BIOMARKERS

original reports

Tumor-Naïve Circulating Tumor DNA as an Early Response Biomarker for Patients Treated With Immunotherapy in Early Phase Clinical Trials

Enrique Sanz-Garcia, MD, PhD[1]; Sofia Genta, MD[1]; Xiaoxi Chen, MSc[2]; Qiuxiang Ou, PhD[2]; Daniel V. Araujo, MD[1,3]; Albiruni R. Abdul Razak, MD[1]; Aaron R. Hansen, MD[1]; Anna Spreafico, MD, PhD[1]; Hua Bao, PhD[2]; Xue Wu, PhD[2]; Lillian L. Siu, MD[1]; and Philippe L. Bedard, MD[1]

abstract

PURPOSE To evaluate early circulating tumor DNA (ctDNA) kinetics using a tumor-naïve assay and correlate it with clinical outcomes in early phase immunotherapy (IO) trials.

METHODS Plasma samples were analyzed using a 425-gene next-generation sequencing panel at baseline and before cycle 2 (3-4 weeks) in patients with advanced solid tumors treated with investigational IO agents. Variant allele frequency (VAF) for mutations in each gene, mean VAF (mVAF) from all mutations, and change in mVAF between both time points were calculated. Hyperprogression (HyperPD) was measured using Matos and Caramella criteria.

RESULTS A total of 162 plasma samples were collected from 81 patients with 27 different tumor types. Patients were treated in 37 different IO phase I/II trials, 72% of which involved a PD-1/PD-L1 inhibitor. ctDNA was detected in 122 plasma samples (75.3%). A decrease in mVAF from baseline to precycle 2 was observed in 24 patients (37.5%) and was associated with longer progression-free survival (hazard ratio [HR], 0.43; 95% CI, 0.24 to 0.77; $P < .01$) and overall survival (HR, 0.54; 95% CI, 0.3 to 0.96; $P = .03$) compared with an increase. These differences were more marked if there was a >50% decrease in mVAF for both progression-free survival (HR, 0.29; 95% CI, 0.13 to 0.62; $P < .001$) and overall survival (HR, 0.23; 95% CI, 0.09 to 0.6; $P = .001$). No differences in mVAF changes were observed between the HyperPD and progressive disease patients.

CONCLUSION A decrease in ctDNA within 4 weeks of treatment was associated with treatment outcomes in patients in early phase IO trials. Tumor-naïve ctDNA assays may be useful for identifying early treatment benefits in phase I/II IO trials.

JCO Precis Oncol 7:e2200509. © 2023 by American Society of Clinical Oncology

INTRODUCTION

Circulating tumor DNA (ctDNA) is a promising noninvasive approach to characterize tumor-related alterations in plasma.[1,2] Tumor-informed assays use results from genome or exome sequencing from tumor tissue to track specific alterations in ctDNA. In contrast, tumor-naïve assays are based on detecting a fixed number of genomic alterations (panels) and do not rely on tumor tissue availability for sequencing. Recent studies have shown that early changes in ctDNA levels over time (kinetics) are associated with long-term treatment outcomes[3] and response to chemotherapy and targeted therapy.[4-7] Similar findings have been observed in patients treated with immunotherapy (IO), including immune checkpoint inhibitors (ICIs) targeting PD-1/PD-L1 in melanoma, lung, and gastric cancers.[8-10] A decrease in ctDNA after 6 weeks of treatment is associated with long-term treatment outcomes in two distinct cohorts of pan-cancer patients treated with ICIs.[11,12]

Early phase clinical trials are a key step in drug development. Many investigational drugs do not move to later stages because of safety concerns or limited efficacy.[13] The majority of anticancer drugs in early phase trials are currently IO agents.[14] However, these trials face new challenges in terms of evaluating dose-limiting toxicities, demonstrating antitumor activity and establishing the recommended phase II dose for further clinical development.[15] There is growing support to move to a model in which selection of the recommended phase II dose is guided not only by the highest tolerable dose but also by pharmacodynamic and antitumor activity (Project Optimus).[16] ctDNA is an attractive noninvasive biomarker that may provide early proof-of-mechanism readouts of biological activity for investigational IO agents.[17]

Unique radiographic response patterns have been observed with IO. Pseudoprogression (PseudoPD) is a late response after an initial radiographic increase in tumor burden.[18] By contrast, hyperprogression (HyperPD)

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on February 10, 2023 and published at ascopubs.org/journal/po on April 7, 2023: DOI https://doi.org/10.1200/PO.22.00509

CONTEXT

Key Objective

Early changes in circulating tumor DNA (ctDNA) are associated with radiographic response to various systemic treatment. We evaluated ctDNA kinetics using a tumor-naïve assay in patients treated with experimental immunotherapy (IO) in early phase clinical trials.

Knowledge Generated

Reductions in ctDNA within the first 21 days of treatment in IO-based early phase clinical trials were associated with radiological response and progression-free and overall survival. These associations were more marked with >50% decrease in ctDNA levels from baseline. Patients with radiological hyperprogression did not show greater increases in ctDNA levels compared with patients who had radiological progression but did not meet criteria for hyperprogression.

Relevance

Our study demonstrates that ctDNA is an early pharmacodynamic response biomarker in early phase IO clinical trials that may be useful to inform drug development.

represents an accelerated tumor growth.[19] There is controversy about whether HyperPD is a real biological phenomenon or an artifact of the variability of radiographic response assessments.[20,21] In addition, there is no clear consensus on how to identify HyperPD.[22,23] Early changes in ctDNA may be useful to anticipate to those radiographic response patterns.

We have previously reported a decrease in ctDNA within the first 3 weeks of treatment in three patients who showed radiological response in IO-based phase I/II clinical trials.[24] We hypothesized that early ctDNA kinetics may identify patients more likely to benefit from treatment before radiological response and that there would be different ctDNA kinetics in patients with HyperPD or pseudoPD.

METHODS

Patients and Samples

From December 2017 to March 2020, we prospectively consented patients with advanced solid tumors in early phase clinical trials at the Princess Margaret Cancer Centre in an institutional liquid biopsy biobanking program (LIBERATE, ClinicalTrials.gov identifier: NCT03702309). For this analysis, we included patients treated with investigational IO therapies, including ICIs, bispecific antibodies, vaccines, or cytokines (either in monotherapy or in combination with other immunomodulatory agents or molecular targeted therapy). Patients with available plasma samples before treatment (baseline) and before second cycle (pre-C2) (usually 3-4 weeks after first dose) were included. This study was approved by the Princess Margaret Cancer Centre Research Ethics Board (no. 18-5815).

Patient demographic, clinical, and treatment outcome data were collected. Treatment outcomes were measured as overall survival (OS) from the date of enrollment in the early phase clinical trial, progression-free survival (PFS), and response rate on the basis of iRECIST version 1.1. The clinical benefit rate was considered complete response, partial response, or stable disease (SD) for >6 months. HyperPD was calculated using two different criteria previously published: Caramella (which analyzes both pretreatment and on-treatment radiological assessments) and Matos (which uses only on-treatment radiological assessments).[25,26]

DNA Extraction, Library Preparation, and Sequencing for ctDNA

Sample processing and genomic profiling were performed as previously described.[24] Briefly, plasma and buffy coat were isolated from whole blood after centrifugation and before cell free DNA extraction using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Germantown, MD). Target enrichment was performed using xGen lockdown probes (Integrated DNA Technologies, Coralville, IA) targeting 425 cancer-associated genes (Data Supplement). The capture reaction was performed with Dynabeads M-270 (Life Technologies, Carlsbad, CA) and the xGen Lockdown hybridization and wash kit (Integrated DNA Technologies) according to the manufacturer's protocols. Captured libraries were on-bead polymerase chain reaction (PCR) amplified with Illumina p5 and p7 primers in KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA), followed by purification using Agencourt AMPure XP beads (Beckman Coulter, Indianapolis, IN). Libraries were quantified by quantitative PCR using a KAPA Library Quantification Kit (KAPA Biosystems). Library fragment size was determined using Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). The target-enriched library was then sequenced on an Illumina sequencing platform (Illumina, San Diego, CA), according to the manufacturer's instructions.

Trimmomatic[27] was used for FASTQ file quality control. Leading and trailing of low-quality (quality reading below 20) or N bases were removed. Pair-end reads were then aligned to the human reference genome-19 using the Burrows-Wheeler Aligner[28] with default parameters. PCR deduplication was performed using Picard V2.9.4 (Broad Institute, Cambridge, MA). Local realignment around indels and base quality score recalibration were performed using the Genome Analysis Toolkit (GATK 3.4.0; Broad Institute,

Cambridge, MA). Somatic single-nucleotide variants were identified using MuTect,[29] and small indels were detected using Scalpel Fang.[30] The cutoff for somatic mutation detection was a variant allele frequency (VAF) of 0.5%, with five supporting reads in plasma samples. Somatic mutations were filtered using matched germline DNA obtained from buffy coat peripheral blood mononuclear cells. If a mutation met the above cutoff in at least one sample, the detection cutoff for the same mutation was dropped in other samples to reduce false negatives. The final list of mutations was annotated using vcf2maf.[39]

Statistical Analysis

Demographics and clinical characteristics are summarized as means, medians, and proportions. Associations between categorical variables were examined using Fisher's exact test. ctDNA was quantified using the mean VAF (mVAF) of all detected mutations in ctDNA. Different variants of the same gene were counted as different mutations. The change between the pre-C2 and baseline ctDNA was defined as mVAF change rate = (pre-C2 mVAF − baseline mVAF)/(baseline mVAF + 0.001%) × 100%. To calculate mVAF change rate, all variants were included regardless of whether were present at both time points or only at a single time point. The association between categorical variables and median mVAF was examined using the Wilcoxon test. Kaplan-Meier curves were compared using the log-rank test for survival analysis. Hazard ratios (HRs) were calculated using a Cox proportional hazards model. A two-sided P value of $<.05$ was considered statistically significant. All statistical analyses were performed using R software (v.4.1.1).[40]

RESULTS

Patient Characteristics

A total of 123 patients were included in the cohort of early phase clinical trials within the LIBERATE program (102 patients received IO) (Fig 1). Plasma samples were available for analysis at baseline and at pre-C2 in 81 patients (79%). The median time from the first dose of treatment to pre-C2 sample collection was 21 days (range 19-55). One patient participated in two trials and other in three trials; the number of different individuals included was 78. The demographic, clinical, and treatment characteristics are summarized in Table 1. The median age was 58 (21-79) years. Patients were diagnosed with 27 different tumor types and enrolled in 37 IO-based early phase trials. Most patients received at least a PD-(L)-1 inhibitor (n = 58, 71.6%). The median follow-up duration was 10.3 months (1.8-46.9 months). The median OS and PFS was 10.3 months (95% CI, 9.5 to 15.1) and 1.9 months (95% CI, 1.8 to 2.6), respectively.

Detection of ctDNA in Plasma Samples

ctDNA profiling was performed on 162 plasma samples. Average coverage depth and quality control are summarized in the Data Supplement. Mutations in ctDNA were identified in 122 samples (75.3%): 60 (74%) at baseline and 62 (76.5%) at pre-C2. At an individual patient level, ctDNA was detected at any time point in 64 patients (79%): 58 at both time points, two at baseline, and four at pre-C2. Overall, ctDNA at baseline was detected in most tumor types, except mesothelioma and sarcoma, where the rates of detection were lower (20% and 33%, respectively). The genomic alterations detected in the ctDNA in ≥3 patients are summarized in the Data Supplement. None of the most frequent mutations detected at baseline predicted PFS or OS (Data Supplement). The median mVAF was 5.1% (range: 0.5%-57.5%) at baseline and 4.1% (range: 0.2%-55.6%) at pre-C2. Baseline ctDNA levels were not associated with differences in OS or PFS (Data Supplement).

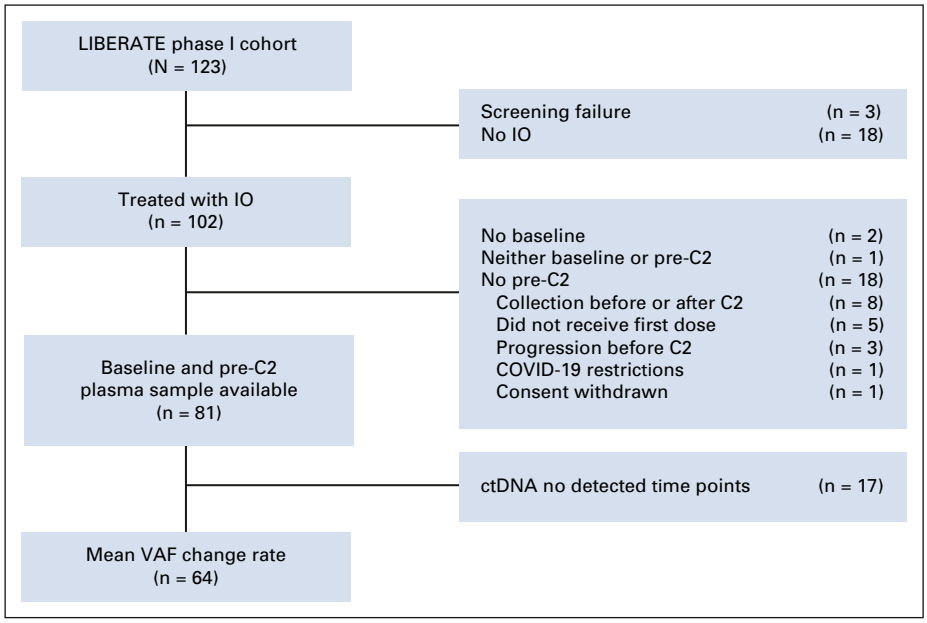

FIG 1. Flow diagram of the patients included in the analysis. ctDNA, circulating tumor DNA; IO, immunotherapy.

TABLE 1. Main Characteristics of Patients Included in the Overall Population (N = 81)

Characteristics	Patients, No. (%)
Sex	
Male	44 (46)
Female	37 (54)
Stage at study entry	
Locally advanced	3 (4)
Metastatic	78 (96)
Tumor type	
Sarcoma	9 (11)
Colorectal carcinoma	8 (10)
Breast carcinoma	7 (9)
Melanoma	6 (7)
Head and neck squamous cell carcinoma	5 (6)
Mesothelioma	5 (6)
Ovary carcinoma	4 (5)
Prostate carcinoma	4 (5)
Biliary duct carcinoma	3 (4)
Endometrium	3 (4)
Pancreatic adenocarcinoma	3 (4)
Renal clear cell carcinoma	3 (4)
Salivary gland carcinoma	3 (4)
Cutaneous adenocarcinoma	3 (4)
Suprarrenal carcinoma	3 (4)
Cervix carcinoma	2 (4)
Esophagus adenocarcinoma	2 (4)
Others (anal carcinoma, conjuctival melanoma, gastric carcinoma, mucoepidermoid carcinoma (head and neck), nasopharyngeal cancer, neuroendocrine tumor, small-cell lung carcinoma, small bowel carcinoma, one each)	8 (10)
Microsatellite stability	
Instable (MSI)	7 (9)
Stable (MSS)	13 (16)
Unknown	61 (75)
No. of drugs	
1	27 (33)
2	51 (63)
3	3 (4)
Therapies	
Monotherapy	25 (31)
Anti–PD-(L)1 monotherapy	5 (6)
Checkpoint inhibitor	14 (17)
Immunomodulator	3 (4)
Vaccines	3 (4)
Combinations	56 (69)
Anti–PD-(L)1 + other checkpoint	22 (27)
Anti–PD-(L)1 + MAPK pathway inhibitor	10 (12)
Anti–PD-(L)1 + immunomodulator	7 (9)
Anti–PD-(L)1 + vaccine	2 (3)
Anti–PD-(L)1 + interleukine	6 (7)
Two anti–PD-(L)1	2 (3)
Anti–PD-(L)1 + immunomodulator + interleukine	2 (3)
Anti–PD-(L)1 + checkpoint inhibitor + immunomodulator	1 (1)
Anti–PD-(L)1 + checkpoint inhibitor + interleukine	1 (1)
Checkpoint inhibitor + immunomodulator	2 (3)
Two checkpoint inhibitors	1 (1)
No. of site of metastases	
Locally advanced	3 (4)
1	28 (34)
2	27 (33)
3	16 (20)
>3	7 (9)
Sites of metastases	
Liver	
Lung	
Peritoneal	
Bone	
Lymph node	
Others (kidney, brain, adrenal, pleural, and skin)	
ECOG	
0	24 (30)
1	57 (70)
Best response	
Complete response	3 (4)
Partial response	5 (6)
Stable disease	23 (28)
Progressive disease	50 (62)
Clinical benefit rate	16 (20)
Hyperprogression	
Yes (Matos criteria)	9 (11)
Yes (Caramella criteria)	4 (5)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; MSI, microsatellite instability; MSS, microsatellite stability.

Mean VAF Change Rate and Treatment Outcome

We evaluated whether the change in ctDNA between baseline and pre-C2 (mVAF change rate) was associated

with treatment outcomes and survival in the 64 patients with ctDNA detection at any time point.

Patients with a decrease in ctDNA showed a longer median OS (10.8 months; 95% CI, 9.53 to NR) compared with those with an increase (9.1 months; 95% CI, 6.2 to 13.1) (HR, 0.54; 95% CI, 0.3 to 0.96; $P = .033$) (Fig 2A). These differences were more marked when molecular response was considered as a decrease >50% from baseline (Fig 2B), with a median OS that was not reached (95% CI, 9.53 to NR) versus 9.6 months (95% CI, 6.7 to 11.9) (HR, 0.23; 95% CI, 0.09 to 0.6; $P = .001$). Similar findings were observed if molecular response was considered a reduction >20% (Fig 2C).

Similarly, median PFS was shorter in patients with an increase in ctDNA compared with a decrease, 1.8 (95% CI, 1.6 to 1.9) versus 2.7 months (95% CI, 1.9 to 5.6); HR 0.43 (95% CI, 0.24 to 0.77); $P = .003$ (Fig 3A). Differences were more marked if molecular response was considered as a reduction >50%: PFS was 1.8 months (95% CI, 1.6 to 1.9) versus 3.5 months (95% CI, 2.8 to NR), HR 0.29 (95% CI, 0.13 to 0.62); $P < .001$ (Fig 3B). This was also observed when there was a decrease in mVAF of >20% (Fig 3C).

mVAF change rate was also associated with radiological response. All patients with radiological response had a decrease in ctDNA compared with only 32.2% of patients who had SD/PD ($P = .001$) (Fig 4A). These differences were more marked when there was a > 50% decrease in ctDNA levels. A mVAF decrease of > 50% was only seen in six (10%) patients who did not experience response (Fig 4B). Similar findings were observed regarding clinical benefit (Data Supplement).

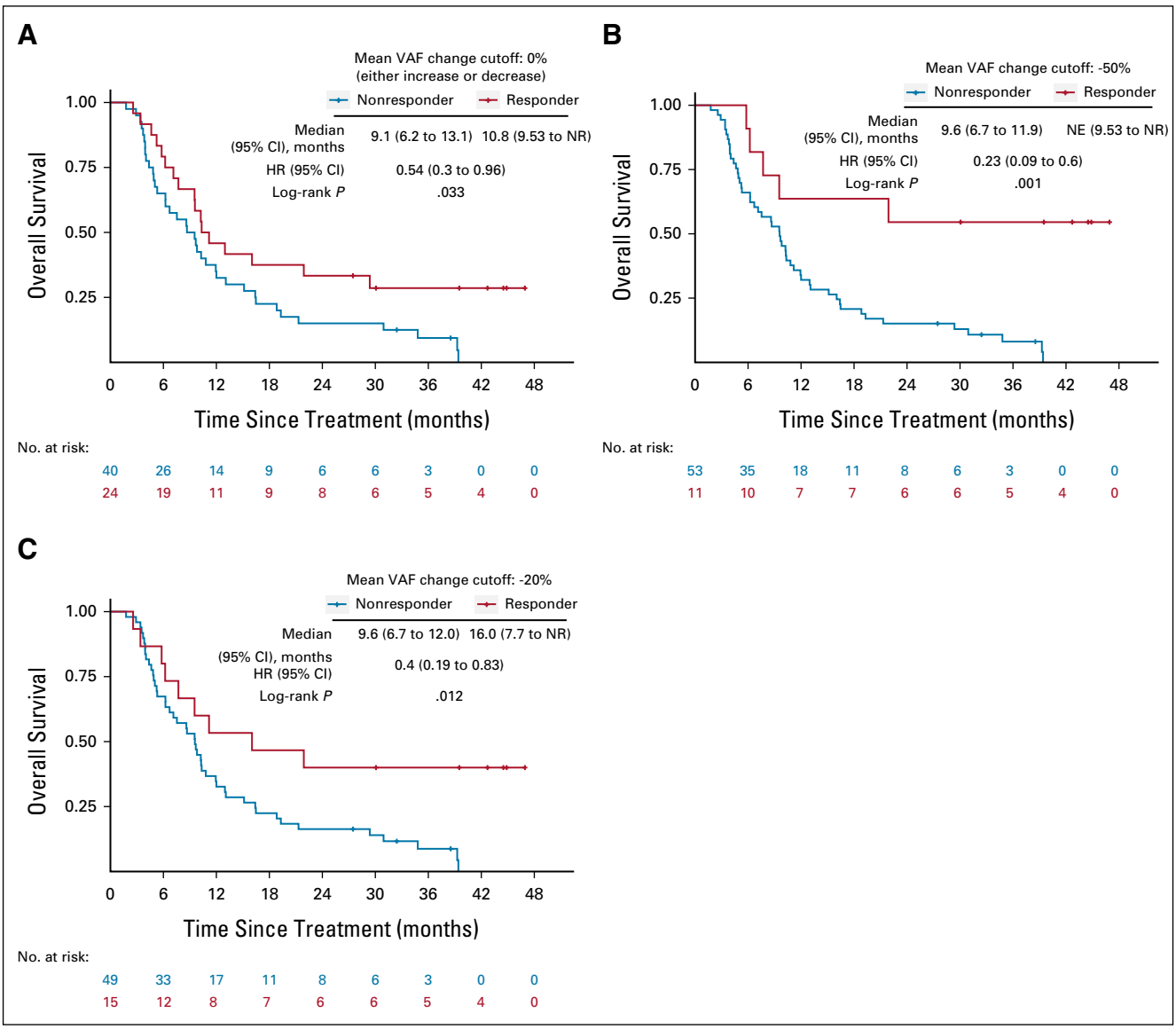

FIG 2. ctDNA change rate and overall survival. ctDNA mean VAF change rate was evaluated at C2 compared with baseline. Responders were defined by mean VAF changes of (A) <0%, (B) <–50%, and (C) <–20%. ctDNA, circulating tumor DNA; HR, hazard ratio.

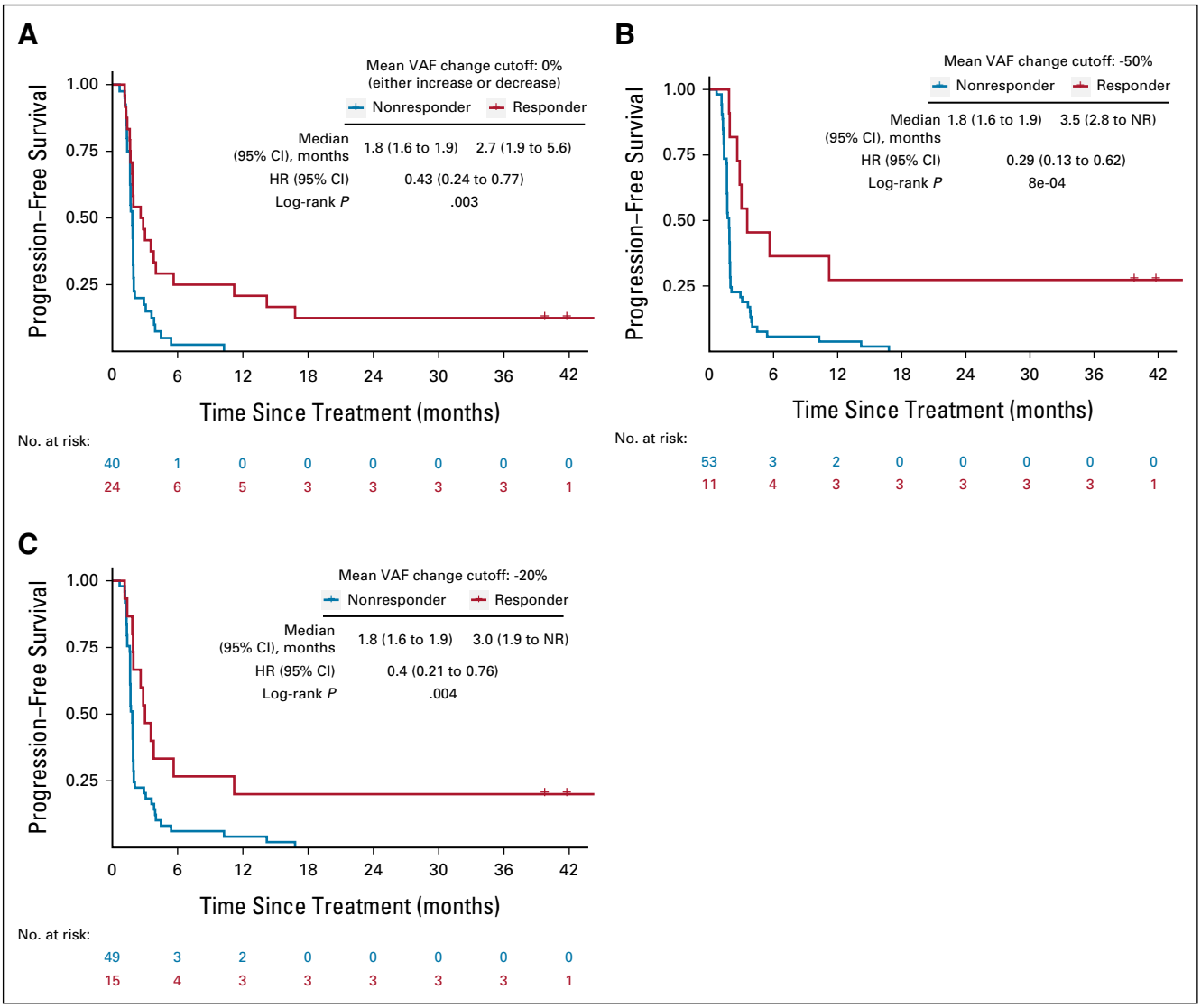

FIG 3. ctDNA change rate and progression-free survival. ctDNA mean VAF change rate was evaluated at C2 compared with baseline. Responders were defined by mean VAF changes of (A) <0%, (B) <–50%, and (C) <–20%. ctDNA, circulating tumor DNA; HR, hazard ratio.

We performed a subanalysis restricting to patients treated with a PD(L)-1 inhibitor alone or in combination with other therapies (n = 58). ctDNA was detected at any of the time points in 45 patients. Baseline ctDNA levels were not associated with PFS or OS. There was a statistically significant difference for longer PFS and OS if ctDNA decreased at any of the prespecified cutoffs (0%, –20%, and –50%) (Data Supplement). Once again, differences were more marked for the cutoff –50%. mVAF change rate was also associated with radiological response and clinical benefit.

Mean VAF Change Rate to Predict Hyperprogression

HyperPD was evaluated in all patients who had PD within 2 months of treatment (n = 49), 43 with ctDNA detection at baseline (87.8%). All patients were evaluable using the Matos criteria. Nine patients (all with ctDNA detected) were classified as having HyperPD (18.4% of patients with PD within 2 months). Three patients were not evaluable according to Caramella criteria because prebaseline radiological assessments were not available. Four patients (all positive for ctDNA at baseline) were considered to have HyperPD (4.9%); two of them were also using Matos criteria. There was a nonstatistically significant trend toward a higher median mVAF change rate in those with HyperPD using Matos Criteria: The median mVAF change rate was +0.41 (95% CI, 0.02 to 1.53) for HyperPD versus +0.24 (95% CI, 0.03 to 1.25) for standard PD (P = .53) (Fig 5A). A similar trend was observed in those using Caramella criteria: The median mVAF change was +0.78 (95% CI, –0.2 to 2.13) in HyperPD versus +0.23 (95% CI, 0.09 to 1.18) in standard PD (P = .5) (Fig 5B). There were no differences in the median mVAF at pre-C2 or baseline between HyperPD and standard PD (Data Supplement). We also compared median mVAF change among HyperPD and the remaining population (standard PD,

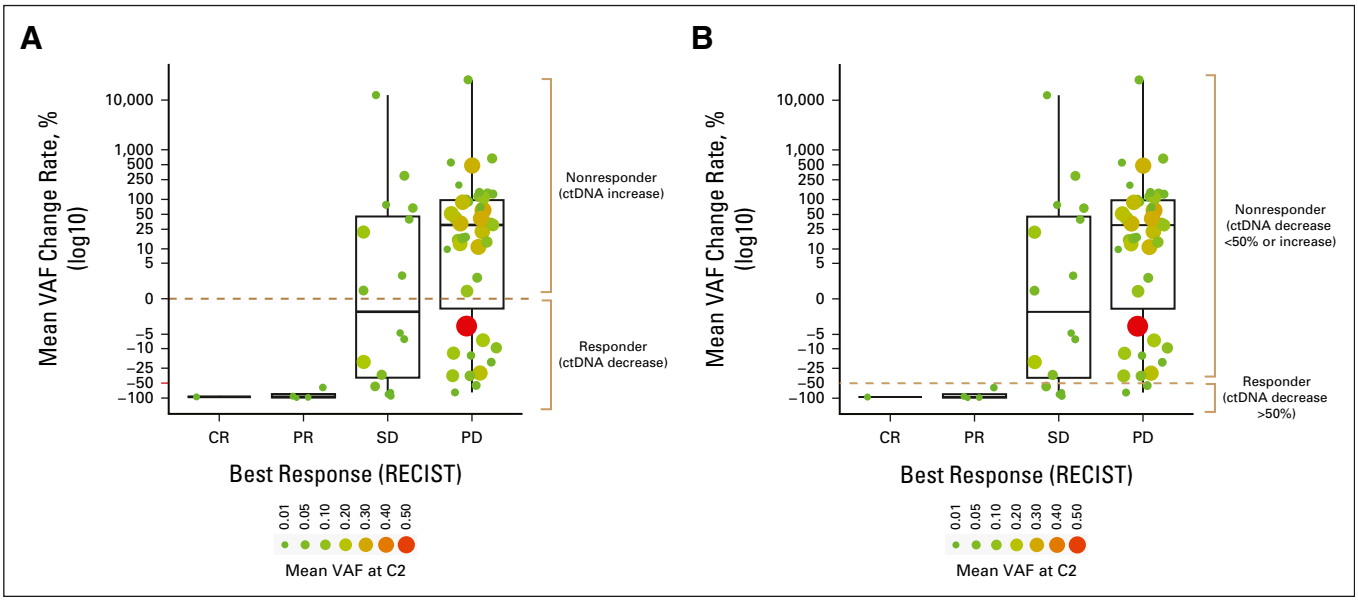

FIG 4. ctDNA change rate and response rate. ctDNA mean VAF change rate was evaluated at C2 compared with baseline. Responders were defined by mean VAF changes of (A) < 0% and (B) < −50%. CR, complete response; ctDNA, circulating tumor DNA; PD, progressive disease; PR, partial response; SD, stable disease.

complete response, partial response, and SD), and we did not see significant differences (Data Supplement). In regard to genomic alterations, there were more patients with baseline *TP53* mutations among HyperPD compared with standard PD (77.8% *v* 32.4%, *P* = .02).

Mean VAF Change Rate to Predict Pseudoprogression

In our cohort, eight patients (9.8%) were treated beyond RECIST progression, but all of them discontinued IO after their subsequent radiological assessment because of further tumor growth. Therefore, none of them can be considered PseudoPD. Interestingly, a patient with MSI-high endometrial carcinoma discontinued treatment because of PD according to iRECIST criteria after 90 days on treatment with a PD-L1 checkpoint inhibitor and MAPK pathway inhibitor. The patient then received palliative radiation to the retroperitoneal lymph nodes. This patient is currently alive (OS = 44.9 months) with no further systemic treatment

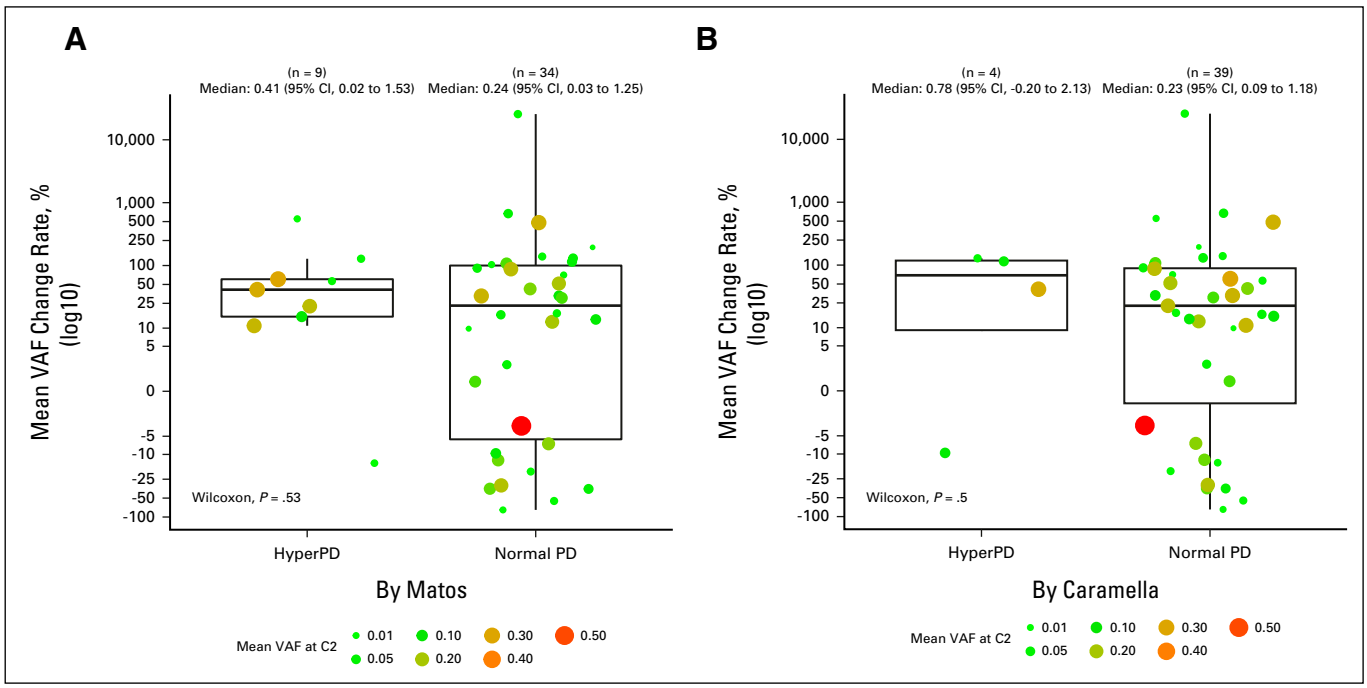

FIG 5. Comparison of mean VAF change rate of HyperPD and non-HyperPD patients. Hyperprogressive disease was determined by two different methods, (A) Matos and (B) Caramella, respectively. PD, progressive disease.

and no evidence of disease. The ctDNA kinetics in this patient between baseline and pre-C2 showed a dramatic decrease in the VAF of all variants (Data Supplement).

DISCUSSION

In this study, we demonstrated that a decrease in ctDNA within the first 3-4 weeks of treatment is associated with radiological response, clinical benefit, and longer survival in patients treated in early phase IO clinical trials. This association was more marked when the decrease in ctDNA was >50%. Differences in median OS and PFS using this cutoff are clinically meaningful compared with any decrease in ctDNA (decrease in ctDNA >0%) where differences in median OS and PFS are only 1.7 and 0.9 months, respectively. Therefore, our data suggest that molecular response should be considered a decrease of ctDNA >50%. This cutoff has been used as a standard for molecular response in patients with advanced non–small-cell lung cancer and melanoma treated with ICIs.[31-33] Early ctDNA kinetics were also recently evaluated in a pan-cancer cohort of patients.[34] A decrease in ctDNA levels within the first 21 days of treatment was predictive of treatment outcomes. Several differences are noted when compared with our analysis. The abovementioned study included patients treated with experimental and standard therapies; only 13% of the patients were treated with IO. That study used a tumor-informed ctDNA assay while we used a tumor-naïve one.

Our results are also consistent with previous studies evaluating anti–PD-(L)1 therapies in patients with different tumor types, where a decrease in ctDNA 6 weeks after the first dose predicted longer survival.[11,12] There are some notable differences between those studies and our work. First, on-treatment detection occurred earlier than in those studies (within 3-4 weeks). Second, our cohort included a broader population of advanced solid tumor types and various investigational IO treatments. Most patients in our cohort received PD-1 or PD-L1 ICIs alone or in combination with other novel drugs. Finally, we used a 425-gene panel tumor-naïve ctDNA panel in contrast to a bespoke tumor-informed ctDNA assay[11] or a smaller 73-gene panel.[12] Large tumor-naïve ctDNA panel sequencing may increase the sensitivity of detection and dynamic ctDNA assessments and may be more broadly applicable across tumor types. In the early phase IO clinical trial field, a study showed that baseline ctDNA detection using shallow whole-genome sequencing was associated with shorter OS.[35] We did not see such differences. The abovementioned study suggested that early increases in ctDNA levels were associated with shorter PFS. We found that any change in ctDNA levels can be associated with OS and PFS.

We were unable to differentiate HyperPD and pseudoPD in our cohort using ctDNA. We observed a similar rate of HyperPD compared with previous studies using the most commonly applied criteria (Matos 12.7% and Caramella 4.9%). However, some of the patients who were considered HyperPD were exposed before to anti–PD-(L)1 therapy, and others did not receive anti–PD-(L)1 therapy in this study so that categorization may not be accurate. We found a higher proportion of *TP53* mutations in the plasma of HyperPD patients. Previous studies using tumor sequencing have reported that *MDM2* amplification, *EGFR* aberrations, *SMARC2* mutations, and *APC* signaling alterations are associated with HyperPD.[36] In our cohort, we did not observe any pseudoPD. However, one of the patients showed an initial PD but the longest OS suggesting a potential pseudoPD. Interestingly, there was a sharp decrease in ctDNA pre-C2. This is consistent with prior studies that have shown that ctDNA may help distinguish pseudoPD in melanoma or non–small-cell lung cancer.[37,38]

Our study had several limitations. This is a retrospective analysis of patients at a single institution although they were prospectively recruited. Our cohort was heterogeneous, with many different tumor types and a variety of experimental IO treatment regimens, including investigational agents that are no longer in clinical development. Some patients could not be evaluated for ctDNA kinetics because ctDNA was not detected at any time point. This was particularly notable for patients with sarcoma and mesothelioma, where the fixed ctDNA panel may not be optimized for plasma mutation detection or where there may be less shedding of ctDNA. Accordingly, there were 64 patients in our cohort with ctDNA detected at any time point, and this relatively small number is reflected in the wide CIs for the median OS and PFS estimates. We also acknowledge that our study was focused on ctDNA values rather than specific variants that may arise before progression and that could be potentially interesting to explore in subsequent studies. Finally, the correlation between ctDNA decrease and radiological response should be interpreted with caution as only eight patients in our cohort experienced radiographic response.

In conclusion, our study suggests that ctDNA kinetics is an early response biomarker in early phase IO clinical trials, as demonstrated in other settings. An early increase in ctDNA after one cycle of treatment may identify patients who do not benefit from experimental IO therapy. This could prompt an earlier radiological response assessment or early discontinuation of ineffective treatment, avoiding continued exposure to ineffective experimental drug treatment. In contrast, a marked decrease in ctDNA may also be reassuring to continue experimental treatment beyond the initial dose limiting toxicity period. This study provides a rationale for integrating ctDNA kinetics in early phase clinical trials as a pharmacodynamic biomarker which can precede changes in radiological imaging. Further validation studies with a prespecified ctDNA cutoff (ie, decrease >50%) and real-time analysis are required before this approach should routinely be used in clinical practice.

AFFILIATIONS

[1]Division of Medical Oncology and Hematology, Department of Medicine, Princess Margaret Cancer Centre, University Health Network, University of Toronto, Toronto, Canada
[2]Geneseeq Technology Inc, Toronto, Canada
[3]Division of Medical Oncology, Hospital de Base, Sao Paulo, Brazil

CORRESPONDING AUTHOR

Philippe L. Bedard, MD, Division of Medical Oncology and Hematology, Department of Medicine, Princess Margaret Cancer Centre, 700 University Ave, Suite 7-723, Toronto, ON M5G 1Z5, Canada; e-mail: philippe.bedard@uhn.ca.

AUTHOR CONTRIBUTIONS

Conception and design: Enrique Sanz-Garcia, Philippe L. Bedard
Financial support: Lillian L. Siu, Philippe L. Bedard
Administrative support: Philippe L. Bedard
Provision of study materials or patients: Albiruni R. Abdul Razak, Aaron R. Hansen, Anna Spreafico, Lillian L. Siu, Philippe L. Bedard
Collection and assembly of data: Enrique Sanz-Garcia, Sofia Genta, Daniel V. Araujo, Aaron R. Hansen, Lillian L. Siu, Philippe L. Bedard
Data analysis and interpretation: Enrique Sanz-Garcia, Xiaoxi Chen, Qiuxiang Ou, Albiruni R. Abdul Razak, Anna Spreafico, Hua Bao, Xue Wu, Lillian L. Siu, Philippe L. Bedard
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Xiaoxi Chen
Employment: Nanjing Geneseeq Technology

Qiuxiang Ou
Employment: Geneseeq Technology Inc

Daniel V. Araujo
Honoraria: Pfizer, MSD, Libbs
Consulting or Advisory Role: MSD Oncology
Travel, Accommodations, Expenses: Pfizer

Albiruni R. Abdul Razak
Consulting or Advisory Role: Adaptimmune, Bayer, GlaxoSmithKline, Medison, Inhibrx
Research Funding: Deciphera, Karyopharm Therapeutics, Pfizer, Roche/Genentech, Bristol Myers Squibb, MedImmune, Amgen, GlaxoSmithKline, Blueprint Medicines, Merck, AbbVie, Adaptimmune, Iterion Therapeutics, Neoleukin Therapeutics, Daiichi Sankyo, Symphogen, Rain Therapeutics
Expert Testimony: Medison

Aaron R. Hansen
Consulting or Advisory Role: Merck, GlaxoSmithKline, Bristol Myers Squibb, Eisai, Novartis, AstraZeneca
Research Funding: Karyopharm Therapeutics (Inst), Merck (Inst), Bristol Myers Squibb (Inst), Boehringer Ingelheim, GlaxoSmithKline (Inst), Roche/Genentech (Inst), Janssen (Inst), AstraZeneca/MedImmune (Inst), Astellas Pharma (Inst), BioNTech (Inst), Pfizer/EMD Serono (Inst), Neoleukin Therapeutics (Inst)

Anna Spreafico
Honoraria: Medison & Immunocore
Consulting or Advisory Role: Merck, Bristol Myers Squibb, Medison & Immunocore
Research Funding: Bristol Myers Squibb (Inst), Novartis (Inst), Merck (Inst), Symphogen (Inst), AstraZeneca/MedImmune (Inst), Bayer (Inst), Surface Oncology (Inst), Janssen Oncology (Inst), Replimune (Inst), Roche (Inst), Alkermes (Inst), Array BioPharma (Inst), GlaxoSmithKline (Inst), Treadwell Therapeutics (Inst), Amgen (Inst), Pfizer (Inst), ALX Oncology (Inst)

Hua Bao
Employment: Nanjing

Xue Wu
Employment: Geneseeq Technology Inc
Leadership: Geneseeq Technology Inc
Travel, Accommodations, Expenses: Geneseeq Technology Inc

Lillian L. Siu
Leadership: Treadwell Therapeutics
Stock and Other Ownership Interests: Agios
Consulting or Advisory Role: Merck, AstraZeneca/MedImmune, Roche, Voronoi Health Analytics, Oncorus, GlaxoSmithKline, Seattle Genetics, Arvinas, Navire, Janpix, Relay Therapeutics, Daiichi Sankyo/UCB Japan, Janssen, Hookipa Pharma, InterRNA, Tessa Therapeutics, Sanofi, Amgen
Research Funding: Bristol Myers Squibb (Inst), Genentech/Roche (Inst), GlaxoSmithKline (Inst), Merck (Inst), Novartis (Inst), Pfizer (Inst), AstraZeneca (Inst), Boehringer Ingelheim (Inst), Bayer (Inst), Amgen (Inst), Astellas Pharma (Inst), Shattuck Labs (Inst), Symphogen (Inst), AVID Radiopharmaceuticals (Inst), Mirati Therapeutics (Inst), Intensity Therapeutics (Inst), Karyopharm Therapeutics (Inst)

Philippe L. Bedard
Consulting or Advisory Role: BMS (Inst), Pfizer (Inst), Seattle Genetics, Lilly, Amgen, Merck, Gilead Sciences
Research Funding: Bristol Myers Squibb (Inst), Sanofi (Inst), AstraZeneca (Inst), Genentech/Roche (Inst), GlaxoSmithKline (Inst), Novartis (Inst), Nektar (Inst), Merck (Inst), Seattle Genetics (Inst), Immunomedics (Inst), Lilly (Inst), Amgen (Inst), Bicara Therapeutics (Inst), Zymeworks (Inst)

No other potential conflicts of interest were reported.

ACKNOWLEDGMENT

We would like to thank the patients and their families for their contribution. This study was performed under the auspices of the LIBERATE study (NCT03702309), an institutional liquid biopsy program at the University Health Network supported by the BMO Financial Group Chair in Precision Cancer Genomics (Chair: Dr Lillian Siu).

REFERENCES

1. Cescon DW, Bratman SV, Chan SM, et al: Circulating tumor DNA and liquid biopsy in oncology. Nat Cancer 1:276-290, 2020
2. Zill OA, Banks KC, Fairclough SR, et al: The landscape of actionable genomic alterations in cell-free circulating tumor DNA from 21,807 advanced cancer patients. Clin Cancer Res 24:3528-3538, 2018
3. Sanz-Garcia E, Zhao E, Bratman SV, et al: Monitoring and adapting cancer treatment using circulating tumor DNA kinetics: Current research, opportunities, and challenges. Sci Adv 8:eabi8618, 2022
4. Hrebien S, Citi V, Garcia-Murillas I, et al: Early ctDNA dynamics as a surrogate for progression-free survival in advanced breast cancer in the BEECH trial. Ann Oncol 30:945-952, 2019
5. Parikh AR, Mojtahed A, Schneider JL, et al: Serial ctDNA monitoring to predict response to systemic therapy in metastatic gastrointestinal cancers. Clin Cancer Res 26:1877-1885, 2020
6. O'Leary B, Hrebien S, Morden JP, et al: Early circulating tumor DNA dynamics and clonal selection with palbociclib and fulvestrant for breast cancer. Nat Commun 9:896, 2018
7. He J, Tan W, Tang X, et al: Variations in EGFR ctDNA correlates to the clinical efficacy of afatinib in non small cell lung cancer with acquired resistance. Pathol Oncol Res 23:307-315, 2017
8. Jin Y, Chen DL, Wang F, et al: The predicting role of circulating tumor DNA landscape in gastric cancer patients treated with immune checkpoint inhibitors. Mol Cancer 19:154, 2020
9. Herbreteau G, Vallée A, Knol AC, et al: Circulating tumor DNA early kinetics predict response of metastatic melanoma to anti-PD1 immunotherapy: Validation study. Cancers (Basel) 13:1826, 2021
10. Ricciuti B, Jones G, Severgnini M, et al: Early plasma circulating tumor DNA (ctDNA) changes predict response to first-line pembrolizumab-based therapy in non-small cell lung cancer (NSCLC). J Immunother Cancer 9:e001504, 2021
11. Bratman SV, Yang SYC, Iafolla MAJ, et al: Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. Nat Cancer 1:873-881, 2020
12. Zhang Q, Luo J, Wu S, et al: Prognostic and predictive impact of circulating tumor DNA in patients with advanced cancers treated with immune checkpoint blockade. Cancer Discov 10:1842-1853, 2020
13. Hobbs BP, Barata PC, Kanjanapan Y, et al: Seamless designs: Current practice and considerations for early-phase drug development in oncology. J Natl Cancer Inst 111:118-128, 2019
14. Tang J, Shalabi A, Hubbard-Lucey VM: Comprehensive analysis of the clinical immuno-oncology landscape. Ann Oncol 29:84-91, 2018
15. Ochoa de Olza M, Oliva M, Hierro C, et al: Early-drug development in the era of immuno-oncology: Are we ready to face the challenges? Ann Oncol 29:1727-1740, 2018
16. Shah M, Rahman A, Theoret MR, et al: The drug-dosing conundrum in oncology—When less is more. N Engl J Med 385:1445-1447, 2021
17. Salawu A, Chen R, Hernando-Calvo A, et al: Impact of pharmacodynamic biomarkers in immuno-oncology (IO) phase 1 clinical trials. J Clin Oncol 39:2653, 2021
18. Borcoman E, Kanjanapan Y, Champiat S, et al: Novel patterns of response under immunotherapy. Ann Oncol 30:385-396, 2019
19. Park HJ, Kim KW, Won SE, et al: Definition, incidence, and challenges for assessment of hyperprogressive disease during cancer treatment with immune checkpoint inhibitors: A systematic review and meta-analysis. JAMA Netw Open 4:e211136, 2021
20. Adashek JJ, Subbiah IM, Matos I, et al: Hyperprogression and immunotherapy: Fact, fiction, or alternative fact? Trends Cancer 6:181-191, 2020
21. Adashek JJ, Kato S, Ferrara R, et al: Hyperprogression and immune checkpoint inhibitors: Hype or progress? Oncologist 25:94-98, 2020
22. Fuentes-Antrás J, Provencio M, Díaz-Rubio E: Hyperprogression as a distinct outcome after immunotherapy. Cancer Treat Rev 70:16-21, 2018
23. Gomes da Morais AL, de Miguel M, Cardenas JM, et al: Comparison of radiological criteria for hyperprogressive disease in response to immunotherapy. Cancer Treat Rev 91:102116, 2020
24. Araujo DV, Wang A, Torti D, et al: Applications of circulating tumor DNA in a cohort of phase I solid tumor patients treated with immunotherapy. JNCI Cancer Spectr 5:2021, 2021
25. Kas B, Talbot H, Ferrara R, et al: Clarification of definitions of hyperprogressive disease during immunotherapy for non-small cell lung cancer. JAMA Oncol 6:1039-1046, 2020
26. Matos I, Martin-Liberal J, García-Ruiz A, et al: Capturing hyperprogressive disease with immune-checkpoint inhibitors using RECIST 1.1 criteria. Clin Cancer Res 26:1846-1855, 2020
27. Bolger AM, Lohse M, Usadel B: Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120, 2014
28. Li H, Durbin R: Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754-1760, 2009
29. Cibulskis K, Lawrence MS, Carter SL, et al: Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol 31:213-219, 2013
30. Fang H, Bergmann EA, Arora K, et al: Indel variant analysis of short-read sequencing data with Scalpel. Nat Protoc 11:2529-2548, 2016
31. Goldberg SB, Narayan A, Kole AJ, et al: Early assessment of lung cancer immunotherapy response via circulating tumor DNA. Clin Cancer Res 24:1872-1880, 2018
32. Nabet BY, Esfahani MS, Moding EJ, et al: Noninvasive early identification of therapeutic benefit from immune checkpoint inhibition. Cell 183:363-376.e13, 2020
33. Forschner A, Battke F, Hadaschik D, et al: Tumor mutation burden and circulating tumor DNA in combined CTLA-4 and PD-1 antibody therapy in metastatic melanoma—Results of a prospective biomarker study. J Immunother Cancer 7:180, 2019
34. Gouda MA, Huang HJ, Piha-Paul SA, et al: Longitudinal monitoring of circulating tumor DNA to predict treatment outcomes in advanced cancers. JCO Precis Oncol 10.1200/PO.21.00512, 2022
35. Galvao V, Matos I, Moreno A, et al: Impact of circulating tumor DNA (ctDNA) detection on survival outcomes of patients (pts) treated with immune-checkpoint inhibitors (ICIs) in early clinical trials. J Clin Oncol 39:2542, 2021
36. Kato S, Goodman A, Walavalkar V, et al: Hyperprogressors after immunotherapy: Analysis of genomic alterations associated with accelerated growth rate. Clin Cancer Res 23:4242-4250, 2017
37. Guibert N, Mazieres J, Delaunay M, et al: Monitoring of KRAS-mutated ctDNA to discriminate pseudo-progression from true progression during anti-PD-1 treatment of lung adenocarcinoma. Oncotarget 8:38056-38060, 2017
38. Lee JH, Long GV, Menzies AM, et al: Association between circulating tumor DNA and pseudoprogression in patients with metastatic melanoma treated with anti–programmed cell death 1 antibodies. JAMA Oncol 4:717-721, 2018
39. vcf2maf package. https://github.com/mskcc/vcf2maf
40. The R Project for Statistical Computing. https://www.r-project.org