Serial ctDNA Monitoring to Predict Response to Systemic Therapy in Metastatic Gastrointestinal Cancers

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ABSTRACT

Purpose: ctDNA offers a promising, noninvasive approach to monitor therapeutic efficacy in real-time. We explored whether the quantitative percent change in ctDNA early after therapy initiation can predict treatment response and progression-free survival (PFS) in patients with metastatic gastrointestinal cancer.

Experimental Design: A total of 138 patients with metastatic gastrointestinal cancers and tumor profiling by next-generation sequencing had serial blood draws pretreatment and at scheduled intervals during therapy. ctDNA was assessed using individualized droplet digital PCR measuring the mutant allele fraction in plasma of mutations identified in tumor biopsies. ctDNA changes were correlated with tumor markers and radiographic response.

Results: A total of 138 patients enrolled. A total of 101 patients were evaluable for ctDNA and 68 for tumor markers at 4 weeks. Percent change of ctDNA by 4 weeks predicted partial response (PR, $P < 0.0001$) and clinical benefit [CB: PR and stable disease (SD), $P < 0.0001$]. ctDNA decreased by 98% (median) and >30% for all PR patients. ctDNA change at 8 weeks, but not 2 weeks, also predicted CB ($P < 0.0001$). Four-week change in tumor markers also predicted response ($P = 0.0026$) and CB ($P = 0.022$). However, at a clinically relevant specificity threshold of 90%, 4-week ctDNA change more effectively predicted CB versus tumor markers, with a sensitivity of 60% versus 24%, respectively ($P = 0.0109$). Patients whose 4-week ctDNA decreased beyond this threshold (≥30% decrease) had a median PFS of 175 days versus 59.5 days (HR, 3.29; 95% CI, 1.55–7.00; $P < 0.0001$).

Conclusions: Serial ctDNA monitoring may provide early indication of response to systemic therapy in patients with metastatic gastrointestinal cancer prior to radiographic assessments and may outperform standard tumor markers, warranting further evaluation.

Introduction

Analysis of circulating tumor DNA (ctDNA), commonly referred to as "liquid biopsy," is a noninvasive way to detect and measure cancer-specific molecular alterations in the blood (1–5). The use of ctDNA is emerging as a useful tool in several settings, including detection of postsurgical residual disease and identifying mechanisms of drug resistance (6–16). Recent data suggest that ctDNA levels within an individual patient correlate with tumor burden over time and that serial assessment of ctDNA may represent a promising approach for monitoring treatment response, with early decreases in ctDNA serving as a predictor of response (17–25). However, further clinical evaluation of ctDNA monitoring as a means of tracking therapeutic response is needed.

Currently, radiographic imaging remains the gold-standard for evaluating treatment response. However, imaging is typically performed several months into therapy, and more frequent radiographic assessment may not be practical or informative. Serum tumor markers (i.e., CEA, CA19-9) have also been used as a means of minimally invasive monitoring of treatment response, but the longer half-lives of these markers and lack of tumor-specificity can limit their performance (26, 27). A more accurate means for early prediction of therapeutic response could be beneficial to distinguish patients most likely to benefit from continued therapy from patients unlikely to benefit, in whom an earlier switch to an alternative therapy may spare toxicity and provide clinical benefit (CB). In this regard, ctDNA represents a promising approach to monitor treatment response and help with early prediction of therapeutic efficacy. ctDNA has the advantages of having a short half-life (∼one hour), high tumor specificity, and can be performed noninvasively at more frequent intervals than imaging (28). However, the utility of serial ctDNA monitoring to predict therapeutic response has not been well characterized.

In this study, we sought to perform a proof-of-concept analysis evaluating the use of serial ctDNA monitoring to predict treatment response in patients with metastatic gastrointestinal cancer receiving systemic therapy. In this prospective cohort, we evaluated whether an early change in ctDNA levels can predict radiographic response to treatment across patients with metastatic gastrointestinal cancer and compared how ctDNA performed relative to standard tumor markers.

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

While prior studies suggest that a directional change in ctDNA levels correlates generally with therapeutic response, it is unclear whether the quantitative ctDNA change might provide an early predictor of response with sufficient accuracy to guide treatment decisions. This proof-of-concept study in patients with metastatic gastrointestinal cancer suggests that the quantitative measure of ctDNA reduction by 4 weeks of therapy provides an accurate prediction of eventual radiographic response and progression-free survival, with favorable performance relative to standard tumor markers. Our study also provides key insights into the optimal timing of ctDNA assessment and the degree of ctDNA reduction corresponding to clinical benefit. While further evaluation in larger studies is needed, serial ctDNA monitoring could facilitate adaptive clinical trial design and help clinicians make more personalized treatment decisions for early adaptation of therapy, limiting the cost and toxicity from ineffective therapies, and allowing a more rapid switch to potentially more effective therapies.

Materials and Methods

Patients and sample collection

Between 2014 and 2018, we enrolled 138 patients with metastatic gastrointestinal cancers. All patients provided informed written consent, and specimens were collected at the Massachusetts General Hospital (MGH) Cancer Center (Boston, MA) according to Institutional Review Board–approved protocols in accordance with the Declaration of Helsinki. Patients were followed during standard-of-care cytotoxic chemotherapy or targeted therapy. Targeted therapies included EGFR-, BRAF-, HER2-, FGFR-, or MET (**Table 1**)-directed therapy. Blood and tumor specimens were obtained. Tumor mutational profiling was performed at MGH (Boston, MA) as part of routine clinical care through a standard clinical institutional next-generation sequencing panel for 104 known cancer genes. Blood was drawn prior to the start of therapy and after initiation of therapy at 2 weeks, 4 weeks, 8 weeks, and then every 8 weeks until progression (**Fig. 1A**). Cell-free DNA was extracted from plasma using QIAamp Circulating Nucleic Acid Kit (Qiagen) and assessed by digital droplet PCR using probes for tumor-specific point mutations (see Supplementary Methods for full ddPCR methods). To improve accuracy, one or more tumor-specific alterations likely to be clonal based on clinical sequencing were identified and used to evaluate ctDNA longitudinally in available plasma specimens (Supplementary Table S1). For patients with multiple assessable mutations, the percent change in mutant allele fraction of up to three mutations ctDNA were averaged. Informative tumor markers, if available during the same timepoints, were also analyzed, and the more dynamic tumor marker was chosen if multiple tumor markers were informative. RECIST 1.1 measurements determined by independent radiological review were used to compare baseline CT scans with first restaging scans during treatment. If unavailable, progression was determined clinically by the investigators (29). Progression-free survival (PFS) on treatment was determined by investigator review.

Table 1. Baseline patient and disease characteristics.

Characteristic	$N = 138$
Median age, years (range)	61 (21–87)
Sex (M)	84 (61%)
Race	
White	122 (88%)
Asian	6 (4.3%)
Black	2 (1.4%)
Unknown	8 (5.8%)
Primary tumor location	
Colorectal	69 (50%)
Pancreas	26 (19%)
Biliary	18 (13%)
Esophagogastric	17 (12%)
Other	8 (5.8%)
Therapy types	
Cytotoxic therapy only	97 (70%)
Targeted therapy only	23 (17%)
Targeted + cytotoxic therapy	18 (13%)
Metastatic at diagnosis	83 (60%)
Median time from metastatic diagnosis to treatment start, months (range)	2.1 (0–205)
Lines of prior metastatic therapy, median (range)	0 (0–6)
First-line therapy	78 (57%)
Second-line therapy	31 (22%)
Third-line therapy or later	29 (21%)

Eligible patients had a histologically confirmed metastatic cancer diagnosis from a gastrointestinal primary, received only systemic therapy before first scan, tumor tissue which was genotyped, a baseline plasma draw within 3-weeks prior to treatment start, and a 4-week plasma draw (average time of 4 weeks drawn between 20–45 days). We evaluated how change in ctDNA levels or change in tumor markers predicted response to treatment and CB (defined as patients who had a PR or an SD primary, had received only systemic therapy as part of their treatment before scan, and had tumor genotyping).

Statistical analyses

We compared the distributions of percent change in ctDNA and in tumor markers at 4 weeks between response categories using Mann–Whitney *U* tests and Kruskal–Wallis tests. For both ctDNA and tumor markers, we identified the cutoff for percent change at 4 weeks yielding approximately 90% specificity (i.e., 90% of patients with CB classified correctly) and compared the sensitivity and positive predictive value associated with the cutoff between ctDNA and tumor markers using Fisher exact test. PFS on treatment was defined as time from treatment start to progression or death (event) or most recent treatment date (censored) and summarized using the Kaplan–Meier method. We compared PFS between patients who did and did not exceed the week four percent change cutoff for ctDNA/tumor markers using the log-rank test and computed the log-rank HR. We evaluated whether clinical characteristics including cancer type, treatment type, and number of prior lines of treatment were confounders in the relationship between outcomes and percent change of ctDNA at 4 weeks using univariate and multivariate logistic (PR and CB outcomes) and Cox proportional hazards (PFS outcome) regression models. We assessed the impact of covariate adjustment on the statistical significance and effect estimate for ctDNA percent change between the univariate and multivariate models for each outcome. As exploratory analyses, we compared the distributions of percent change in ctDNA at 2 weeks and 8 weeks between response categories as described above and performed subgroup analyses by tumor type and treatment type. All analyses were conducted among patients with available data for the

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specified variable and time point. The two-sided significance level was 0.05 for all comparisons.

Results

Overall, 138 patients met eligibility criteria and were enrolled—50% colorectal cancer, 29% pancreatic cancer, 13% biliary cancers, 12% esophagogastric cancer, and 6% other gastrointestinal primaries (**Table 1**). A total of 70% were treated with cytotoxic chemotherapy, 17% with targeted therapy, and 13% with targeted therapy in combination with cytotoxic chemotherapy (**Table 1**; Supplementary Table S2). In 101 patients, we identified at least one mutation that could be tracked in ctDNA that was detectable at baseline (Supplementary Table S3). A subset of 68 patients had evaluable tumor

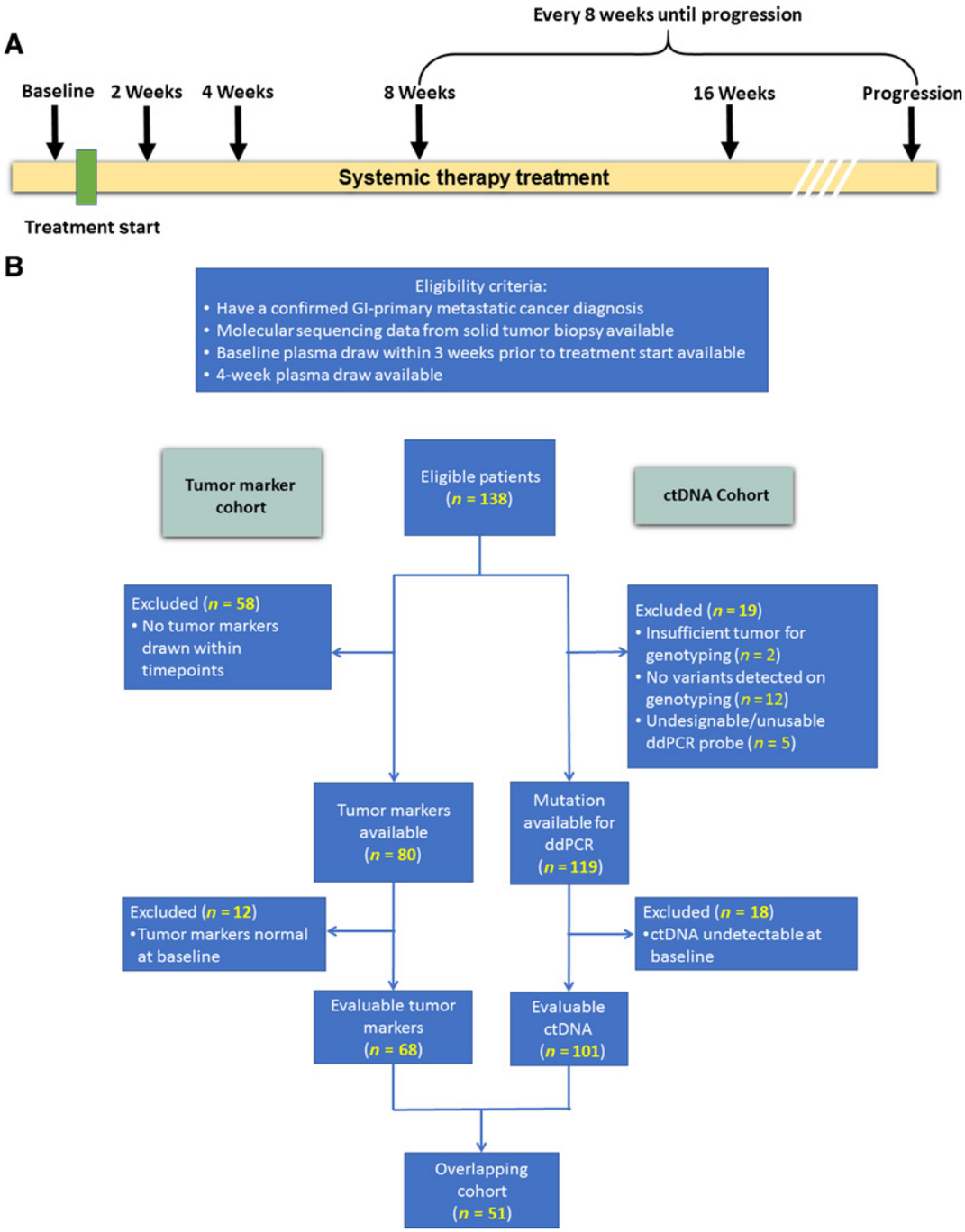

Figure 1.
Draw schedule (**A**) and CONSORT diagram (**B**).

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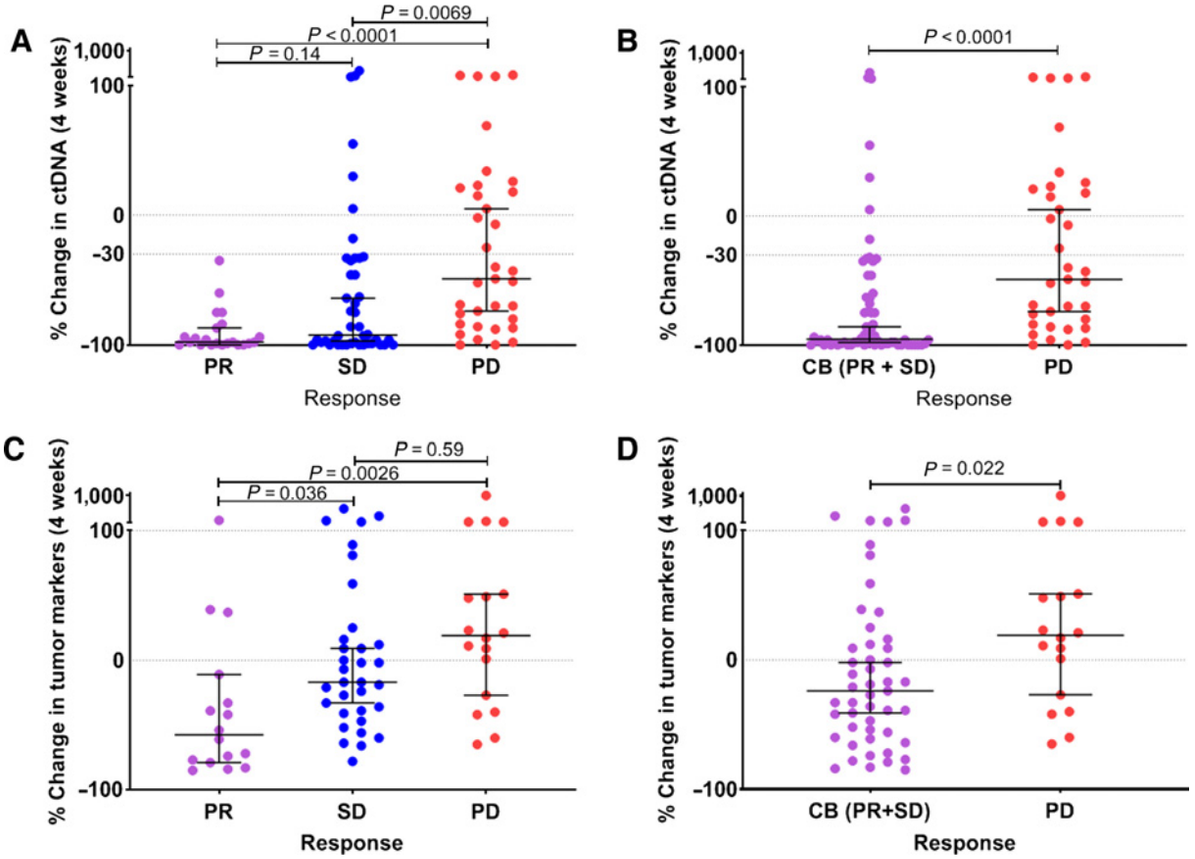

Figure 2.

Changes in ctDNA (**A**, **B**) and tumor markers (**C**, **D**) at 4 weeks are shown for patients grouped by radiographic response by RECIST1.1 criteria. Each data point represents the percent change in ctDNA or tumor markers at 4 weeks relative to baseline for a single patient. Horizontal bars represent the median, and error bars indicate 95% CI. SD: stable disease.

markers within the specified timepoints (Supplementary Table S4). A total of 51 patients had both tumor markers and ctDNA that were evaluable at 4 weeks (**Fig. 1B**). Of the patients with evaluable ctDNA, 27 patients had 2-week draws and 85 patients had 8-week draws. For ctDNA, the average time from treatment start until the 4-week blood draw for ctDNA analysis was 29.9 days ± 4.8 (standard deviation) while the average time to first restaging scan was 55.4 days ± 19.8 (standard deviation).

We observed that the percent change in ctDNA mutant allele fraction (MAF) at 4 weeks predicted radiographic PR and CB (PR or SD). Patients achieving PR had a median ctDNA decrease at 4 weeks of 98.0% compared with patients with progressive disease (PD) who had a median decrease of 49.0% ($P < 0.0001$; **Fig. 2A**). Notably, all patients with PR had a decrease of ctDNA of >30%. ctDNA change was also predictive of CB, with a median decrease of 95.5% observed in these patients (**Fig. 2B**). Patients with PR or SD also had a significantly greater decrease of standard tumor markers (median −57.50% and median −7.00%, respectively) compared with those with PD (median 21.0%, $P = 0.0026$ for PR vs. PD; **Fig. 2C**). The change in tumor markers was also predictive of CB ($P = 0.022$; **Fig. 2D**).

We next assessed the sensitivity and positive predictive value (PPV) for predicting CB of ctDNA and tumor markers at a clinically relevant specificity threshold of approximately 90%, at which no more than one out of every 10 patients who would achieve CB from therapy would fail to be identified with each respective assay. Interestingly, all patients achieving PR exhibited ctDNA decreases beyond this threshold, which equated to a ctDNA decrease of 30% or greater. Of 28 patients who did not have a ctDNA decrease reaching this threshold, all but 2 (26 pts, 93%) developed PD and discontinued treatment within 4 months of starting therapy. At this threshold, the sensitivity of ctDNA for predicting CB was 60% and PPV was 75%. In contrast, a change in tumor markers at a similar threshold yielding approximately 90% specificity for CB had a sensitivity of only 24% with a PPV of 44%. The difference between sensitivity of ctDNA versus tumor markers was statistically significant (0.0109; Supplementary Table S5.)

Furthermore, we performed additional exploratory analyses of ctDNA at this 90% specificity threshold. A >30% decrease in ctDNA also predicted PFS (HR, 3.29; 95% CI, 1.55–7.00; $P < 0.0001$). Patients whose ctDNA decreased by >30% had a median survival of 175 days, while patients whose ctDNA did not had a median survival of 59.5 days (**Fig. 3A**). Similarly, patients with a ctDNA decrease of >30% after 8 weeks of therapy also showed improved PFS (HR, 4.34; 95% CI, 1.69–11.11; $P < 0.0001$; median survival 183 days vs. 64 days, respectively; **Fig. 3B**).

Interestingly, we observed possible differences in the association of ctDNA changes with PFS based on tumor type. Most notably, ctDNA change showed a striking association with PFS in colorectal

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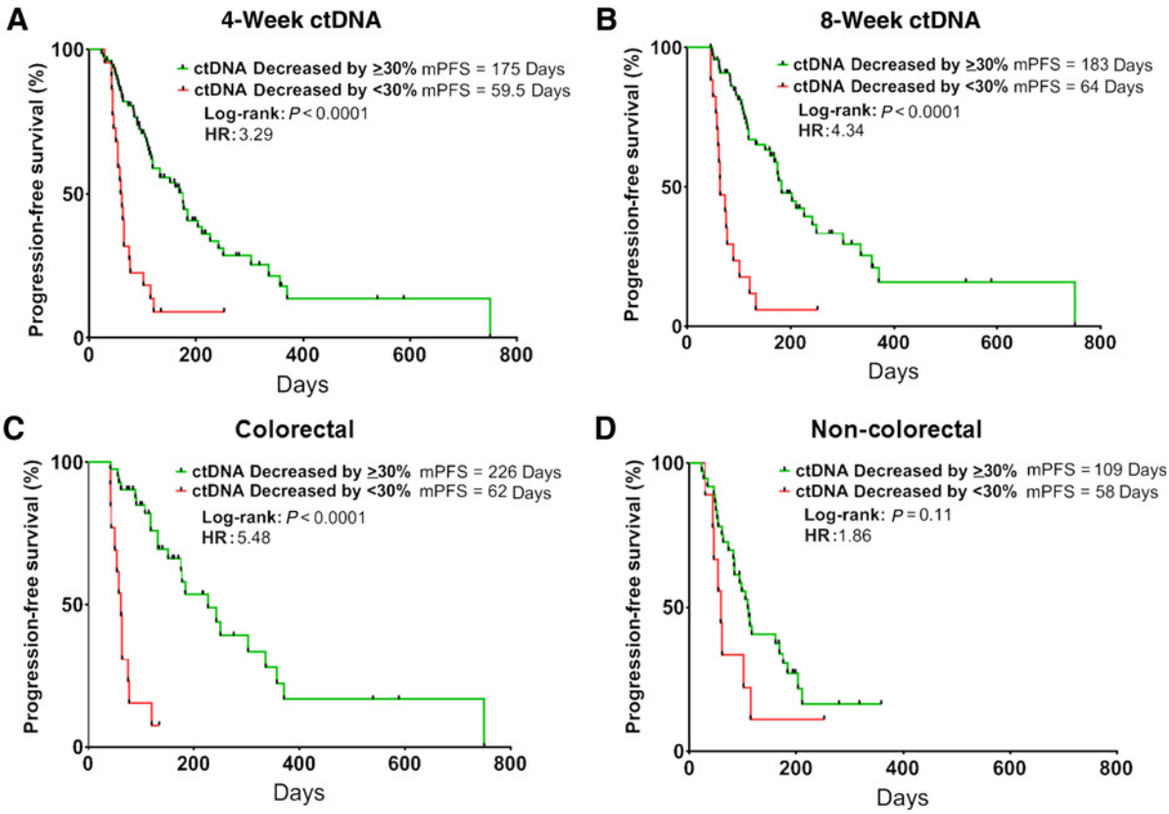

Figure 3.

Kaplan–Meier curves showing PFS by percent change in ctDNA for all patients at 4 weeks (**A**) and 8 weeks (**B**) from treatment initiation, or for patients with colorectal cancer only (**C**) or noncolorectal cancer at 4 weeks (**D**). mPFS: median progression-free survival.

cancer ($n = 55$), where patients whose ctDNA decreased by >30% had a median survival of 226 days on treatment, compared with just 62 days (HR, 5.484; 95% CI, 1.69–17.78; $P < 0.0001$). Conversely, while a clear trend was noted, the association of ctDNA change with PFS did not reach statistical significance in patients with noncolorectal cancer patients ($n = 46$; $P = 0.11$; **Fig. 3C** and **D**). Similarly, while ctDNA change predicted response (PR vs. PD) as well as CB in patients with colorectal cancer ($P < 0.0001$), this association did not reach statistical significance in patients with noncolorectal cancer ($P = 0.085$, $P = 0.086$, respectively), although a similar trend was observed (Supplementary Fig. S1A–S1D).

However, we observed that ctDNA change did predict PFS across tumor types in patients receiving targeted therapy with or without chemotherapy (HR, 3.49; 95% CI, 1.19–10.30; $P = 0.0002$) and in patients receiving chemotherapy alone (HR, 2.95; 95% CI, 1.06–8.22; $P = 0.0017$; Supplementary Fig. S2A and S2B). Similarly, ctDNA was predictive of CB for patients receiving targeted therapy ($P < 0.0001$) and chemotherapy alone ($P = 0.043$), although ctDNA was better able to predict radiographic response in patients receiving targeted therapy ($n = 34$) versus cytotoxic chemotherapy alone ($n = 67$; $P = 0.0003$ vs. $P = 0.17$, respectively; Supplementary Fig. S2B–S2F). We also evaluated patients based on line of therapy and found that ctDNA change was predictive of CB across different lines of therapy (Supplementary Fig. S3A–S3C).

Given the heterogeneity of tumor types, treatment types, and lines of therapy, we performed a multivariate analysis adjusting for these variables and found that a ctDNA change of at least 30% remained significantly associated with both CB (OR = 6.9; 95% CI, 2.304–20.732;

Table 2. Unadjusted and adjusted associations between 30% decrease in ctDNA at 4 weeks and clinical outcomes.

	Unadjusted (univariate)		Adjusted (multivariate)[a]	
Outcome	**OR/HR[b] (95% CI)**	***P***	**OR/HR[b] 95% CI**	***P***
Partial response	—[c]	—	—[c]	—
Clinical benefit	OR = 6.321 (2.256–17.717)	0.0005	OR = 6.912 (2.304–20.732)	0.0006
Progression-free survival	HR = 0.277 (0.161–0.479)	<0.0001	HR = 0.324 (0.183–0.571)	0.0001

[a]Adjusted for diagnosis, treatment type, and number of prior lines of therapy.
[b]ORs/HRs represent odds/risk of outcome for decrease ≥30% in week 4 ctDNA compared with change > −30%.
[c]Cannot be estimated because all patients with PR had ≥30% decrease in week 4 ctDNA.

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P = 0.006) and PFS (HR, 0.324; 95% CI, 0.183–0.571; P = 0.0001; **Table 2**). In addition, the unadjusted and adjusted ORs and HRs were similar in magnitude, providing further evidence that our unadjusted results are not confounded by the heterogeneity in clinical characteristics.

Finally, we performed an exploratory analysis assessing the optimal timing for ctDNA assessment for prediction of CB. While earlier prediction of therapeutic response would certainly have advantages, we observed that at 2 weeks, change in ctDNA did not show a statistically significant correlation with treatment

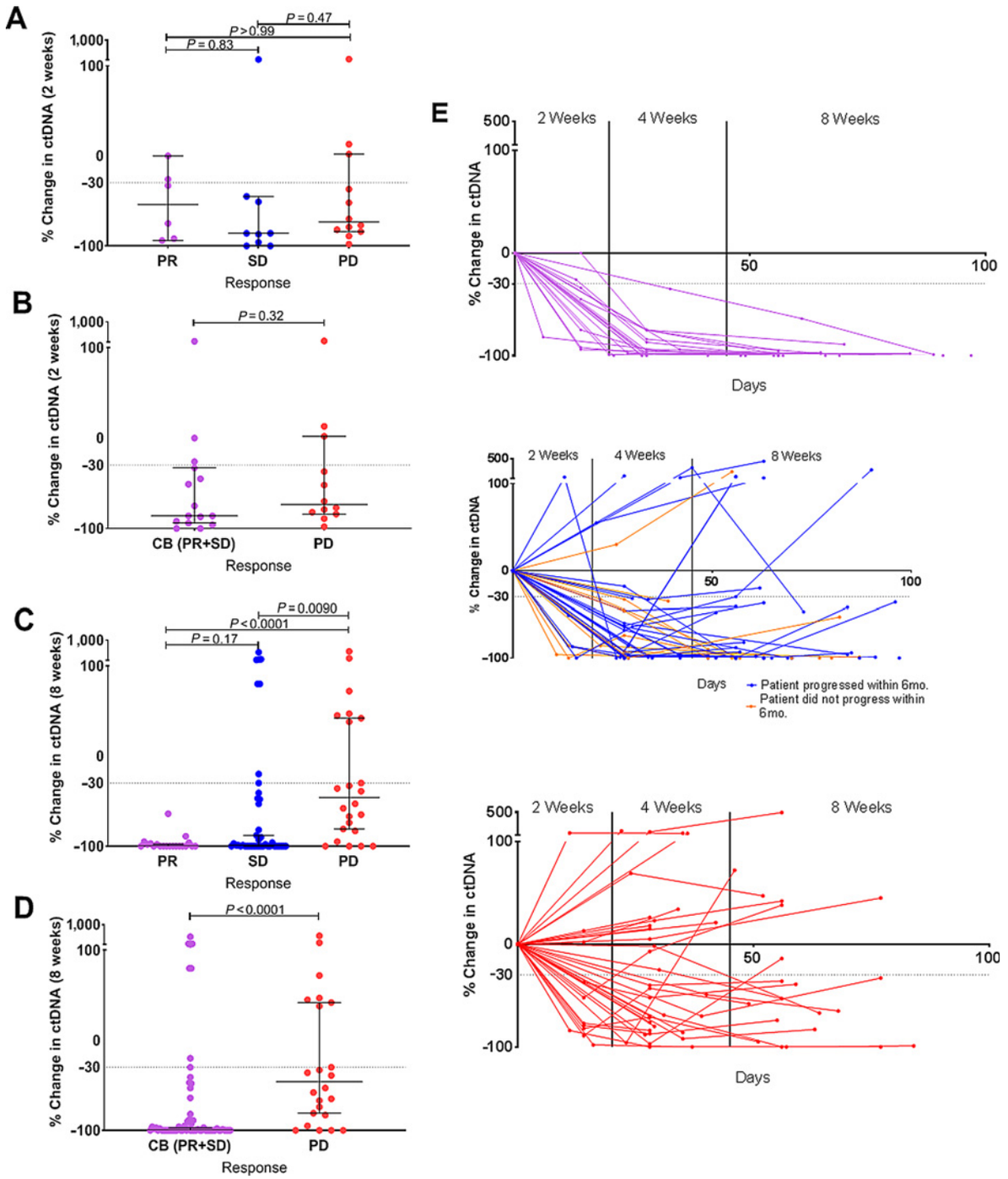

Figure 4.

Changes in ctDNA at 2 weeks (**A**, **B**) or 8 weeks (**C**, **D**) of treatment by RECIST1.1 criteria in patients achieving PR, SD (stable disease), and PD (**A**, **C**) or CB and PD (**B**, **D**). **E,** Longitudinal ctDNA changes during the first 100 days of therapy in patients with PR (top, purple lines), SD (middle, blue and orange lines), and PD cases (bottom, red lines). For SD patients, blue lines represent patients with PFS < 6 months and orange lines represent patients with PFS > 6 months. Horizontal bars represent the median, and error bars indicate 95% CI.

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response or CB, but at 8 weeks a similar degree of statistical significance observed at 4 weeks remained between patients achieving PR (median −100%) vs. PD (median −46.0%; $P < 0.0001$) as well as SD (median −99.0%) and PD ($P = 0.0090$; **Fig. 4A–C**). At 8 weeks, ctDNA change also predicted CB ($P < 0.0001$; **Fig. 4D**). Even when the analysis was restricted to the 27 patients who had 2-week draws (all had 4-week draws, and all but 5 patients who progressed prior to 8 weeks had 8-week draws), we found that 4-week and 8-week ctDNA change remained a statistically significant predictor of CB, whereas 2-week ctDNA change did not show a statistically significant association. (Supplementary Fig. S4A–S4D). We observed continued evolution of ctDNA levels from 2 weeks to 4 weeks in many patients (**Fig. 4E**). For patients achieving PR at the first set of scans, ctDNA decline was remarkably consistent (**Fig. 4E**). Most patients with PR had benefit beyond 6 months, with only 6 patients with PR progressing within 6 months. Interestingly, for patients achieving SD, over time a rise in ctDNA levels was seen in many patients who developed PD within 6 months (**Fig. 4E**, blue), whereas ctDNA levels remained suppressed in most patients remaining on therapy for more than 6 months prior to developing PD (**Fig. 4D**, orange). The changes highlight the ability of ctDNA detection to predict CB longitudinally and the ability to detect a dynamic increase in ctDNA levels at progression (Supplementary Fig. S5; Supplementary Table S4).

Discussion

This proof-of-concept study suggests that serial ctDNA monitoring may provide an early and reliable predictor of treatment response and CB to systemic therapy. We observed a rapid and consistent decline in ctDNA levels during the first 4 weeks of systemic therapy in patients achieving PR or CB. All patients who achieved a PR had a decrease in ctDNA levels by 4 weeks of treatment of at least 30%, with most exhibiting a near complete decline (median 98% decrease). Importantly, the decrease in ctDNA levels by 4 weeks in patients achieving PR or CB was significantly greater than in patients with PD. Notably, all patients achieving PR had decreases in ctDNA beyond 30% and of the 28 patients who did not have a ctDNA decrease reaching this threshold, 93% of patients developed PD and discontinued treatment within 4 months of starting therapy (Supplementary Fig. 5). ctDNA decrease of 30% or more also predicted a stark difference in PFS, median PFS of 175 days versus 59.5 days (HR, 3.29; 95% CI, 1.55–7.00; $P < 0.0001$).

These data suggest that further evaluation of ctDNA monitoring and its potential for early prediction of response or lack of therapeutic benefit is warranted and that serial ctDNA monitoring could offer early insight into whether a patient is responding to a given therapy and should therefore continue that therapy, or whether a patient is unlikely to respond. Early identification of patients who are not responding to therapy would allow a switch to an alternative therapy sooner, increasing the chance of potential benefit, and reducing unnecessary toxicity from an ineffective therapy. Thus, serial ctDNA monitoring could increase the efficiency of personalized therapeutic decisions for individual patients and allow for adaptive clinical trials where therapy might be modulated on the basis of ctDNA.

Notably, change in standard serum tumor markers—CEA and CA19-9—by 4 weeks also exhibited an association with response and CB, although with more limited statistical confidence than for ctDNA ($P = 0.021$ tumor markers vs. $P < 0.0001$ for ctDNA). Therefore, we conducted an exploratory analysis comparing the effectiveness of ctDNA versus tumor markers in predicting CB. We assessed the predictive power of ctDNA or tumor makers at a specificity threshold of 90%. This threshold was selected as a potential clinically relevant specificity cutoff, such that if a treatment were to be discontinued or changed due to lack of a sufficient decrease in either marker, then no more than 1 of 10 patients who would go on to derive some CB would have treatment discontinued on the basis of this result. At this 90% specificity threshold, the sensitivity for predicting CB was 60% for ctDNA versus only 24% for tumor markers ($P = 0.0109$). These results suggest that ctDNA monitoring may potentially outperform standard tumor markers at this early time point and ctDNA monitoring may offer advantages over the current standard of care. It is possible that the shorter half-life and increased tumor specificity of ctDNA may provide advantages over standard tumor markers for monitoring of treatment response (30, 31).

We also explored how early after treatment initiation ctDNA could predict response. An earlier ability to predict whether a patient is benefiting, may allow an earlier switch of a nonresponding patient to a more effective therapy, increasing the chance of benefit and limiting unnecessary exposure. However, our initial analysis suggests that if ctDNA is assessed too early after the initiation of therapy, its predictive power is more limited. At 2 weeks after the start of therapy, we did not observe a significant association between change in ctDNA levels and response or CB, as we did at 4 and 8 weeks, although the number of patients with available 2-week plasma samples was limited. One potential explanation may be due to the kinetics of ctDNA release during therapy. ctDNA levels may increase acutely after initiation of therapy, due to release of tumor DNA as a result of tumor cell death, before decreasing in parallel with a reduction in tumor burden (32). Indeed, additional evolution of ctDNA levels was observed between 2 weeks and 4 weeks (**Fig. 4E**), and specifically, further decreases in ctDNA were observed by 4 weeks in patients achieving PR. These data suggest that further optimization of the timing of ctDNA assessment following therapeutic initiation will be critical.

While we observed a highly consistent decrease in ctDNA in patients achieving PR, the change in ctDNA levels by 4 weeks in patients with SD or PD was more variable. For SD patients, this may reflect the fact that SD, as defined by RECIST 1.1, includes patients who achieve some degree of tumor shrinkage not reaching criteria for PR, as well as patients whose tumors increase in size but not by enough to meet criteria for PD. In SD patients achieving a PFS of >6 months, a far more consistent decrease in ctDNA levels by 4 weeks was observed (**Fig. 4E**). Conversely, SD patients with PFS <6 months typically exhibited a rise or rebound in ctDNA by 8 weeks. Similarly, PD patients exhibiting an initial reduction in ctDNA levels by 4 weeks, also showed a rise or rebound in ctDNA levels by 8 weeks. Likewise, in patients with PR or prolonged SD, a consistent rebound in ctDNA levels was observed as patients developed eventual disease progression (Supplementary Fig. S3). These findings support the potential importance of serial monitoring of ctDNA to gain further insight into the evolution of a patient's response over time.

This study does have several limitations. First, while over 100 patients were evaluated, the overall sample size is still limited, and not all cases had both serial tumor marker and ctDNA assessments. Second, we were unable to evaluate ctDNA in several patients (**Fig. 1B**). In some cases, this was because no mutations were detected upon clinical tumor sequencing. This issue could be overcome by performing more broad-based tumor sequencing facilitating the identification of trackable DNA mutations in more patients. In other cases, a customized ddPCR probe could not be designed for specific mutations or patients did not have detectable baseline levels of ctDNA. While the proportion of patients with unevaluable ctDNA was similar to patients whose baseline tumor markers were in the normal range and thus also unevaluable, it is possible that different ctDNA

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technologies, including larger next-generation sequencing panels, customized multiplexed mutation assays, multiple mutation tracking or including methylation markers could be more effective for tracking ctDNA in more patients (33–35). In this study, individualized ddPCR was utilized as a means of establishing clinical proof-of-concept for serial ctDNA monitoring, and is not necessarily the optimal approach. Third, several patients had very low levels at baseline meaning small fluctuations in ctDNA levels over time could lead to large calculated percent changes, potentially affecting the accuracy of response prediction. Indeed, outlier values were often observed in patients with very low baseline levels of ctDNA (Supplementary Table S3), and lower levels of baseline ctDNA observed in patients with noncolorectal cancer (Supplementary Fig. S1E and S1F) may be one explanation for why ctDNA change was less effective in predicting response and PFS in patients with noncolorectal versus colorectal cancer (**Fig. 3C** and **D**; Supplementary Fig. S1A–S1D). In future studies, the use of more sensitive technologies or determination of a minimum basal level of ctDNA for accurate interpretation may be important to overcome this potential issue and to define effective thresholds for clinical decision-making.

In summary, these data suggest that serial monitoring of ctDNA has the potential to provide an early indication of treatment response and CB across a range of gastrointestinal cancers receiving an array of systemic cytotoxic and/or targeted therapies. While larger and more comprehensive studies are needed to define the optimal timing of ctDNA assessments, to determine the most accurate thresholds for response prediction, and to evaluate the most suitable and cost-effective technologies for ctDNA measurement, serial ctDNA monitoring has the potential to help guide clinicians in making more personalized treatment decisions and to facilitate early adaptation of therapy to limit the cost and toxicity from ineffective therapies and to allow a more rapid switch to a potentially more effective therapy. Moreover, serial monitoring of ctDNA could be used as an early marker of efficacy or lack of efficacy to facilitate adaptive clinical trial strategies. Thus, further prospective assessment of serial ctDNA monitoring as a means of predicting therapeutic response is warranted.

Disclosure of Potential Conflicts of Interest

A.R. Parikh is an employee/paid consultant for Foundation Medicine, Eli Lilly, and Puretech, and reports receiving other commercial research support from Bristol-Myers Squibb, Guardant Health, Array, Novartis, Macrogenics, Plexxicon, and Tesaro. D.P. Ryan is an employee/paid consultant for MPM Capital, Gritstone, and Oncorus; holds ownership interest (including patents) in MPM Capital and Acworth Pharma; and reports receiving other remuneration from Uptodate, Johns Hopkins University Press, and McGraw-Hill. L. Goyal is an employee/paid consultant for Taiho Pharmaceuticals, Debiopharm, Klus Pharmaceuticals, Agios Pharmaceuticals, QED, Alentis Pharmaceuticals, and Incyte Pharmaceuticals. E. Roeland is an employee/paid consultant for Napo Pharmaceuticals, Heron Therapeutics, BASF, Oragenics, Asahi Kasei Pharma, Vector Oncology, Imuneering, and Prime Oncology. A.X. Zhu is an employee/paid consultant for AstraZeneca, Bayer, Eisai, Lilly, Merck, Exelisis, and Roche/Genentech. A.J. Iafrate is an employee/paid consultant for Repare Therapeutics, reports receiving commercial research grants from Sanofi, and holds ownership interest (including patents) in ArcherDx. T.S. Hong is an employee/paid consultant for Synthetic Biologics and Novocure, and reports receiving other commercial research support from AstraZeneca, Taiho, Bristol-Myers Squibb, and Intraop. R. B. Corcoran is an employee/paid consultant for Amgen, Array Biopharma, Astex, Avidity Biosciences, Bristol-Myers Squibb, C4 Therapeutics, Chugai, Elicio, FOG Pharma, Found Therapeutics/Kinnate Biopharma, Genentech, Guardant Health, LOXO, Merrimack, N-of-One, Novartis, nRichDx, Revolution Medicines, Roche, Roivant, Shionogi, Spectrum Pharmaceuticals, Symphogen, Taiho, and Warp Drive Bio; reports receiving commercial research grants from Lilly, Asana, and AstraZeneca; and holds ownership interest (including patents) in Avidity Biosciences, C4 Therapeutics, nRichDx, and Found Therapeutics/Kinnate Biopharma. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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