ctDNA as a Potential Surrogate Biomarker for Response to Combination Vilastobart and Atezolizumab in Heavily Pretreated Microsatellite Stable (MSS) Metastatic Colorectal Cancer (mCRC) (NCT04896697)

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SCAN ME

Abstract 541

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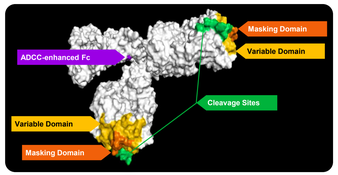
BACKGROUND

MSS mCRC Remains a Significant and Growing Unmet Need

- In the US, CRC is 2nd in cancer mortality and the leading cause of death from cancer in young men (<50 years), often presenting late-stage[1]
- Response rates for FDA-approved agents in third line+ MSS mCRC (trifluridine + tipiracil, regorafenib and fruquintinib) are low (0 to 6%)[2-5] and approved ICIs (pembrolizumab / nivolumab / ipilimumab) in MSI-H CRC have minimal efficacy in patients with MSS mCRC (<5% ORR)[6, 7]
- Next generation anti-CTLA-4 agents in development have activity in MSS mCRC without liver metastases.[8-10] However, tolerability is a limitation of systemically active, unmasked agents, including high imAEs and/or discontinuation rates[8]

Vilastobart: Tumor-Activated, High Affinity Binding, Fc-Enhanced Anti-CTLA-4 in Phase 2 Development



- High affinity binding resulted in 10x potency of ipilimumab in preclinical studies[11]
- Fc mutations provided enhanced effector function (ADCC), improved T cell priming and Treg depletion[11]
- On-treatment biopsies in Phase 1 monotherapy demonstrated >70% activated molecule in tumor with <15% activated molecule in periphery[12]
- Vilastobart, in Phase 1 monotherapy and Phase 2 combination with atezolizumab (Tecentriq®), was generally well tolerated with a safety profile consistent with tumor-activated design[13, 14]
- In heavily pretreated patients with MSS mCRC without liver metastases, vilastobart in combination with atezolizumab demonstrated an ORR of 26%, including 7 PR (6 confirmed) per RECISTv1.1[14]
- One patient in Phase 1C with MSS mCRC had full resolution of a liver metastasis with PR (confirmed) ongoing on therapy for 14+ months[14]

ctDNA as a Potential Surrogate Biomarker for Response

- ctDNA offers a dynamic, minimally invasive method to monitor treatment response by quantifying tumor-derived cell-free DNA by measuring methylation levels at tumor-specific genomic loci using plasma
- Highly sensitive ctDNA monitoring and its correlation with radiographic response in patients with MSS mCRC treated with vilastobart in combination with atezolizumab has not been reported before; hence, we explored the utility of ctDNA kinetics as a potential surrogate biomarker for response to treatment in the Phase 2 study (NCT04896697)

STUDY DESIGN AND METHODS

PHASE 1A/B
Monotherapy Dose-Escalation and Expansion
Advanced Solid Tumors (n=39)
Vilastobart monotherapy RP2D established as 150 mg Q6W

PHASE 1C
Vilastobart + Atezolizumab Dose-Escalation
Advanced Solid Tumors (n=26)
Vilastobart dose levels up to 150 mg Q6W cleared

PHASE 2
Vilastobart + Atezolizumab
Metastatic MSS mCRC (n=44)
Vilastobart 100 mg Q6W + atezolizumab 1200 mg Q3W

KEY PHASE 2 ELIGIBILITY

- At least 1 prior chemotherapy regimen for mCRC
- No prior ICI
- Must be assessed for MSI-H or dMMR status; patients with MSI-H/dMMR tumors are excluded

- In this exploratory biomarker analysis, plasma ctDNA was serially analyzed using the Guardant Infinity assay, which is a multi-omic assay that identifies somatic alterations across >750 cancer-related genes and measuring thousands of DMRs to produce a TF score at each timepoint
- In Phase 2, among 27 patients with MSS mCRC without liver metastases, 25 had evaluable ctDNA kinetics and response data for association analysis, and 23 had post-baseline target lesion and ctDNA data for correlation analysis. Statistical analyses (Spearman's rank correlation, Wilcoxon rank sum, Fisher's Exact) explored the relationship between ctDNA changes and responses

PATIENT POPULATION

Phase 2 Enrolled Heavily Pretreated Patients with MSS mCRC With and Without Liver Metastases, Including Patients with Peritoneal Metastases

Patient Characteristics	Total (n=44)
Age, median (range)	55 (25-82)
Female	22 (50%)
ECOG PS 0	18 (41%)
ECOG PS 1	26 (59%)

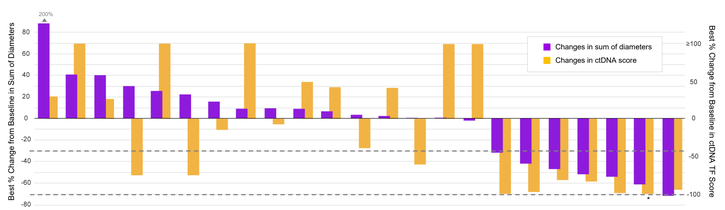
Prior Lines of Anti-Cancer Treatment	
Median	**4 (1-8)**
1	4 (9%)
2	5 (11%)
3	11 (25%)
4	9 (21%)
5	6 (14%)
6 or more	9 (20%)

80% of patients had 3 or more prior lines of treatment

Tumor Types	Total (n=44)
MSS mCRC	**44**
with liver metastases	17
without liver metastases	27
with peritoneal metastases	*8*

Treatment Status	Total (n=44)
Continuing on Treatment	**14**
Discontinued Treatment	**30**
Disease Progression	23
Adverse Events	4
Investigator Decision	1
Other	2

RADIOGRAPHIC RESPONSES WERE ACCOMPANIED BY MEANINGFUL REDUCTIONS IN ctDNA

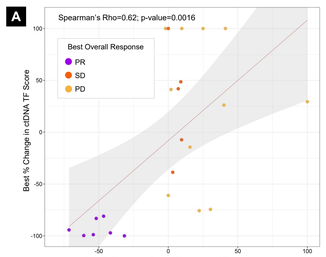


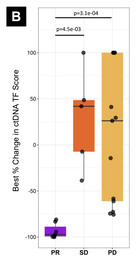
*Patient with undetectable ctDNA for >20 weeks.
Dodged waterfall plot showing best % change from baseline in the sum of diameters of target lesions (left y-axis; purple bars) and best % change from baseline in ctDNA TF score (right y-axis; yellow bars). Each pair of bars corresponds to the same patient (x-axis).

- ORR of 26% in heavily pretreated patients with MSS mCRC without liver metastases, including 7 PRs (6 confirmed) per RECIST v1.1[14]

ctDNA CHANGES WERE SIGNIFICANTLY ASSOCIATED WITH BEST OVERALL RESPONSE

Association Between Best % Changes in ctDNA and Best Overall Response in Patients with MSS mCRC Without Liver Metastases Treated with Vilastobart in Combination with Atezolizumab





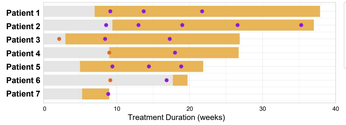
- A statistically significant positive correlation was observed between best percentage changes in ctDNA TF scores and the sum of target lesion diameters (Spearman's Rho=0.62; p=0.0016, n=23)
- ctDNA TF score best percentage changes were significantly associated with best overall response status (PR vs. SD: p=4.5x10^{-3}; PR vs. PD: p=3.1x10^{-4})

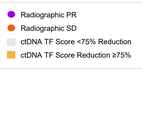
A. Scatter plot showing correlation between best % change in ctDNA TF score (y-axis) and best % change of sum of diameters (x-axis) in Phase 2 patients without liver metastases and with available post-baseline measurements (n=23). Individual subjects are represented as colored points according to best overall response. Data points with extreme values (≥100%) were truncated for visualization but actual values were used in the correlation analysis.

B. Box plot displaying best % change in ctDNA TF scores (y-axis) stratified by best overall response status (x-axis) in Phase 2 patients without liver metastases and (n=25). P-values from Wilcoxon rank sum test comparing ctDNA changes between response groups are shown. Data points with extreme values (≥100%) were truncated for visualization but actual values were used in the correlation analysis.

DEEP REDUCTIONS IN ctDNA GENERALLY OCCURRED BEFORE RADIOGRAPHIC RESPONSES

Longitudinal Analysis of ctDNA Dynamics and Overall Response among Patients with MSS mCRC Without Liver Metastases who Responded to Treatment with Vilastobart in Combination with Atezolizumab





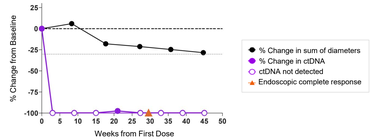
- All patients with a PR had significant reductions in ctDNA TF scores of ≥75% from baseline (Fisher's Exact p=1.7x10^{-5}; positive predictive value=87.5%; specificity=94%; sensitivity=100%)

Swimmer plot showing treatment duration, cDNA TF score reduction, and response dynamics in patients achieving PR (n=7). Horizontal bars represent individual patient trajectories. Bar width indicates the treatment duration. Bar color changes from gray to yellow at the first time point where a cDNA TF score reduction from baseline of ≥75% was recorded. Points indicate response status at the time of assessment (purple, PR; orange, SD).

ENDOSCOPIC COMPLETE RESPONSE WITH ctDNA CLEARANCE WHILE LESIONS PERSISTED ON RADIOGRAPHIC ASSESSMENT

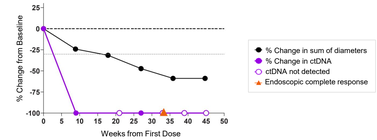
38-year-old male with locally advanced MSS rectal cancer (Phase 1C)

- Prior treatment with capecitabine (neoadjuvant), FOLFOX (adjuvant), and FOLFIRI + panitumumab
- Received vilastobart 150 mg Q6W + atezolizumab 1200 mg Q3W
- Patient had complete clearance of ctDNA by 4 weeks. PR by RECISTv1.1 was reached at ~45 weeks with complete resolution of diffusion abnormalities on MRI (suggestive of fibrosis)
- At 30 weeks, endoscopic complete response with negative biopsies followed by 3 consecutive cycles of undetectable ctDNA



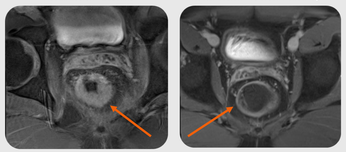
35-year-old female with peritoneal lesions and KRAS mutant disease

- Prior treatment with FOLFIRI, sotorasib and panitumumab
- Received vilastobart 100 mg Q6W + atezolizumab 1200 mg Q3W
- Patient had complete clearance of ctDNA by 10 weeks. PR by RECISTv1.1 was reached at ~18 weeks and remained PR since
- At 34 weeks, endoscopic evaluation showed limited residual tumor in the proximal transverse colon with negative malignancy on biopsy followed by 2 consecutive cycles of undetectable ctDNA



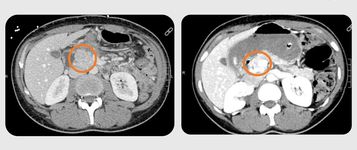
Figures display percent change in sum of diameters of target lesions (RECISTv1.1) and percent change in ctDNA score (Guardant360 Liquid (Infinity) assay) over time (weeks from the first dose of vilastobart in combination with atezolizumab), with an orange marker indicating timing of endoscopic complete response. Data as of Oct 20, 2025.

Rectal Lesion - Baseline | **Rectal Lesion - 18 Week Follow-Up**



Near resolution of rectal wall thickening

Abdominal Lesion - Baseline | **Abdominal Lesion - 18 Week Follow-Up**



Significant shrinkage of abdominal lesion

CONCLUSIONS

- Vilastobart in combination with atezolizumab demonstrated deep reductions in ctDNA (≥75%) that were significantly associated with response in heavily pretreated patients with MSS mCRC without liver metastases
- Endoscopic complete response while radiographic findings persist, suggest ctDNA may be a reliable early marker of treatment benefit, and supports ctDNA as a potential surrogate biomarker for immunotherapy response
- Encouraging clinical data to date suggest vilastobart has the potential to be an anti-CTLA-4 combination agent of choice compatible with a range of existing and next-generation agents

ABBREVIATIONS: ADCC: antibody-dependent cell-mediated cytotoxicity; ctDNA: circulating tumor DNA; CTLA-4: cytotoxic T-Lymphocyte Associated Protein 4; dMMR: deficient mismatch repair; DMRs: differentially methylated regions; ECOG PS: Eastern Cooperative Oncology Group Performance Status; Fc: fraction crystallizable; ICI: immune checkpoint inhibitors; imAE: immune-mediated adverse events; mCRC: metastatic colorectal cancer. MRI: magnetic resonance imaging; MSI-H: microsatellite instability-high; MSS: microsatellite stable; ORR: objective response rate; PD: progressive disease; PR: partial response; RECISTv1.1: Response Evaluation Criteria in Solid Tumor version 1.1; RP2D: recommended Phase 2 dose. SD: stable disease; Q3W: every 3 weeks; Q6W: every 6 weeks; TF: tumor fraction; Treg: regulatory T-cell.

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REFERENCES: **1.** Siegel, CA Cancer J Clin.2023;73:233. **2.** Grothey. Lancet. 2013;381:303. **3.** Mayer, N Engl J Med. 2015;372:1909. **4.** Li, JAMA. 2018;319:2486. **5.** Prager, N Engl J Med 2023;388:1657-67. **6.** Sahin, Am Soc Clin Oncol Educ Book. 2022:42:1. **7.** Overman, ASCO 2016. **8.** Bullock, Nature Medicine 2024: 30 2558–2567. **9.** Li, ASCO 2025. **10.** Fakih, ASCO GI 2025. **11.** Jenkins, J ImmunoTherapy Cancer 2023. **12.** Davar, ESMO IO 2023. **13.** Davar, SITC 2024. **14.** Fakih, ASCO 2025.

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