Feasibility of personalized circulating tumor DNA detection in stage II and III melanoma

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The objective of this study was to evaluate the feasibility of developing personalized, tumor-informed assays for patients with high-risk resectable melanoma and examine circulating tumor DNA (ctDNA) levels in relation to clinical status. Pilot prospective study of clinical stage IIB/C and resectable stage III melanoma patients. Tumor tissue was used to design bespoke somatic assays for interrogating ctDNA in patients' plasma using a multiplex PCR (mPCR) next-generation sequencing (NGS)-based approach. Plasma samples for ctDNA analysis were collected pre-/post-surgery and during surveillance. Out of 28 patients (mean 65 years, 50% male), 13 (46%) had detectable ctDNA prior to definitive surgery and 96% (27/28) tested ctDNA-negative within 4 weeks post-surgery. Pre-surgical detection of ctDNA was significantly associated with the later-stage ($P = 0.02$) and clinically evident stage III disease ($P = 0.007$). Twenty patients continue in surveillance with serial ctDNA testing every 3–6 months. With a median follow-up of 443 days, six out of 20 (30%) patients developed detectable ctDNA levels during surveillance. All six of these patients recurred with a mean time to recurrence of 280 days. Detection of ctDNA in surveillance preceded the diagnosis of clinical recurrence in three patients, was detected concurrent with clinical recurrence in two patients and followed clinical recurrence in one patient. One additional patient developed brain metastases without detection of ctDNA during surveillance but had positive pre-surgical ctDNA. Our results demonstrate the feasibility of obtaining a personalized, tumor-informed mPCR NGS-based ctDNA assay for patients with melanoma, particularly in resectable stage III disease.

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Introduction

Melanoma is the deadliest type of skin cancer, with over 325 000 people diagnosed and 57 000 deaths per year, globally [1]. While localized melanomas have a high survival rate, melanoma-specific survival (MSS) decreases significantly in patients diagnosed with regional and metastatic disease [2]. Patients with thicker primary melanomas, with and without sentinel lymph node involvement, and patients with clinically evident regional metastatic disease, are at a higher risk of metastasis [3]. On the basis of the 8th edition of the American Joint Committee on Cancer staging criteria for melanoma, MSS varies significantly across higher-risk stage II and III patients. When divided into substages, 5-year MSS ranges from 94 to 82% for stage IIA–IIC and 93 to 32% for stage IIIA–IIID [3]. Tumor histologic and patient clinical features are incorporated into staging criteria and used to stratify patients for risk of metastasis. There currently is no reliable, patient tumor-informed, blood-based biomarker to assess the real-time risk of recurrence during surveillance or to identify resistance to treatment. While physical exam detects some skin and superficial nodal recurrences, deeper nodal recurrences and organ metastases often remain hidden until the patient reports clinical symptoms or the disease is large enough for detection on radiologic imaging.

Circulating tumor DNA (ctDNA) consists of cell-free DNA fragments released into the bloodstream from apoptotic tumor cells [4]. Serial testing for the presence of ctDNA is minimally invasive and has the potential to detect relapse early by identifying minimal/molecular residual disease

(MRD) after surgery. Recent studies on the clinical utility of ctDNA detection in melanoma have found a significant association between the detection of ctDNA and poor prognosis [5–8]. Most of the published studies have employed digital droplet PCR (ddPCR), which is designed to detect prespecified mutations, typically alterations in *BRAF*, *TERT*, *NRAS*, or *KIT* [9]. While effective, this type of assay is not useful for monitoring tumors that lack these mutations.

The primary aim of this study was to evaluate the feasibility of detecting ctDNA using a bespoke multiplex PCR (mPCR) next-generation sequencing (NGS)-based assay [10] in patients with clinical stage IIB/C and fully resectable stage III melanoma. The secondary aims were to assess the relationship between ctDNA status and disease stage, receipt of adjuvant therapy, and clinical status before surgery and during surveillance. Previous studies utilizing the bespoke mPCR NGS-based assays in non-small cell lung cancer, colorectal cancer, muscle-invasive bladder cancer, and breast cancer have shown positive predictive values for relapse ranging from 93 to 100% with an average lead time of 2.8–9.5 months compared to radiographic detection [10–13]; however, the utility of the platform in high-risk resectable melanoma is currently unknown.

Methods

Study population

In this nonrandomized, prospective pilot study, patients with clinical stage IIB/C and resectable stage III melanoma were recruited at the Huntsman Cancer Institute and Intermountain Medical Center in Salt Lake City, Utah. Per standard of care, patients underwent wide local excision with sentinel lymph node biopsy or therapeutic node dissection, depending on their clinical stage. Patient pathologic stage, after surgery and complete analysis of wide excision and sentinel node/therapeutic node dissection, was recorded and updated if different from the clinical stage. Tissue for whole-exome sequencing (WES) and ctDNA assay generation was obtained from either the primary tumor biopsy, recurrent tumor biopsy at study entry, or from the surgically resected lymph node metastasis/residual disease in wide excision specimen depending on clinical stage and tissue availability. Blood samples were collected within a few days prior to, or immediately preceding, surgery and within 4 weeks after surgery. After enrollment of the initial pilot cohort, an amendment was added to continue the collection of blood samples every 3–6 months in surveillance. Initial blood samples were collected in one 6 ml EDTA tube and two 10 ml Streck (Omaha, Nebraska, USA) tubes. All subsequent blood draws were collected in two 10 ml Streck tubes. Surveillance blood draws were coordinated with routine clinic visits and radiographic studies. Blood was collected up to every 6 months for stage II patients and every 3–4 months for stage III patients. Demographic, pathologic, clinical status, and treatment information was collected prospectively at research visits and through medical chart review. Investigators obtained informed consent from all patients enrolled in the trial (NCT05736523) and the study was approved by the Institutional Review Board at the University of Utah prior to patient enrollment.

Personalized and tumor-informed circulating tumor DNA assay

A personalized, tumor-informed ctDNA assay (Signatera bespoke mPCR NGS assay; Natera, Inc., Austin, Texas, USA) was used for the detection and quantification of ctDNA, as previously described [10]. Briefly, WES was performed on tumor tissue and matched normal blood samples. Matched normal sequencing was performed to computationally remove putative germline and clonal hematopoiesis of indeterminate potential mutations. Following sequencing, a set of up to 16, somatic nucleotide variants (SNVs) specific to the tumor DNA of each patient was identified for mPCR testing. The SNVs are prioritized based on optimized design parameters that ensure uniqueness in the human genome, amplicon efficiency, and primer interaction. Following plasma cfDNA extraction and library preparation, mPCR was conducted on an aliquot of the cfDNA library, followed by amplicon-based sequencing and to an average NGS depth per amplicon of more than 100 000× on an Illumina platform (San Diego, California, USA). Blood samples with at least two out of 16 variants detected above a predefined algorithmic confidence threshold were considered positive for ctDNA and ctDNA concentration was reported in mean tumor molecules per ml of plasma (MTM/ml). Patients were excluded if there was insufficient tumor tissue to complete WES. Available tumor tissue was limited by Clinical Laboratory Improvement Amendments (CLIA) requirements for diagnostic tissue retention.

Statistical analysis

The primary objective of the pilot study was to assess the feasibility of generating ctDNA assays after sample acquisition and processing pre and postoperative samples within a clinically reasonable timeframe. Specifically, we evaluated the percentage of patients with pre and postoperative ctDNA results received within 6 weeks of collecting the postoperative plasma sample and acquiring tissue for assay generation. The 6-week time point was determined as a clinically reasonable amount of time to coordinate tissue acquisition, generate the tumor-informed assay, and complete the initial sample analysis. We hypothesized that sample acquisition, processing, and return of results from all three ctDNA analyses would occur within 6 weeks of sample acquisition of the postoperative serum sample for greater than 75% of patients. The assay would be deemed feasible if this proportion is greater than 75%. A one-sided sample exact binomial test was performed at the 0.10 significance level to test this hypothesis, with the null hypothesis being that this proportion is 0.75 or less. A total of 30 evaluable patients were required to provide 82% power to reject the null hypothesis assuming the true proportion is 90%. Descriptive statistics were calculated to summarize the primary demographic, clinicopathologic,

disease status, and time to positive ctDNA result in surveillance. Associations between clinicopathologic characteristics and pre-surgical ctDNA status were compared using the chi-squared test or Fisher's exact test depending on the combined variable count. Statistical analysis was performed using IBM SPSS Statistics, for Windows (Version 28.0; IBM Corp., Armonk, New York, USA) and *P* values less than 0.05 were considered significant. The datasets generated during the current study are not publicly available due to containing information that could compromise research participant privacy and consent.

Results

Generation of circulating tumor DNA assay

Between November 2020 and December 2021, 43 patients consented, 28 of whom were eligible for enrollment and evaluation of the primary endpoint. All 28 eligible patients [mean age, 65 years; 14 (50%) male] completed pre and postoperative blood draws (Table 1).

Table 1 Study cohort

Characteristics	*n* = 28
Age (years), median (25th–75th percentile)	66 (58–74)
Sex, *n* (%)	
Male	14 (50)
Female	14 (50)
Race/ethnicity, *n* (%)	
African American or Black	1 (3)
American Indian or Alaskan Native	1 (3)
Non-Hispanic White	26 (93)
Clinical stage at diagnosis and study enrollment (AJCC 8th edition), *n* (%)	
IIB	7 (25)
IIC	6 (21)
III	15 (54)
Primary tumor location[a], *n* (%)	
Head/neck	2 (7)
Trunk	9 (32)
Upper extremity	5 (18)
Lower extremity	10 (36)
Melanoma of unknown primary	2 (7)
Histologic subtype, *n* (%)	
Nodular	10 (36)
Superficial spreading	6 (21)
Acral lentiginous	4 (14)
Desmoplastic	1 (4)
Unspecified/metastatic	7 (25)
Breslow depth, mm [median (25th–75th percentile)]	3.9 (2.4–5.0)
Ulceration, *n* (%)	13 (46)
Microsatellites, *n* (%)	4 (14)
Pathologic stage at diagnosis (AJCC 8th edition), *n* (%)	
IIB	4 (14)
IIC	5 (18)
IIIB	2 (7)
IIIC	15 (54)
IIID	2 (7)
Adjuvant therapy, *n* (%)	
Anti-PD1	17 (61)
Combined Anti-PD1/anti-CTLA-4	1 (4)
None	10 (36)
Follow-up time (days), median (25th–75th percentile)	
Surveillance cohort (*n* = 20)	443 (365–548)

AJCC, American Joint Committee on Cancer.
[a]Patients at study enrollment had either a primary tumor with/without concurrent regional metastatic disease or recurrent regional metastatic disease. This section describes where their associated primary tumor was located, either at study presentation or from treatment prior to relapse.

About half (13/28) were patients with clinical stage IIB/C melanoma who underwent wide excision with sentinel node biopsy. The remaining 15 patients had clinical stage III melanoma and underwent resection of clinically evident nodal/in-transit disease. All but two (26/28, 92.8%) pre-surgical ctDNA reports were generated and received within 6 weeks of tissue receipt with a median time of 3.4 weeks.

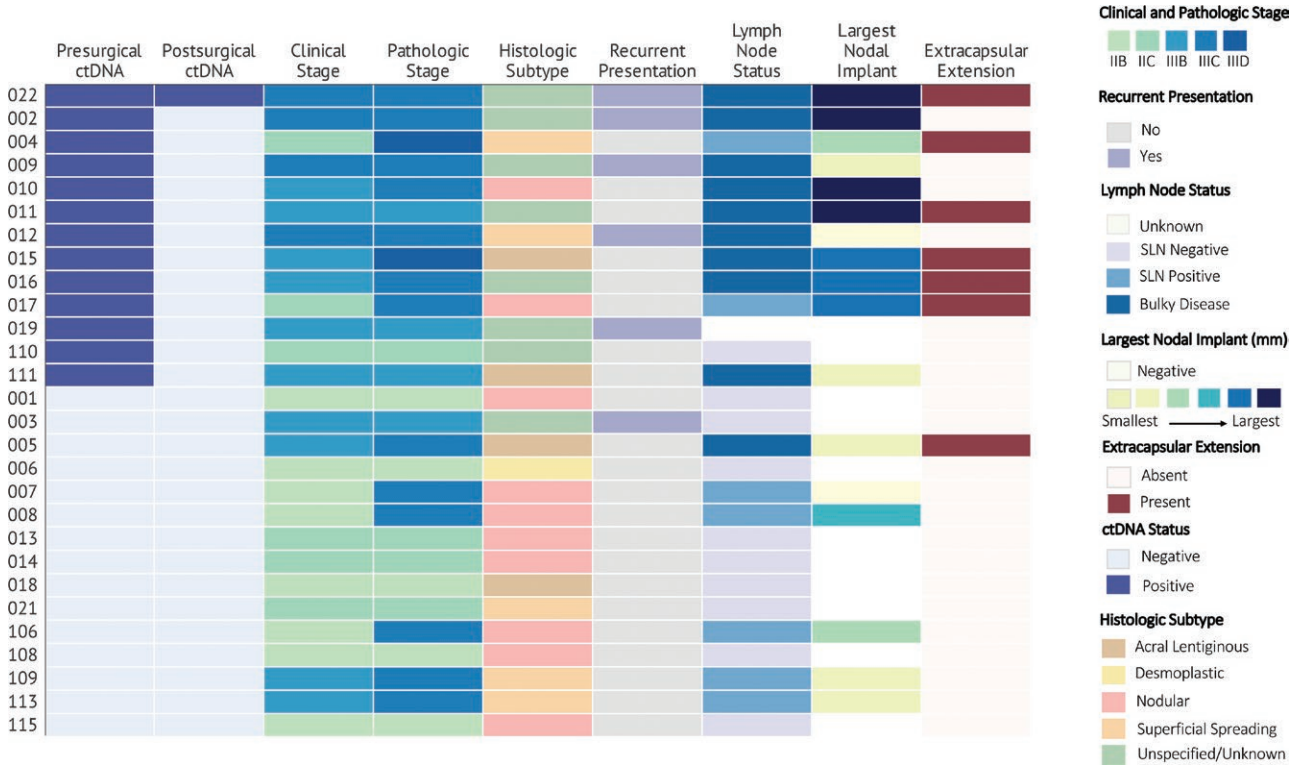
Of the 15 patients excluded, over half were due to insufficient tissue for assay development (8/15, 53%). All patients excluded due to insufficient tissue were pathologic stage IIB with a mean Breslow depth of 3.4 mm and accounted for two-thirds of all stage IIB patients initially enrolled (8/12, 66%). For many of these patients, available tumor tissue was limited by CLIA requirements for diagnostic tissue retention. Out of the remaining patients, six were excluded for inability to obtain blood or additional tissue samples within a clinically reasonable time frame for inclusion and one patient declined to proceed with the study after initial enrollment. Several blood samples were not able to be collected or shipped within the necessary time frame due to standard protocols being interrupted by the coronavirus disease 2019 pandemic. The inability to obtain tissue samples within a reasonable timeframe occurred in patients who had their initial biopsy performed outside of our hospital system and despite multiple attempts, we were unable to obtain the tissue block.

The majority (89%, 8/9) of patients with pathological stage IIB/C did not receive adjuvant systemic therapy as the study predates Food and Drug Administration approval of immunotherapy for this group of patients. In contrast, most (89%, 17/19) pathologic stage III patients received immunotherapy. Systemic immunotherapy was held for two pathologic stage IIIB patients due to a history of Guillain–Barre in one patient and a history of multiple autoimmune conditions in the other patient.

Preoperative and postoperative detection of circulating tumor DNA

Among patients with preoperative samples (n = 28), nine patients were pathologic stage IIB/C and 19 patients were pathologic stage III. Overall pre-surgical ctDNA detection was 46% (13/28) and was observed to be higher in patients with stage III (63%, 12/19) compared to patients with stage IIB/C disease (11%, 1/9) (Fig. 1). Eleven of the 12 (92%) of the pathologic stage III patients with detectable ctDNA prior to surgery had clinically evident disease at study enrollment, and the single patient with pathologic stage II melanoma with detectable preoperative ctDNA was stage IIC. Pre-surgical ctDNA detection was significantly associated with pathologic stage, clinically evident disease, number of involved lymph nodes, and extracapsular extension (Table 2). No significant association was observed between preoperative ctDNA status

Fig. 1



Clinicopathologic characteristics. Thirteen out of 28 (46%) had detectable ctDNA prior to definitive surgery and 96% (27/28) tested ctDNA-negative within 4 weeks post-surgery. Pre-surgical detection of ctDNA was significantly associated with later-stage and clinically evident stage III disease. ctDNA, circulating tumor DNA.

and sex, age, Breslow thickness, ulceration status, or the size of nodal metastasis. In the postoperative setting, all except one patient cleared their ctDNA within 4 weeks post-surgery. The one patient with detectable ctDNA in the postoperative setting had pathologic stage IIIC melanoma. The ctDNA concentration of this patient dropped substantially from a preoperative level of 447.63 MTM/ml to a postoperative level of 0.35 MTM/ml, suggesting early detectable MRD in this high-risk patient.

Detection of circulating tumor DNA in surveillance setting

Over half the cohort (n = 20) is continuing with surveillance blood draws every 3–6 months, depending on the pathologic stage. The clinical course for each patient, including the detection of ctDNA is outlined in Fig. 2. With a median follow-up of 443 days, six out of 20 (30%) patients had detectable ctDNA levels during surveillance. All six patients recurred with a mean time to recurrence of 280 days post-surgery. Detection of ctDNA in surveillance preceded the diagnosis of clinical recurrence in three patients (50%), was detected concurrent with clinical recurrence in two patients, and followed clinical recurrence in one patient. One additional patient developed recurrence without detection of ctDNA during surveillance.

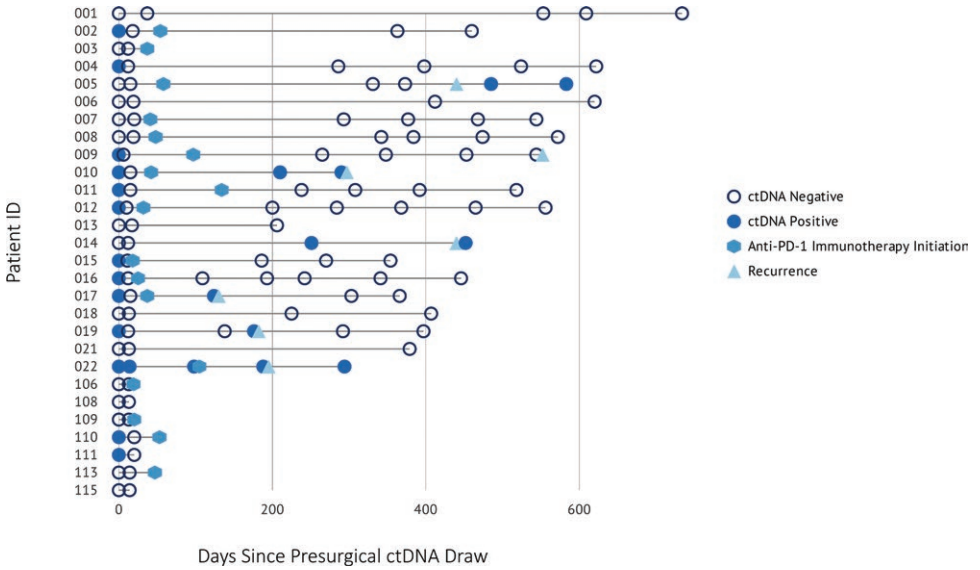
The positive ctDNA result preceded clinical or radiological recurrence by 78 days, 189 days, and 174 days in three patients (IDs 010, 014, and 022, respectively). Two of the patients (ID 010 and 022) had stage IIIC melanoma and received adjuvant pembrolizumab prior to recurrence. The other patient (ID 014) had stage IIC melanoma and did not receive adjuvant immunotherapy. The first patient (ID 010) developed neurologic symptoms about two and a half months after testing positive for ctDNA in surveillance, and an MRI identified metastatic disease to the brain as the initial site of recurrence. The time to recurrence was 288 days from surgery. The initial positive result was 0.34 MTM/ml at 210 days from surgery and the subsequent positive result was 6.57 MTM/ml at 294 days from surgery. The patient ultimately died of metastatic disease at 459 days post-surgery. The second patient (ID 014) developed a biopsy-confirmed distant metastasis to the lower pole of the kidney at 440 days post-surgery. The initial positive result was 0.12 MMT/ml at 251 days from surgery and the subsequent positive result was 31.81 MTM/ml at 452 days from surgery. The third patient (ID 022) has had persistently detectable

Table 2 Clinicopathologic characteristics of study cohort and relation to pre-surgical ctDNA status

Characteristic	Total, *n* (%)	Pre-surgical ctDNA, *n* (%)		
	n = 28	Negative (*n* = 15)	Positive (*n* = 13)	*P* value*
Sex				0.71
Female	14 (50)	7 (47)	7 (54)	
Male	14 (50)	8 (53)	6 (46)	
Age[a]				0.45
<66 years	14 (50)	9 (60)	5 (39)	
≥66 years	14 (50)	6 (40)	8 (61)	
Pathologic stage				0.02
II	9 (32)	8 (53)	1 (8)	
III	19 (68)	7 (47)	12 (92)	
Breslow thickness[a]				0.69
<3.9 mm	10 (36)	6 (40)	4 (31)	
≥3.9 mm	13 (46)	9 (60)	4 (31)	
Unknown[b]	5 (18)	0 (0)	5 (38)	
Ulceration				0.69
Absent	10 (36)	6 (40)	4 (31)	
Present	13 (46)	9 (60)	4 (31)	
Unknown[b]	5 (18)	0 (0)	5 (18)	
Number of involved lymph nodes				0.02
0	11 (39)	9 (60)	2 (15)	
1–2	10 (36)	5 (33)	5 (39)	
≥3	7 (25)	1 (7)	6 (46)	
Extracapsular extension				0.03
No	21 (75)	14 (93)	7 (54)	
Yes	7 (25)	1 (7)	6 (46)	
Largest nodal metastasis[a,c]				0.3
<5.5 mm	10 (36)	5 (83)	5 (46)	
≥5.5 mm	7 (25)	1 (17)	6 (54)	
Clinical status				0.007
SLN negative	9 (32)	8 (53)	1 (8)	
SLN biopsy positive stage III	4 (14)	3 (20)	1 (8)	
Clinically evident stage III	15 (54)	4 (27)	11 (85)	

ctDNA, circulating tumor DNA; SLN, sentinel lymph node.
[a]Categories defined by median.
[b]Excluded from *P* value calculation.
[c]Includes SLN-positive and clinically evident nodal disease.
**P* values calculated using chi-square or Fisher's exact test depending on count.

Fig. 2



Timeline of ctDNA detection, immunotherapy, and clinical recurrence. All six patients (005, 010, 014, 017, 019, 022) with detectable ctDNA in surveillance developed recurrence. One patient (009) without detectable ctDNA in surveillance developed brain metastases as the initial site of recurrence. ctDNA, circulating tumor DNA.

ctDNA since surgery that increased from 0.35 MTM/ml at 14 days post-surgery to 19.01 MTM/ml at 98 days post-surgery to 946.39 MTM/ml at 188 days post-surgery. Concurrent with the third post-surgical ctDNA draw, the patient was found to have multiple new in-transit lesions on clinical exam and computed tomography (CT) imaging as well as new pulmonary nodules, mediastinal and lower cervical adenopathy, consistent with metastatic disease.

Detection of ctDNA was concurrent with clinical recurrence in two patients. One patient (ID 017) with stage IIIC melanoma receiving adjuvant pembrolizumab developed regional nodal and in-transit recurrences detected clinically and with CT imaging at 139 days from surgery. The ctDNA level measured at the same time point was 14.21 MTM/ml. The other patient (ID 019) had stage IIIB melanoma and developed in-transit nodules that were identified clinically at 180 days from surgery. The ctDNA level measured at the same time point was 0.06 MTM/ml.

The patient (ID 005) with clinical recurrence proceeding ctDNA detection had stage IIIC melanoma and received adjuvant pembrolizumab. They developed regional nodal recurrence detected clinically at 440 days post-surgery. The ctDNA level measured following the detection of clinical recurrence was positive at 1.32 MTM/ml. The patient (ID 009) with clinical recurrence without positive ctDNA during surveillance had stage IIIC melanoma and had positive preoperative ctDNA (1.74 MTM/ml). The patient developed new neurologic symptoms at 544 days post-surgery and was found to have new distant metastases to the brain on imaging.

Discussion

Within the evolving treatment paradigm for melanoma patients, determining who will benefit most from treatment, the timing of therapy, and the ideal interval for imaging during surveillance are critical for optimizing patient outcomes. As a minimally invasive and easily repeatable test, serial monitoring with ctDNA during surveillance shows promise as a biomarker of recurrence risk prediction to further inform clinical decision-making for patients with high-risk diseases. While previous studies have demonstrated the clinical utility of ctDNA detection in melanoma, most of the published studies have employed ddPCR designed to detect prespecified mutations (Table 3). We demonstrated the feasibility of generating a personalized, tumor-informed ctDNA assay (bespoke mPCR NGS-based assay) for high-risk stage IIB/C and resectable stage III melanoma patients in a timeframe reasonable for assistance with real-time clinical surveillance. We found that in these high-risk patients, pre-surgical ctDNA was frequently present and was most common in patients with clinically evident stage III disease. Our preoperative ctDNA detection of 46% (11% in stage II and 63% in stage III) performed similarly to the reported overall detection by ddPCR for stage II and outperformed ddPCR for stage III [6–8]. The ability to detect ctDNA preoperatively may imply a higher risk of concurrent, occult micro-metastatic disease for a subset of patients and could have implications on future recurrence risk and therapeutic decisions.

Additionally, our initial surveillance results replicate previous findings that ctDNA is associated with clinical recurrence, and a positive result will precede clinical recurrence. All patients who went on to develop clinical recurrence had detectable ctDNA either preoperatively or during surveillance. This is consistent with previous findings, where detection of ctDNA prior to surgery is independently predictive of shorter MSS, and post-surgical ctDNA-positivity is associated with inferior disease-free survival (Table 3) [6–8]. The reliability of our approach is further supported by the consistent detection of ctDNA in serial blood samples and the strong correlation with clinical status. Surgical intervention was the only time point where ctDNA clearance was achieved, which is not unexpected. In contrast to previous studies, the majority of our stage III cohort received adjuvant therapy. To date, the impact of adjuvant therapy on ctDNA status in melanoma patients is unknown, and further surveillance data is needed. In addition, two of our patients who recurred initially developed brain metastases (ID 009 and 010). One was preceded by the detection of ctDNA in plasma during surveillance (ID 010) while the other patient (ID 009) had positive preoperative ctDNA, but undetectable levels in surveillance (ID 009). Our findings are consistent with previous studies that have concluded that plasma ctDNA detection has more limited use for the detection of metastatic disease in the central nervous system due to the blood–brain barrier [5,22].

While our results are promising, this study is limited by its small sample size, nonrandomized design, and inconsistent timing of post-surgical surveillance blood collection across patients. Additionally, two-thirds of patients enrolled with stage IIB melanoma were ineligible due to insufficient tissue to undergo WES for assay generation. The small amount of pathologic tissue available in some primary melanoma biopsies may limit the utilization of this approach in some clinical stage II patients, and further investigation and optimization of personalized ctDNA generation in this scenario are needed; however, because WES was being performed as part of a research protocol, institutional CLIA requirements for diagnostic tissue retention restricted tumor block depletion. In standard workflows outside of research, more of the primary tumor would possibly be available for tissue assay generation.

Our higher ctDNA detection rate suggests that a personalized, tumor-informed approach may offer greater sensitivity for ctDNA detection in patients with advanced-stage melanoma, and initial results suggest

Table 3 Overview of recent ctDNA studies in melanoma

Source	Patient cohort	Stage	Technique	Markers	Results
Ashida *et al.* (2017) [14]	*n* = 5	IV	ddPCR	*BRAF*, *NRAS*	Response to treatment with nivolumab corresponded to a reduction in ctDNA levels
Bratman *et al.* (2020) [15]	*n* = 12	IV	Tumor-informed ctDNA assay	16 clonal somatic mutations	Baseline ctDNA level and early clearance of ctDNA were associated with a good prognosis
Forschner *et al.* (2019) [16]	*n* = 35	IV	ddPCR	*BRAF*, *NRAS*, *GNAQ*, *CDK4*, *STAT1*	High tumor mutation burden and undetectable ctDNA in follow-up were associated with response to immunotherapy and improved survival
Gray *et al.* (2015) [17]	*n* = 48	IV	ddPCR	*BRAF*, *NRAS*	Monitoring ctDNA level provides insight into response to kinase inhibitor therapy and can be used to monitor for early evidence of resistance to treatment
Lee JH *et al.* (2017) [18]	*n* = 86	IV	ddPCR	*BRAF*, *NRAS*, *KIT*	Persistently elevated ctDNA levels despite immunotherapy predicted a poor prognosis
Lee JH *et al.* (2019) [7]	*n* = 174	IIIB/C/D	ddPCR	*BRAF*, *NRAS*, *KIT*	Presence of ctDNA prior to surgery independently predicted worse survival in patients receiving no adjuvant therapy
Lee RJ *et al.* (2018) [6]	*n* = 161	IIB/C, III	ddPCR	*BRAF*, *NRAS*	Post-surgical detection of ctDNA was predictive of relapse and decreased survival in high-risk resected melanoma patients
Marczynski *et al.* (2020) [5]	*n* = 19	III, IV	ddPCR	*BRAF*, *NRAS*, *TERT*	Patients with detectable ctDNA had a significantly increased risk of progression
Marsavela *et al.* (2020) [19]	*n* = 110	IV	ddPCR	*BRAF* or custom *NGS* panel if *BRAF WT*	Low concentration of ctDNA prior to initiation of immunotherapy predicted longer progression-free survival
Rowe *et al.* (2018) [20]	*n* = 127	IIB–IV	BEAMing digital PCR	*BRAF*, *NRAS*	Visceral metastases and increased tumor burden were associated with the detection of ctDNA
Seremet *et al.* (2019) [21]	*n* = 85	IV	ddPCR	*BRAF*, *NRAS*	Baseline and interval ctDNA levels reflected tumor burden and were predictive for clinical response to immunotherapy
Tan *et al.* (2019) [8]	*n* = 126	III	ddPCR	*BRAF*, *NRAS*, *TERT*	Pre and postoperative ctDNA detection correlated with more advanced sub-stage and was associated with a higher probability of recurrence

BEAMing, beads, emulsions, amplification, magnetic; ctDNA, circulating tumor DNA; ddPCR, digital droplet PCR; NGS, next-generation sequencing.

this should be investigated further to assist in surveillance, especially for patients lacking more common mutations required for fixed mutation ddPCR assays. Larger prospective studies should assess the clinical applications and utility of ctDNA monitoring in high-risk melanoma patients. Specifically, this includes investigating pre-clinical evidence of recurrence to inform surveillance imaging protocols, detection of MRD and risk stratification for receipt of adjuvant therapy, and evaluating response/resistance to systemic therapy.

Conclusion

Our results demonstrate that the successful design of a personalized, tumor-informed mPCR NGS-based ctDNA assay is feasible for patients with melanoma, particularly in resectable stage III disease. Research tissue requirements may have impaired evaluation of the Stage IIB patient population, and conclusions regarding use in this subgroup of patients must be taken with caution. This evidence supports further investigation into the use of the platform to assess the clinical utility of ctDNA in high-risk melanoma patients. Larger scale prospective studies are warranted to evaluate the use of serial ctDNA monitoring for early detection of recurrence, and its potential impact on surveillance imaging protocols and adjuvant therapy decisions in high-risk melanoma.

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Conflicts of interest

K.G. was employed by HCI for the extent of his involvement in this study; however, he left HCI in August 2021 and currently works for Merck Pharmaceuticals. G.B. and R.R. are both employed by Natera, Inc. T.B. is the PI for cancer clinical trials sponsored by Genentech, Amgen, and Replimune, but receives no direct compensation for these roles. For the remaining authors, there are no conflicts of interest.

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