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ORIGINAL ARTICLE

Circulating tumor DNA-based molecular residual disease detection for treatment monitoring in advanced melanoma patients

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Abstract

Background: Immune checkpoint inhibitors (ICIs) have substantially improved overall survival in patients with advanced melanoma; however, the lack of biomarkers to monitor treatment response and relapse remains an important clinical challenge. Thus, a reliable biomarker is needed that can risk-stratify patients for disease recurrence and predict response to treatment.

Methods: A retrospective analysis using a personalized, tumor-informed circulating tumor DNA (ctDNA) assay on prospectively collected plasma samples (n = 555) from 69 patients with advanced melanoma was performed. Patients were divided into three cohorts: cohort A (N = 30), stage III patients receiving adjuvant ICI/observation; cohort B (N = 29), unresectable stage III/IV patients receiving ICI therapy; and cohort C (N = 10), stage III/IV patients on surveillance after planned completion of ICI therapy for metastatic disease.

Results: In cohort A, compared to molecular residual disease (MRD)-negative patients, MRD-positivity was associated with significantly shorter distant metastasis-free survival (DMFS; hazard ratio [HR], 10.77; p = .01). Increasing ctDNA levels from the post-surgical or pre-treatment time point to after 6 weeks of ICI were predictive of shorter DMFS in cohort A (HR, 34.54; p < .0001) and shorter progression-free survival (PFS) in cohort B (HR, 22; p = .006). In cohort C, all ctDNA-negative patients remained progression-free for a median follow-up of 14.67 months, whereas ctDNA-positive patients experienced disease progression.

See editorial on pages 000–000, this issue.

Conclusion: Personalized and tumor-informed longitudinal ctDNA monitoring is a valuable prognostic and predictive tool that may be used throughout the clinical course of patients with advanced melanoma.

KEYWORDS
advanced melanoma, circulating tumor DNA (ctDNA), immune checkpoint inhibitors (ICI), molecular residual disease (MRD), treatment monitoring

INTRODUCTION

Melanoma has the highest rate of mortality among all skin cancers, accounting for >80% of skin cancer-related deaths.[1,2] In recent years, the clinical adoption of immune checkpoint inhibitors (ICIs), as well as BRAF/MEK inhibitors have led to substantial improvement in the overall survival (OS) rates of patients with advanced melanoma.[3-7] The 5-year survival rate has improved from <10% in patients treated with chemotherapy to 26%–52% in patients receiving immuno- and/or targeted therapies.[5,8-10]

Adjuvant systemic therapy (nivolumab, pembrolizumab, or BRAF targeted therapy) is currently recommended for patients with resectable advanced melanoma.[11] For patients with unresectable melanoma, the available first-line regimens include anti–PD-1 monotherapy, anti–PD1-based combination immunotherapy, or BRAF-targeted therapy for tumors with BRAF V600 mutations.[11] However, treatment resistance with targeted therapies and limited response to immunotherapies may be observed.[8,12-14] Additionally, high cost and treatment-induced toxicity, as well as the phenomenon of ICI-induced pseudoprogression cause significant challenges in the successful adoption of these therapies.[15-17]

National Comprehensive Cancer Network guidelines currently recommend periodic imaging and/or clinical assessment to determine therapeutic efficacy or disease progression.[11] However, imaging-based surveillance suffers from limitations in how frequently it may be performed, false positivity, and misinterpretation of results that can lead to expensive and unnecessary procedures.[18–20] Although plasma lactate dehydrogenase (LDH) levels can be prognostic in melanoma, only 30%-40% of patients with stage IV disease have elevated LDH at baseline and it can frequently become elevated due to treatment toxicity or other reasons unrelated to disease state.[21,22] Currently, there are no other clinically useful blood-based biomarkers available that can assess treatment response and/or disease progression in real-time and help optimize subsequent treatment strategies.[23]

Recent studies have demonstrated the potential of plasma circulating tumor DNA (ctDNA) as a prognostic and predictive biomarker in melanoma independent of baseline clinical parameters.[24] In patients with advanced melanoma, undetectable or low levels of ctDNA at baseline, before any therapy, may be indicative of longer progression-free survival (PFS) and OS.[25] The postoperative detection of ctDNA may also be predictive of disease relapse in patients with stage III melanoma.[26,27] Additionally, monitoring ctDNA dynamics longitudinally could identify disease progression earlier than radiologic assessment, because it may be a predictor of response to treatment.[24,28,29] However, these studies have used digital droplet polymerase chain reaction (ddPCR) or similar methodologies for ctDNA analysis that only followed one known tumor mutation, and thus could not be used in a significant percentage of patients without common melanoma mutations. Notably, mutations in *BRAF*, *NRAS*, and *KIT* are reported to occur in approximately 38%–45%, 20%, and 10% of patients with melanoma, respectively, and detection of ctDNA containing *BRAF* and *NRAS* mutations is applicable in only approximately 50% of melanoma patients.[24,30,31]

Furthermore, tracking multiple tumor mutations per patient may also increase the efficacy of a ctDNA assay in predicting clinical outcomes. Therefore, we sought to evaluate the predictive and prognostic value of a personalized, tumor-informed ctDNA assay for the detection of molecular residual disease (MRD) after curative surgery and to assess treatment response in patients with stage III–IV melanoma.

MATERIALS AND METHODS

Subjects and study design

This study represents a retrospective analysis of real-world data from prospectively collected, longitudinal plasma samples (n = 555) from stage III–IV melanoma patients treated between April 2020 to March 2022. Patients were divided into three different cohorts (Figure 1): 1) cohort A (N = 30, stage III): adjuvant setting, wherein post-resection patients were either initiated on anti–PD-1 therapy or observation, with samples on ICI collected approximately every 4 weeks; 2) cohort B (N = 29, stage III/IV): ICI treatment setting, wherein patients with unresectable melanoma received first-line PD-1 inhibitor-based treatment, with samples collected every 3 or 4 weeks depending on ICI regimen; and 3) cohort C (N = 10, stage III/IV): post-ICI surveillance setting, wherein patients with complete response (CR) at the end of the first year or with stable disease (SD)/partial response (PR) at the end of second year were monitored after anti–PD-1 therapy. For patients on surveillance in all three cohorts, samples were collected every 12 weeks at the time of radiographic imaging (computed tomography [CT] or positron emission tomography [PET]/CT). All clinical data were abstracted and interpreted by the clinical team. The study was approved by the corresponding ethical and independent review services and was conducted in accordance with the Declaration of Helsinki.

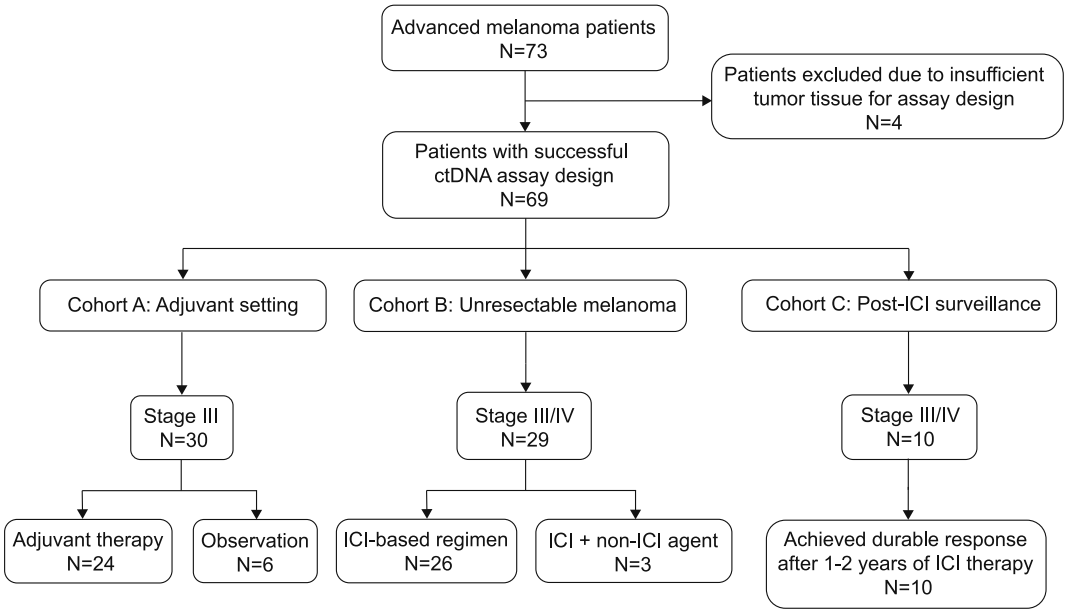


FIGURE 1 CONSORT diagram showing enrollment of patients into three subcohorts. For cohort C, durable response with the previous line of therapy was defined as complete response for 1 year or stable disease or partial response for 2 years.

Personalized ctDNA assay using multiplex PCR-based next-generation sequencing workflow

For all patients, blood specimens (two 10-mL Streck tubes) were collected at the time of each ICI treatment or at the time of scans for patients on surveillance, with an average of nine samples per patient (range, 1–19). All biological specimens were processed following a Clinical Laboratory Improvement Amendments-validated standard operating procedure at Natera. Personalized, tumor-informed ctDNA assays (Signatera) were designed as previously described.[32] Briefly, whole-exome sequencing (WES) was performed on formalin-fixed and paraffin-embedded tumor tissue along with matched normal blood samples from each patient. A set of up to 16 high-ranked, patient-specific, somatic, single nucleotide variants (SNVs) from WES were selected for multiplex PCR (mPCR) testing. The mPCR primers targeting the personalized SNVs were used to track ctDNA in the respective patients' plasma. Plasma samples with two or greater SNVs detected above a predefined algorithm's confidence threshold were considered ctDNA-positive. ctDNA concentration (levels) was reported as mean tumor molecules (MTM) per mL of plasma.

Genomic analysis to estimate tumor mutational burden and identify driver variants

Whole exome VCF files were used to calculate tumor mutational burden (TMB) based on all somatic SNVs per Mb for all 69 patients as previously described.[33] A subanalysis of 39 patients with measurable disease was performed to determine the predictive value of TMB in response to ICI therapy. The prevalence of driver mutations known to be associated with melanoma (listed in Figure 6) was evaluated.[34–38]

Statistical analysis

The ctDNA statistical analysis plan was developed before the reconciliation of the laboratory and de-identified clinical data. Samples were analyzed by the data assessors at Natera who were blinded to patient outcomes. Statistical significance was evaluated using Fisher's exact test for categorical variables. For stage III patients, the primary outcome measure was distant metastasis-free survival (DMFS), assessed between the date of resection and radiologic and/or clinical distant relapse and/or death. For unresectable melanoma patients, the primary outcome measure was PFS, assessed between the date of the first blood draw and the date of radiologic and/or clinical disease progression. The Kaplan–Meier method was used for estimating the survival distributions. Log-rank test or Cox proportional hazards model was used for comparing two survival distributions with $p \leq .05$ being considered significant. Statistical analyses were performed in STATA v16.1.

RESULTS

Patient and tumor characteristics

Of the 73 patients in the study, personalized ctDNA assays were successfully designed for 69 (94.5%) patients, since four patients had insufficient tumor tissue. In cohort A ($N = 30$), 24 (80%)

patients received adjuvant nivolumab treatment and six (20%) underwent observation. Of these six patients, five had received ICI therapy before relapse and/or surgery and one had underlying comorbidity. A total of 233 plasma samples were collected starting 4 weeks post-surgery and during follow-up for a median of 19.6 months (range, 0.4–24.2). In cohort B (N = 29), all patients received first-line anti–PD-1 treatment for advanced disease; 12 patients received nivolumab alone, 14 received ipilimumab/nivolumab, and three received anti–PD-1 combined with a non-ICI agent. Baseline (before treatment) plasma samples were collected from 28 patients and longitudinal on-treatment plasma samples (n = 232) were collected from 29 patients. The median follow-up time was 14.2 (range, 0.2–20.8) months. In post-ICI cohort C (N = 10), a total of 62 plasma samples were collected with a median follow-up of 14.7 (range, 14.1–18.2) months. Patient characteristics for all cohorts are summarized in Table 1.

Genomic analysis

TMB was calculated with a median of 14.4 mutations per megabase (Mb) (range, 0.03–184 mutations/Mb) (Figure S1A) across all 69 patients. Pathogenic and likely pathogenic somatic mutations in *NF1*, *TERT*, *RB1*, *BRAF*, *BRCA1*, and *BRCA2* genes were observed in >50% of the patients whereas mutations in *IDH1*, *MEK1*, *MEK2*, *PTEN*, *CDK4*, and *CDK6* genes were observed in <10% of the patients (Figure S1B).

Cohort A: ctDNA detection at the MRD time point is prognostic of distant metastasis-free survival

Of the 30 patients in cohort A (adjuvant setting), 29 had plasma samples available at the MRD time point (after resection and before adjuvant therapy). Of the 29 patients, five (17%) were MRD-positive and 24 (83%) were MRD-negative (Figure 2A). Of the five MRD-positive patients, three (60%) experienced distant relapse within 4 months after surgery. In contrast, only one patient (4%) from the MRD-negative cohort experienced distant relapse, 12 months after surgery. In seven instances of relapse so far (one patient had multiple relapses), ctDNA was either detectable post-surgery or became detectable during serial monitoring in five instances. In two relapse cases—one local subcentimeter dermal metastasis (Patient 29) and a small adrenal metastasis (Patient 27)—molecular evidence of relapse was not observed. Therefore, sensitivity was 83% for distant relapses, with specificity of 96%. ctDNA analysis allowed an average lead time of 3 months over standard imaging (PET/CT or CT) (Figure 2B). Compared to the baseline MRD-negative patients, the MRD-positive patients had a significantly shorter DMFS (median 4 months vs. not reached; HR, 10.77; p = .01) (Figure 2C).

Next, we evaluated the effect of adjuvant immunotherapy on MRD-positive patients' outcomes. The MRD-positive patients who did not receive adjuvant therapy (observation; N = 2) relapsed within 4 months (100%), whereas one of the three (33%) patients receiving adjuvant ICI therapy relapsed within the same period. (Figure 2A). DMFS stratified by the presence or absence of adjuvant ICI in the MRD-positive patients is shown in Figure S2.

Cohort A: ctDNA status during ICI therapy is prognostic of patient outcomes

Compared to ctDNA-negative patients, ctDNA-positivity observed at week 4 (N = 24) and week 6 (N = 27) of adjuvant treatment was significantly associated with an inferior DMFS (week 4: median, 11.5 months if ctDNA-positive vs not reached; HR, 6.9, p = .025; Figure 3A; and week 6: median, 3.5 months if ctDNA-positive vs. not reached; HR, 34.54, p < .0001; Figure 3B). Week 6 ctDNA status appeared to have a stronger correlation with clinical outcomes than week 4 status. We also assessed the association of ctDNA dynamics with DMFS. Patients who became ctDNA-positive (N = 2, p = .025) or remained persistently positive (N = 2; p < .0001) during treatment had significantly worse DMFS compared to ctDNA-negative patients (Figure 3C). In contrast, those who became ctDNA-negative (N = 2) or remained negative (N = 25) had similar outcomes, superior to those of the ctDNA-positive patients (Figure 3C).

Cohort B: ctDNA is detectable in advanced melanoma and its dynamics are predictive of response to first-line ICI treatment

Of the 29 patients in Cohort B (advanced melanoma on first-line ICI), baseline ctDNA was detected in 26 (90%) patients before the start of first-line ICI treatment (Figure 4A). Four patients had baseline ctDNA levels above the 75th percentile (median ctDNA level, 2965 MTM/mL, range, 138–25,858 vs. cohort median 9.8 MTM/mL, range, 7–25,859). Notably, three of these four patients died very early in their ICI therapy regimen (median, 1 month; treatment range, 0.27–1.86 months), with accelerated disease progression.

For patients with baseline positivity and serial ctDNA testing, we then evaluated the correlation between ctDNA status/dynamics and response to first-line ICI treatment (N = 19). (Three patients who received non-ICI with ICI combination regimens were excluded from this analysis due to the potential synergistic effects of the non-ICI agents on ctDNA kinetics.) ctDNA-positivity at weeks 3–11 (cycle [C] 2–4) of first-line ICI treatment was associated with worse PFS (p = .015) (Figure 5A) compared to ctDNA-negative patients. In addition, patients with any increase in ctDNA level at this time point had a significantly shorter PFS, compared to patients with a decrease in ctDNA levels (median, 5.7 months vs. not reached; HR, 22; p = .006)

TABLE 1 Patient demographics and baseline characteristics.

	Patients, No. (%)		
Patient characteristics	**Cohort A (*N* = 30)**	**Cohort B (*N* = 29)**	**Cohort C (*N* = 10)**
Age (median), years (range)	72 (21–90)	64 (39–89)	66 (51–85)
Gender			
Male	16 (53)	20 (69)	7 (70)
Female	14 (47)	9 (31)	3 (30)
AJCC clinical stage (V8)			
IIIA	–	–	–
IIIB	15 (50)	–	–
IIIC	15 (50)	1 (3)	3 (30)
IIID	–	–	–
IV	–	28 (97)	7 (70)
Ulceration			
Absent	14 (47)	5 (17)	2 (20)
Present	11 (36)	7 (24)	1 (10)
Unknown	5 (17)	17 (59)	7 (70)
BRAF V600 status by targeted sequencing			
Wild-type	19 (64)	19 (66)	7 (70)
Mutated	10 (33)	9 (31)	3 (30)
Unknown	1 (3)	1 (3)	–
BRAF V600 status by WES			
Mutated	10 (33)	9 (31)	3 (30)
LDH level (baseline)			
Normal	23 (77)	18 (62)	3 (30)
Elevated	4 (13)	11 (38)	7 (70)
Unknown	3 (10)	–	–
Type			
Cutaneous	28 (94)	22 (76)	5 (50)
Acral	1 (3)	1 (3)	1 (10)
Lentigo	–	1 (3)	–
Mucosal	–	1 (3)	1 (10)
Unknown	1 (3)	4 (15)	3 (30)
Treatment			N/A
Adjuvant nivolumab	24 (80)	–	–
Adjuvant observation	6 (20)	–	–
1st-line nivolumab	–	12 (41)	–
1st-line ipilimumab/nivolumab	–	14 (48)	–
1st-line ICI + agent	–	3 (10)	–
No. of metastatic sites	N/A		N/A
0		1 (3)	
<3		13 (45)	
≥3		15 (52)	

Abbreviations: AJCC, American Joint Committee on Cancer; ICI, immune checkpoint inhibitor; LDH, lactate dehydrogenase; N/A, not applicable; WES, whole-exome sequencing.

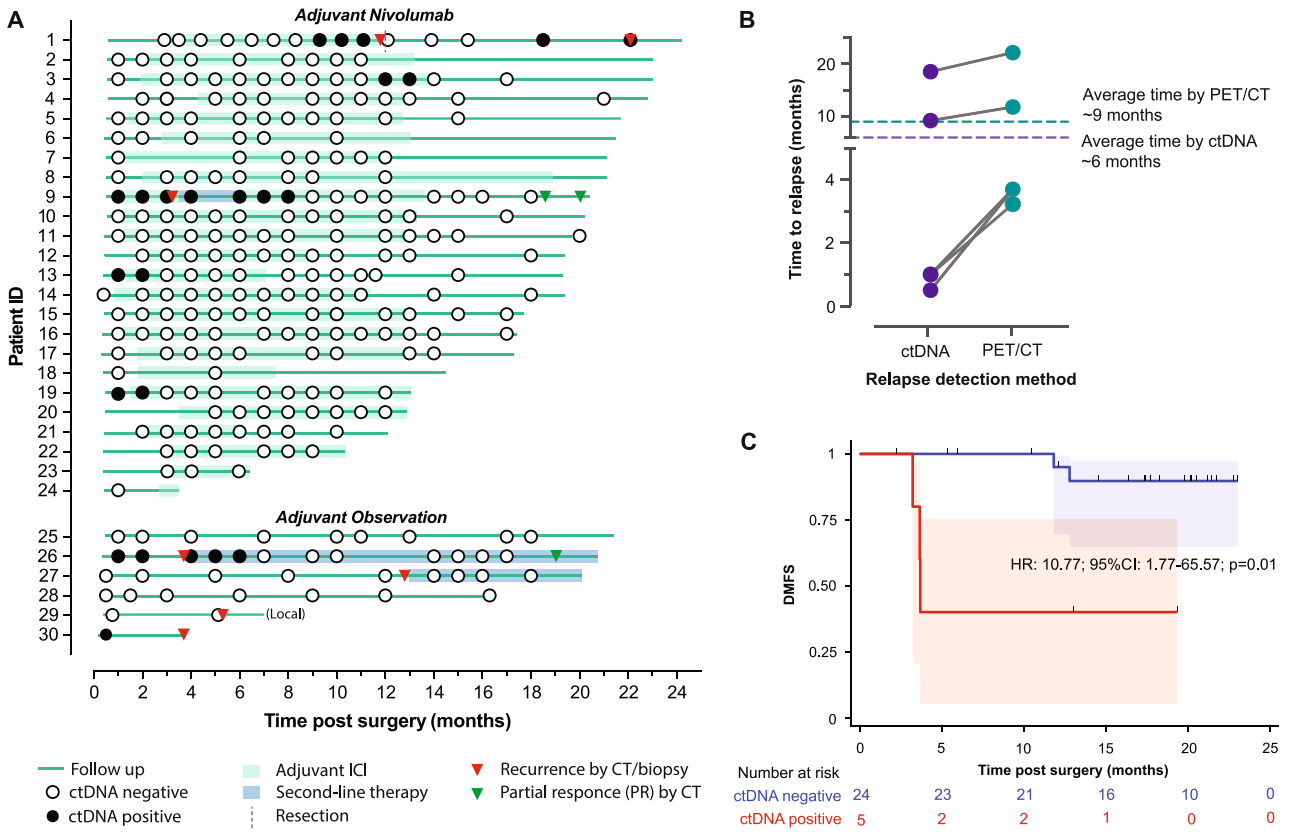


FIGURE 2 In the adjuvant setting (cohort A), ctDNA detection is predictive of DMFS. (A) Swimmer's plot shows clinical outcomes, duration of adjuvant therapy, and longitudinal ctDNA analysis for each patient. Of the six patients in the observation arm, five patients had received prior systemic therapy before surgery and enrollment (except patient 29). (B) Average lead time to relapse. (C) Patients stratified by ctDNA status at MRD time point, with median DMFS for the ctDNA-positive group of 4 months and not reached for the ctDNA-negative group. Shaded areas in the Kaplan–Meier plots indicate 95% CIs. CIs indicate confidence intervals; ctDNA, circulating tumor DNA; DMFS, distant metastasis-free survival; MRD, molecular residual disease.

(Figure 5B). Similar findings were observed at 6 weeks of first-line ICI treatment, with significantly worse PFS in patients with increasing ctDNA at this time point versus decreasing and/or undetectable ctDNA (median, 7.5 months vs. not reached; HR, 18; $p = .013$) (Figure S3).

Next, the association of ctDNA status at weeks 3–11 (C2–4) with Response Evaluation Criteria in Solid Tumors (RECIST)-based response was investigated. ctDNA was detected during serial monitoring in all four patients who experienced progressive disease (PD) by C4, whereas 10 of 15 (67%) patients with PR/CR were ctDNA-negative (Figure 5C). All patients (10 of 10) who were ctDNA-negative at this time point had evidence of radiographic response (PR/CR). Similarly, ctDNA dynamics at this time was associated with response, as 100% (4 of 4) of patients who experienced PD had an increase in ctDNA at this time, whereas 100% (15 of 15) of patients with a ctDNA decrease had a radiographic response (PR/CR) (Figure 5D).

Although ctDNA clearance was associated with a PR or CR by imaging (Figure 5E), an early increase in ctDNA levels was associated with primary resistance to first-line ICI treatment (Figure 5F). One patient, demonstrating transient ctDNA clearance, eventually experienced disease progression over 12 months after initiating ICI treatment, suggesting acquired resistance, and the patient's ctDNA became detectable 5.4 months before PD. (Figure 5G). In addition, compared to radiographic imaging, ctDNA dynamics correctly identified a patient with true progression (Patient 40, Figure 4A) and pseudoprogression (Patient 58, Figure 4A).

Cohort C: ctDNA-positivity precedes clinical progression post-ICI treatment

Of the 10 patients in the post-ICI completion surveillance setting, nine (90%) were ctDNA-negative at the time of enrollment and the majority (7 of 10, 70%) remained ctDNA-negative (Figure 4B). All (7 of 7) ctDNA-negative patients remained progression-free until the last follow-up (median, 14.67; range, 14.13–18.23 months). Of the three ctDNA-positive patients, two patients subsequently progressed, whereas the third died shortly after the positive result, clinically from cancer recurrence. For these patients, ctDNA-positivity preceded clinical progression by a median of 3.34 (range, 0.6–6.9) months. Two of the three ctDNA-positive patients benefited from subsequent radiotherapy or targeted therapy resulting in ctDNA clearance and showed continued treatment response for at least 6 and 9 months. Overall, these results indicate that

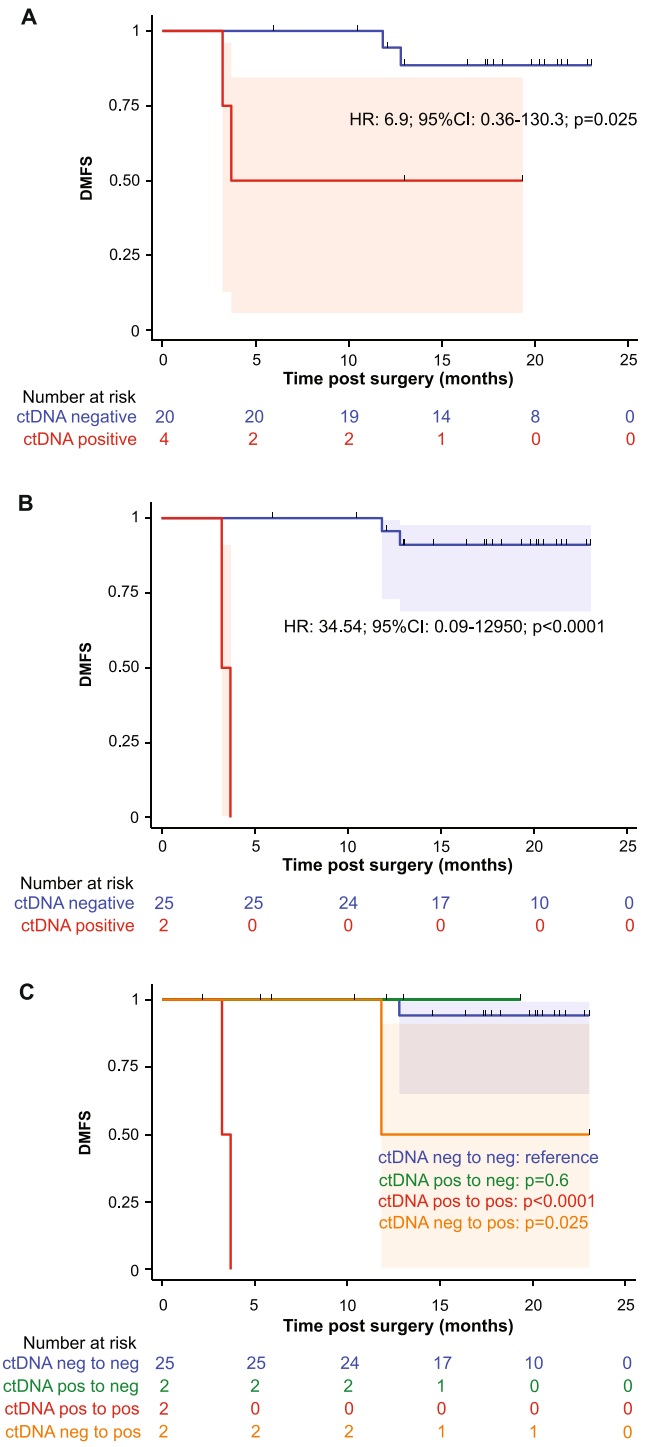


FIGURE 3 Association of ctDNA status with clinical outcome for patients in cohort A. For the patients in the observation arm, the median treatment start time of patients receiving adjuvant therapy was considered as the start time. (A) Patients with stage III melanoma stratified by ctDNA status at week 4 of adjuvant therapy, with median DMFS of 11.5 months for ctDNA-positive group and not reached for the ctDNA-negative group. (B) Patients with stage III melanoma stratified by ctDNA status at week 6 of adjuvant therapy, with median DMFS of 3.5 months for the ctDNA-positive group and not reached for the ctDNA-negative group. (C) Patients stratified by ctDNA clearance pattern, with median DMFS not reached for ctDNA negative-to-negative and ctDNA positive-to-negative groups, 3.5 months for ctDNA positive-to-positive group, and 17.4 months for ctDNA negative-to-positive group. ctDNA indicates circulating tumor DNA; DMFS, distant metastasis-free survival.

undetectable serial ctDNA post-ICI treatment was associated with a favorable outcome.

Change in ctDNA combined with TMB status is prognostic of patient outcomes

In a subanalysis of patients ($N = 39$; cohorts B and C), the predictive value of TMB was evaluated in response to ICI therapy. In the cohort of patients with measurable disease, 25% (3 of 12) of those with low TMB status (≤14.7 mutations/Mb) presented with PD during treatment with ICI, whereas 18.5% (5 of 27) of patients with high TMB (>14.7 mutations/Mb) did not respond to treatment (Figure 6). Next, we compared ctDNA status and dynamics at weeks 3–11 (C2–4) with TMB in stage IV patients receiving first-line ICI treatment. As demonstrated in Figure 5A, a strong association between change in ctDNA at C2–4 and PFS was observed ($p = .015$); however, TMB alone was not predictive of PFS ($p = .26$) (Figure S4A). On combining ctDNA status with TMB status, compared to patients with high TMB or ctDNA-negativity, patients with low TMB and positive ctDNA status had a significantly worse PFS ($p = .039$) (Figure S4B). These data suggest that ctDNA status and/or dynamics could add value to patient prognostication.

DISCUSSION

This study demonstrates the feasibility of performing a personalized, tumor-informed ctDNA assay for the detection of MRD and monitoring response to ICI treatment in a real-world cohort of patients with stage III-IV melanoma. Although previous studies have shown the prognostic and predictive value of ctDNA monitoring in patients with advanced melanoma, these studies have primarily focused on ctDNA methodologies designed to detect or monitor single hotspot mutations in *BRAF*, *NRAS*, *KIT*, and/or *TERT* promoter. Therefore, a ctDNA testing methodology that tracks mutations beyond common driver variants and tracks multiple patient-specific mutations may be more useful and sensitive to tumor dynamics in clinical practice.

This personalized, tumor-informed ctDNA assay, which tracks up to 16 somatic, clonal SNVs, detected distant melanoma relapse with a sensitivity of 83% and specificity of 96% in the adjuvant stage III setting. These rates were higher than observed in two prior studies of ctDNA in post-surgery melanoma patients using ddPCR to track a single mutation in *BRAF*, *NRAS*, or *TERT*, one with a sensitivity of 20% and specificity of 95% for distant relapse, the other with sensitivity of 55% and specificity of 94% for relapse at 12 months.[26,39] The

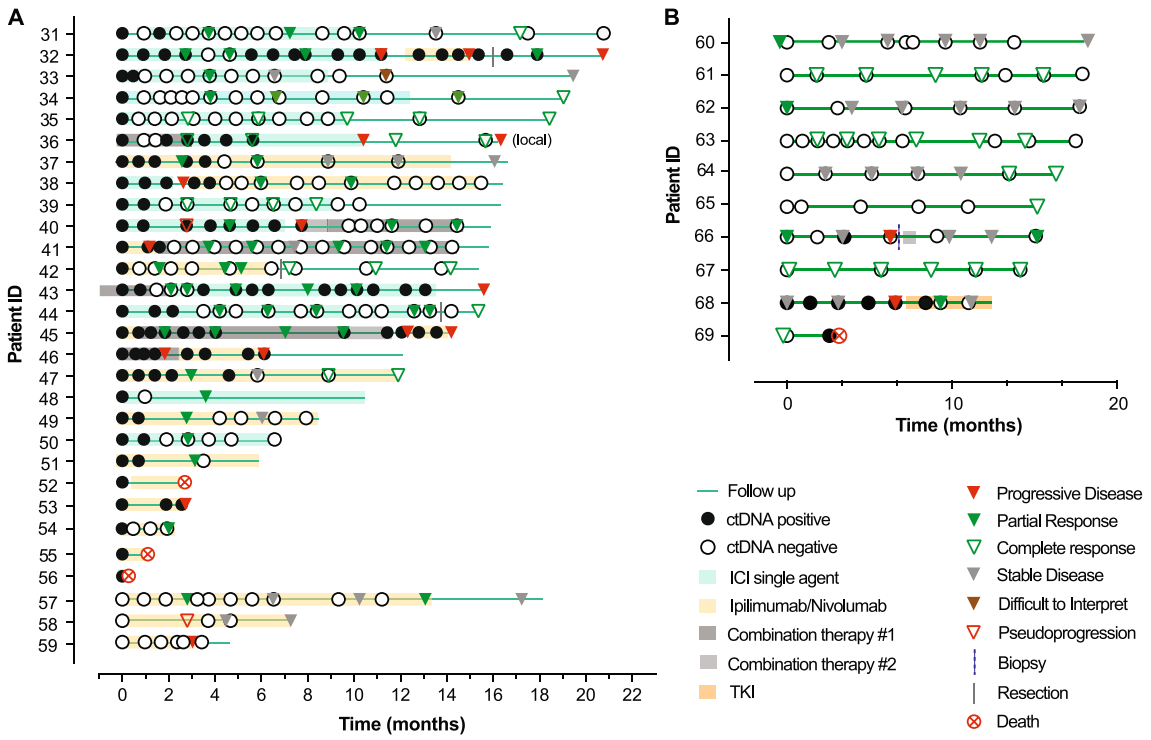


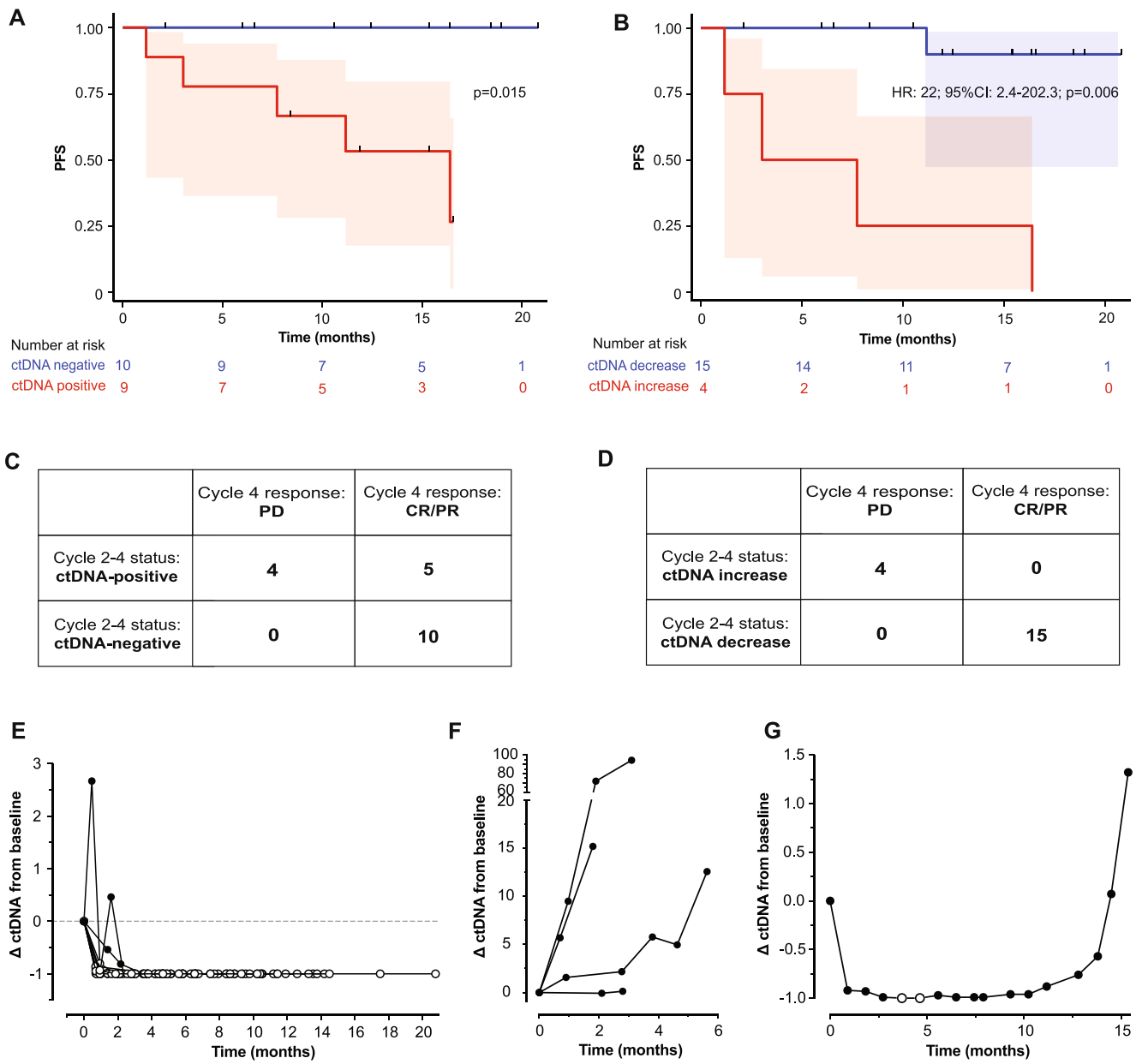
FIGURE 4 Swimmer's plot shows clinical outcomes, duration of systemic therapy, and longitudinal ctDNA analysis for patients in (A) cohort B and (B) cohort C. ctDNA indicates circulating tumor DNA.

correlation with worse DMFS of post-surgical ctDNA detection before adjuvant ICI (MRD time point) is also consistent with the CheckMate 915 trial where detection of ctDNA before adjuvant ICI predicted poorer DMFS.[40] Although longitudinal ctDNA analyses from that trial were not reported, in our study increasing ctDNA levels after 6 weeks of adjuvant anti-PD-1 were also predictive of significantly shorter DMFS, with a stronger correlation than observed at the MRD time point, highlighting the value of monitoring ctDNA dynamics. There was also a preliminary trend suggesting that ctDNA-positive patients receiving adjuvant anti–PD-1 may experience better outcomes compared to those under observation. Although such findings have been reported in urothelial carcinoma and colorectal cancer,[33,41] prospective studies would be needed to determine whether changes in adjuvant systemic therapy in patients with elevated ctDNA levels can lower the subsequent risk of relapse.

In the cohort of patients with advanced melanoma on first-line ICI, three of four patients with baseline ctDNA levels higher than 75th percentile died within 2 months of starting ICI. Similar observations have been reported where worse clinical outcomes were noted for patients with high ctDNA levels (>500 copies/mL) at baseline,[12] because a very high ctDNA concentration at baseline may be prognostic of relapse. A prior study also explored the predictive value of the personalized, tumor-informed ctDNA assay in 12 metastatic melanoma patients treated with pembrolizumab, where changes in ctDNA levels from baseline after three cycles of ICI correlated with PFS.[29] In this cohort, we found that an increasing ctDNA concentration at 6 weeks on anti–PD-1 therapy in metastatic melanoma was associated with a lack of response and significantly reduced PFS. These results highlight the predictive value of ctDNA status and early dynamics in advanced melanoma patients treated with ICI, as well as the potential to identify high-risk patients ahead of radiologic and/or clinical progression who may benefit from modification of their therapeutic regimen.

Furthermore, lack of ctDNA detection on surveillance post-ICI completion in metastatic melanoma was associated with improved survival, because ctDNA-positivity was observed to precede radiographic progression by a median of 3.3 months during longitudinal monitoring of patients. Although all ctDNA-negative patients remained progression-free during follow-up, these data support the potential use of ctDNA as a noninvasive biomarker that can be used at shorter intervals compared to radiographic imaging and provide a real-time assessment of the disease progression at a molecular level before clinical relapse. An important future step will be to evaluate prospectively if an earlier change in treatment based on ctDNA increase will improve clinical outcomes.

In recent years, TMB has been explored as a genomic biomarker to predict response to ICI treatments in patients with melanoma because studies have shown conflicting results on the association of high TMB with OS benefit on ICI.[42–45] In this cohort, we were unable to demonstrate a correlation between TMB alone and PFS in patients with advanced melanoma treated with first-line ICI therapy. However, the data suggest that combining ctDNA with TMB or ctDNA alone appears to be better predictive biomarkers than TMB alone in melanoma patients treated with ICI.



C

	Cycle 4 response: **PD**	Cycle 4 response: **CR/PR**
Cycle 2-4 status: **ctDNA-positive**	**4**	**5**
Cycle 2-4 status: **ctDNA-negative**	**0**	**10**

D

	Cycle 4 response: **PD**	Cycle 4 response: **CR/PR**
Cycle 2-4 status: **ctDNA increase**	**4**	**0**
Cycle 2-4 status: **ctDNA decrease**	**0**	**15**

FIGURE 5 (A and B) Kaplan–Meier estimates of PFS for patients with stage IV melanoma stratified by (A) for patients stratified by ctDNA status at cycles 2–4 of first-line ICI, median PFS was 16.4 months for ctDNA positive group and not reached for ctDNA negative group; and (B) for patients stratified by ctDNA dynamics from baseline at cycle 2–4, median PFS 5.7 months for ctDNA increasing group and not reached for ctDNA decreasing group. (C and D) Risk groupings according to early clinical response and ctDNA status. (C) Cycle 4 RECIST groupings (columns) and cycles 2–4 ctDNA status (C; rows) or ctDNA dynamics (D; rows) are shown for 19 patients. (E–G) ctDNA trajectories. (E) Patients with a complete or partial response by RECIST criteria. (F) Patients with PD to first-line ICI with primary resistance. (G) Patient with PD to first-line ICI with acquired resistance. The y-axis in (E)–(G) represents a positive or negative fold change in ctDNA levels compared to the baseline. Filled circles represent ctDNA-positivity, and open circles represent ctDNA-negativity. ctDNA indicates circulating tumor DNA; ICI, immune checkpoint inhibitor; PD, progressive disease; PFS, progression-free survival; RECIST, Response Evaluation Criteria in Solid Tumors.

Although these findings are potentially applicable to clinical practice, there are important limitations including the limited sample size and duration of follow-up, and variations in the treatment patients received. In summary, this study demonstrates the predictive and prognostic value of personalized and tumor-informed ctDNA testing in patients with advanced melanoma treated with ICI and suggests that early ctDNA dynamics may indicate tumor response to ICI and detect disease progression during surveillance. The utility of treatment change in melanoma based on ctDNA dynamics will need to be validated in future clinical trials.

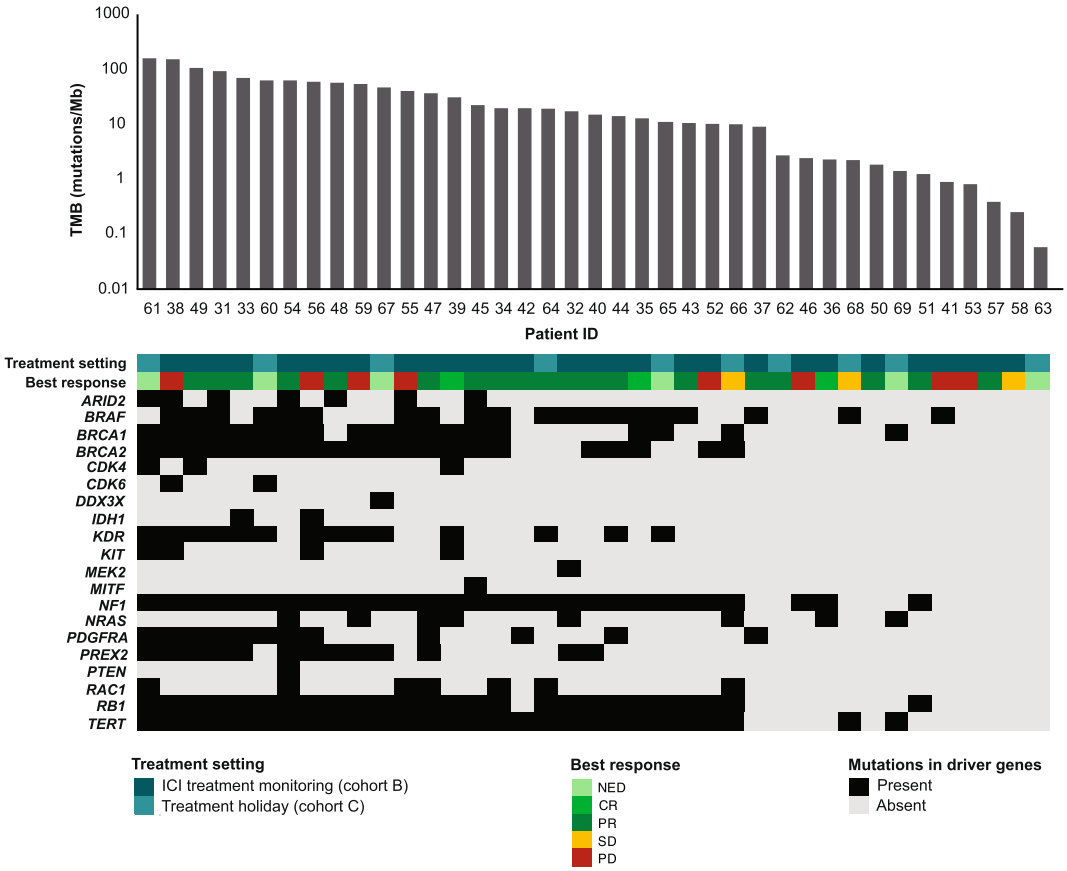


FIGURE 6 Onco-plot summarizing clinical and genomic features of 39 patients from cohorts B and C with measurable disease who received first-line ICI treatment. The tumors are arranged in descending order by total SNVs per megabase using a semi-logarithmic scale. Tumor status for the presence or absence of mutations in known melanoma-associated genes with driver mutations has been highlighted. ICI indicates immune checkpoint inhibitor; SNVs, single nucleotide variants.

AUTHOR CONTRIBUTIONS

Zeynep Eroglu: Conceptualization, data curation, investigation, project administration, resources, validation, supervision, and writing–review, and editing. **Shifra Krinshpun:** Conceptualization, methodology, project administration, validation, and writing–review, and editing. **Ekaterina Kalashnikova:** Conceptualization, methodology, resources, formal analysis, software, visualization, validation, writing–original draft, and writing–review, and editing. **Sumedha Sudhaman:** Conceptualization, Formal analysis, software, resources, visualization, writing–original draft, and writing–review, and editing. **Turkan Ozturk Topcu:** Data curation and writing–review and editing. **Matt Nichols:** Data curation and writing–review and editing. **Justin Martin:** Data curation and writing–review and editing. **Katherine M. Bui:** Data curation and writing–review and editing. **Joseph Markowitz:** Data curation and writing–review and editing. **Nikhil I. Khushalani:** Data curation and writing–review and editing. **Ahmad A. Tarhini:** Data curation and writing–review and editing. **Jane L. Messina:** Data curation and writing–review, and editing. **Charuta C. Palsuledesai:** Visualization, project administration, writing–original draft, and writing–review and editing. **Meenakshi Malhotra:** Conceptualization, visualization, project administration, writing–original draft, and writing–review, and editing. **Perry Olshan:** Conceptualization, project administration, supervision, and writing–review, and editing. **Alexey Aleshin:** Conceptualization, project administration, methodology, resources, supervision, and writing–review, and editing.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

Moffitt Cancer Center holds the sole responsibility for the accuracy of the clinical data and can provide data on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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