/10.1158/1538-7445.AM2020-1992

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