ORIGINAL ARTICLE

Individualized Cell-Free DNA Monitoring With Chromosomal Junctions for Mesothelioma

Kaushal Parikh, MD,[a] Faye R. Harris, MS,[a] Giannoula Karagouga, MS,[a,†] Amy Schrandt, CRA,[a] Jay Mandrekar, PhD,[a] Sarah Johnson, MS,[a] Alexa McCune, BS,[a] Dorsay Sadeghian, MD,[a] Debarshi Roy, PhD,[a] Katarzyna Polonis, PhD,[a] Athanasios Gaitatzes, MS,[a] Aaron O. Bungum, BS,[a] Eric S. Edell, MD,[a] Mitesh J. Borad, MD,[b] Tobias Peikert, MD,[a] Farhad Kosari, PhD,[a] John Cheville, MD,[a] George Vasmatzis, PhD,[a] Aaron S. Mansfield, MD[a,*]

[a]*Mayo Clinic, Rochester, Minnesota*[a]
[b]*Mayo Clinic, Phoenix, Arizona*

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ABSTRACT

Introduction: The spatially complex nature of mesothelioma and interventions like pleurodesis, surgery, and radiation often complicate imaging-based assessment. Further, cell-free DNA (cfDNA) based monitoring strategies are inadequate for mesothelioma, given the presence of a few recurring nonsynonymous somatic variants. However, patient-specific chromosomal rearrangements are commonly found in mesothelioma. Our study objective was to develop an individualized cfDNA assay to enable blood-based monitoring using circulating tumor DNA (ctDNA) in mesothelioma. We hypothesized that the unique chromosomal rearrangement junctions found in mesothelioma could be employed for individualized ctDNA detection and disease monitoring.

Methods: DNA was extracted from tumor specimens for whole genome sequencing. Chromosomal junctions, prioritized by highest allele frequency and low homology to the rest of the genome, were selected for detection. Primers and Taqman probes were designed to span the junctions, forming personalized junction panels. Patient plasma obtained before therapy and at response assessment was tested for the presence of personalized junctions via quantitative polymerase chain reaction.

Results: Our study included nine patients, four with peritoneal and five with pleural mesothelioma. 763 chromosomal junctions were identified in the tumors of all cases. We selected three to five junctions per sample for quantitative polymerase chain reaction. We detected 25/30 (83%) of selected junctions in the plasma of seven out of nine patients (78%). Cell-free junction detection at follow-up was concordant with disease status: cfDNA junctions were detected in three patients with persistent disease, and not detected in a patient with no evidence of disease after surgery.

Conclusions: With further validation, individualized ctDNA junction assays could supplement imaging for disease monitoring in mesothelioma.

Keywords: Mesothelioma; Cell-free DNA; Chromosomal rearrangements

Introduction

Mesothelioma is a rare and aggressive malignancy that is often incurable. Most patients are frequently

†Deceased.

*Corresponding author.

Address for correspondence: Aaron S. Mansfield, MD, Mayo Clinic Medical Oncology, 200 First Street SW, Rochester 55905200, MN. E-mail: Mansfield.Aaron@mayo.edu

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diagnosed with advanced disease.[1] Mesotheliomas are also spatially complex cancers that are not easily measured with standard imaging techniques. The standard response evaluation criteria in solid tumors do not adequately capture the responses seen in this disease, prompting the development of modified pleural response evaluation criteria.[2] Even these criteria have limitations given the many pitfalls of interpretation. A critical barrier to effective clinical management of mesothelioma and clinical trial design is the lack of real-time biomarkers of recurrence and therapeutic efficacy.

The recent advances in cell-free DNA (cfDNA) testing for solid tumors have not been applicable to patients with mesothelioma. Despite the decades of asbestos exposure associated with most cases of this disease, there are relatively few recurrent, nonsynonymous somatic variants detected in these tumors.[3,4] The largest genomic profiling study of mesothelioma to date showed that of the few somatic variants present, 85% of the detected variants were novel and had not been reported in the Catalogue of Somatic Mutations in Cancer.[3] The most commonly mutated genes identified were tumor suppressors or chromatin remodelers (such as *BAP1*, *NF2*, *SETD2*, *DDX3X*, etc.). Except for rare cases of *ALK* rearrangements in a few patients with peritoneal mesothelioma,[5] no targetable oncogenes driving this malignancy have been identified and approved for use in this setting. In Addition, the most commonly mutated genes in mesothelioma do not currently have therapeutic potential with Food and Drug Administration-approved therapies and are not prioritized for inclusion on commercially available cfDNA assays. Furthermore, some mutations involving *BAP1* are difficult to detect with standard sequencing approaches.[6] The only study to date of cfDNA for mesothelioma detected tumor-specific somatic variants in the plasma of merely 30% (three out of 10) of patients.[7] This low sensitivity is inadequate for the widespread adoption of cfDNA for mesothelioma to detect disease recurrence and to monitor responses to systemic therapies.

Chromosomal rearrangements are a defining molecular feature of mesothelioma. In our prior work, we reported that mesothelioma is frequently involved with complex patterns of chromosomal rearrangements,[8,9] such as chromothripsis or chromoplexy.[6] In prior work, our group used whole genome sequencing to detect chromosomal rearrangements in endometrial and ovarian cancers and then designed primers to detect the junctions of these rearrangements in blood.[10,11] While single nucleotide variants are often present as one genome copy[12] and are thus found in plasma at the threshold of sequencing error,[13] there are often increased copy numbers of chromosomal rearrangements in cancers which could increase the sensitivity of their detection in plasma or tissue with appropriate technique.[12] Given the limitations for the detection of genes commonly mutated in mesothelioma, we sought to determine whether the approach of individualized circulating tumor DNA (ctDNA) junctions could be used for monitoring in mesothelioma.

Methods

Seven patients with biopsy-proven pleural or peritoneal mesothelioma were prospectively enrolled and consented to this institutional review board (IRB) approved study (IRB no. 20-001460). Participants consented and enrolled at Mayo Clinic in Rochester, MN between September 2020 and September 2021. Two patients were retrospectively pulled from our tissue biorepository (IRB No. 13-000942). All patients had sufficient tumor specimens available for sequencing, and baseline blood draws were collected for ctDNA testing. Following our previous study establishing the value of tumor junction burdens in predicting response and survival with immune checkpoint inhibitors, we investigated whether ctDNA junctions could be detected in patients with mesothelioma (Fig. 1).[9]

Blood and tissue were collected and processed as described previously.[11] Briefly, to construct a junction testing panel for each patient, three to five junctions were initially selected for plasma screening (Supplementary Table 1) with an emphasis on multi-copy junctions when possible and precise breakpoint identification. Tumor purity, fragment counts, bridged coverage, and tumor ploidy were assessed for sequencing quality control. (Supplementary Table 2). Primers and Taqman probes were designed for all cases, with a mean amplicon size of 85 base pairs (range 66–110 base pairs). Cycle thresholds (Ct's) obtained following quantitative polymerase chain reaction (qPCR) with pre-amplification were interpreted semi-quantitatively due to the lack of a standard curve. When ctDNA was plotted on linear graphs, qPCR Ct's were normalized as described below. As previously described,[10,11] an amplicon corresponding to N-acetylglucosamine kinase (a housekeeping gene)[14] was used as an amplification control present in all cfDNA.

Collection and Processing of Tumor Tissue and Blood

The tumor was identified in the pleural or peritoneal samples by gross and frozen section microscopic examination in the Mayo Clinic Frozen Section Laboratory. A fresh tumor in excess of diagnostic clinical material was placed in a plastic cassette and snap-frozen in isopentane. A secondary review by an experienced surgical pathologist was performed using a toluidine blue-stained frozen section. Sufficient tumor cellularity was obtained through the use of macrodissection.[15] DNA was extracted with the Qiagen AllPrep DNA/RNA mini kit (Qiagen,

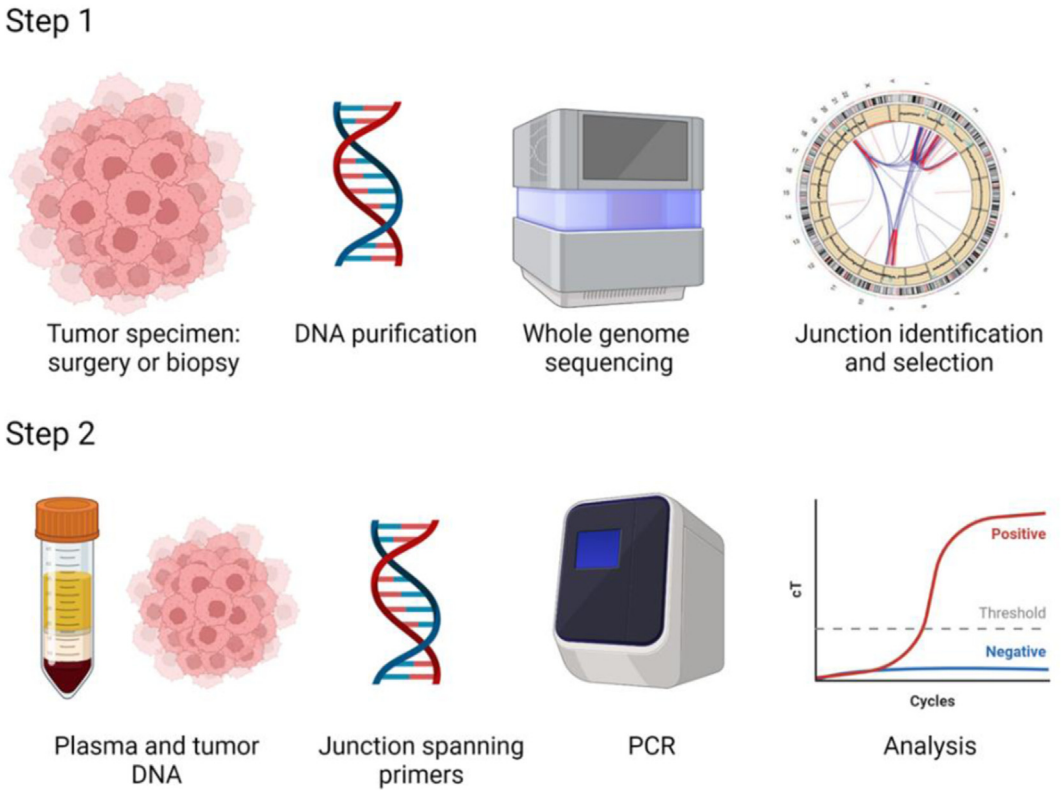

Figure 1. Study design. Our study involved detecting junctions that result from chromosomal rearrangements, insertions and deletions in mesothelioma in Step 1, then designing primers and probes that span these junctions to detect them in plasma in Step 2. Figure created with BioRender at BioRender.com.

Valencia, CA; catalog No. 80204) or Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA; catalog No. 69504) following the manufacturer's protocol.

At each blood draw, whole blood was collected in ethylenediamine tetraacetic acid tubes and processed for platelet-poor plasma as described previously,[11] within four hours and stored at −80°C. Between 3 mL to 10 mL of plasma (mean 5.3 mL) was processed using the QIAamp circulating nucleic acid kit (Qiagen, Valencia, CA; catalog No. 55114) with a concentration step using Zymogen columns (Zymo, Irvine, CA; catalog No. D4013) using eluted volumes as specified in (Supplementary Table 3). Processed cfDNA was stored at −20°C. Concentrations of total cfDNA yield were measured with a Qubit 2.0 using the dsDNA HS assay kit (Thermo Fisher Scientific, #Q32854). Germline DNA was isolated from each patient using the buffy coat with Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA; catalog No. 69504) and tested with each junction to ensure selected junctions were somatic.

Tumor Sequencing and Bioinformatic Processing

Whole genome sequencing was performed on all tumor tissue samples, using either the Nextera Mate-Pair Kit (Illumina, #FC-132-1001) or whole genome NEB ultra II library prep (New England Biolabs, #E7645). All libraries were sequenced at an average depth of 69.33 bridged coverage, sufficient for junction calling.

The sequencing data were mapped by the binary indexing mapping algorithm,[16] which simultaneously maps both fragment reads to the GRCh38 reference genome. Tumor junctions were identified by structural variant analysis tools tools,[17] which use a minimum of three supporting reads for junction detection. Three to five somatic junctions were selected per tissue sample for plasma screening. The following criteria were used for junction selection: (1) Junctions with higher supporting read numbers were selected for increased confidence and increased tumor representation, (2) junctions associated with replicating copy gains for greater sensitivity of detection, (3) junctions with precise breakpoints for primer designing, (4) low likelihood of germline rearrangement, (5) not located in highly repetitive regions of the genome to minimize off-target signals and (6) ability to design primers with amplicon size of 80 to 100 base pairs.[11] These junctions were selected to allow detection at greater specificity than single nucleotide variants and decrease the likelihood of false positives.

Polymerase Chain Reaction and qPCR of Personalized Junction Assays

Polymerase chain reaction (PCR) was performed with an initial multiplexed 10-cycle pre-amplification step using Easy A Taq Polymerase (Agilent, Santa Clara, CA; catalog No. 600404) in a 100 μl total reaction to amplify target junctions. The 100μl reaction was concentrated using Zymogen columns (Zymo, Irvine, CA; catalog No. D4013) to a total elution volume to enable 13 μl to 15 μl per junction used for each multiplexed PCR reaction. Following pre-amplification, qPCR was performed in a non-multiplexed manner. For the qPCR, Taqman Gene Expression Master Mix (ThermoFisher Waltham, MA; catalog No. 4369016) was used 2x in the reaction. qPCR was run on Applied Biosystems ViiA-7 or QuantStudio 7 (Applied Biosystems/ThermoFisher) using standard protocols as described previously with technical duplicates for all Taqman qPCRs. Longitudinal follow-up blood draws were normalized by N-acetylglucosamine kinase measurement at baseline to account for variable DNA concentration input. Germline control, a normal control comprised of pooled plasma processed for cfDNA using the Circulating Nucleic Acid kit standard protocol, and a No Template Control of RNase/DNAse free water were used for confirming specificity. Patient-specific buffy coat DNA was also used as a negative control. Patient-specific tumor DNA was used in each case as a positive control. The presence of junctions in tumor DNA and their absence in negative controls were confirmed prior to testing in patient plasma. Junction primers demonstrating poor specificity or lacking sensitivity were not tested in plasma cfDNA samples (Supplementary Table 4).

Descriptive statistics were used to summarize the patient characteristics and rates of ctDNA detection. Biorender was used to create Figure 1. R was used to generate the bar plot (Fig. 2) and the alluvial plot was made with the R package gg alluvial (Fig. 3).

Results

Patient Characteristics

We included 9 patients in this study, 5 with pleural mesothelioma and 4 with peritoneal mesothelioma. The median age of the participants was 66 years, ranging from 47 to 76 years. Five participants were males (2 with peritoneal and 3 with pleural) and four were females (2 with peritoneal and 2 with pleural). Histologically, there were five patients with epithelioid mesothelioma, one with sarcomatoid disease, and three with biphasic disease. Four of nine patients were alive at

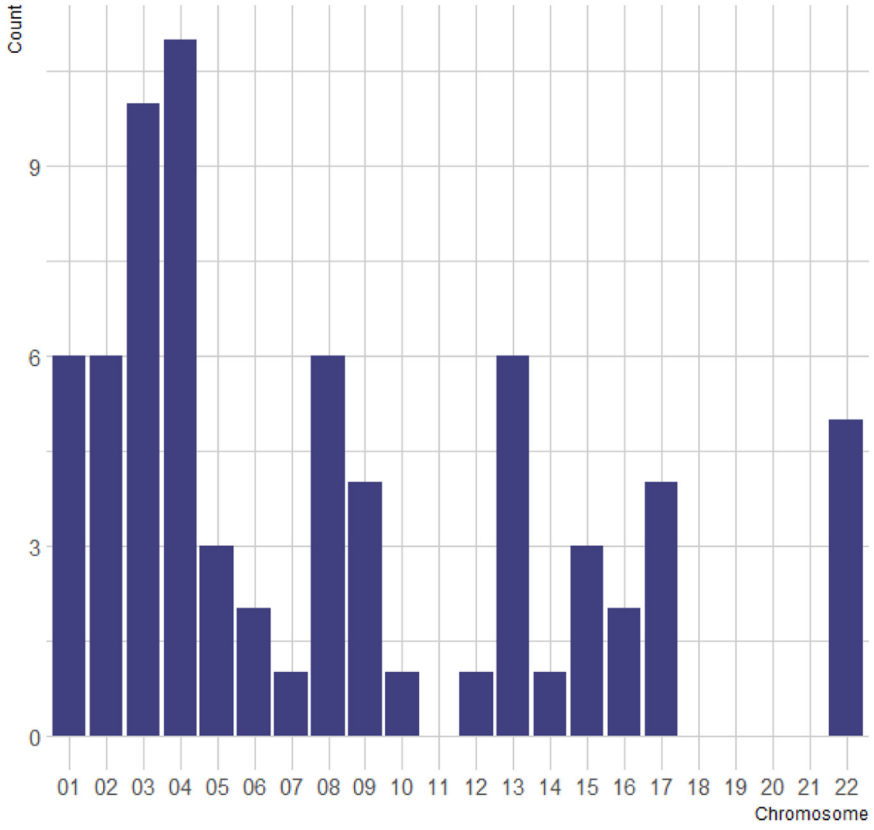

Figure 2. Chromosomes involved with selected junctions. The x-axis represents chromosomes 1 through 22 and the y-axis represents the number of junctions selected involving each chromosome.

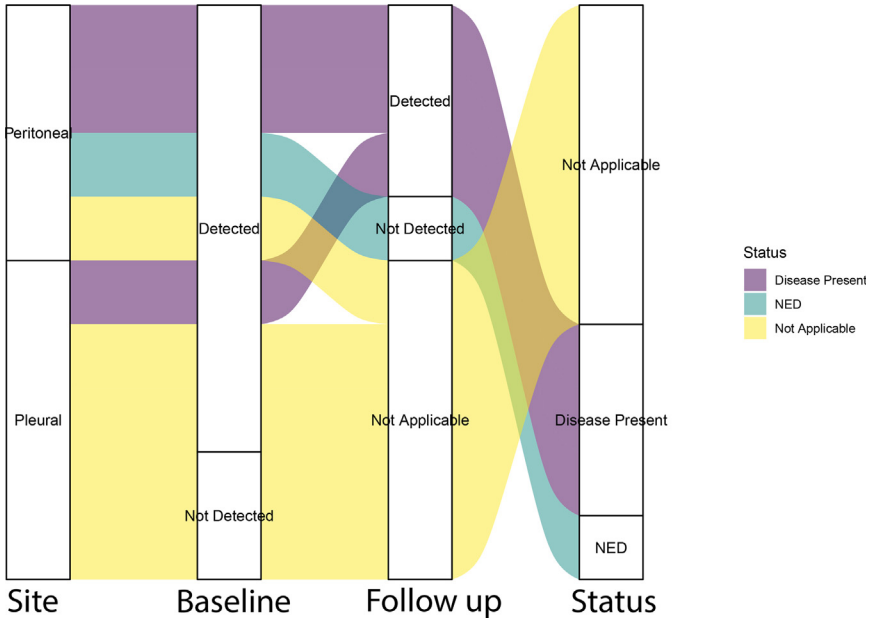

Figure 3. The first column of this alluvial plot demonstrates the primary site of the mesothelioma. The second column represents whether any circulating tumor DNA was detected. The third column represents whether circulating tumor DNA was detected after treatment. The fourth column represents whether there was persistent disease by imaging or not. NED, no evidence of disease.

the last follow-up, and one of them was alive after surgery without definitive recurrence on imaging. Among the four living patients, one was diagnosed with biphasic histology while the other three were diagnosed with epithelioid histology. Only one of the living patients was diagnosed with peritoneal mesothelioma (Table 1).

Whole Genome Sequencing and Junction Selection

Whole genome sequencing performed on tumor DNA detected multiple inter- and intra-chromosomal rearrangements, deletions, and amplifications in each specimen as observed in our prior projects (Representative example in Fig. 4, all specimens Supplementary Figs. 1–8).[8,9,18] In nine tumor tissue specimens, we detected a total of 763 chromosomal junctions (median 79, range 22–199). Out of 763 junctions among the 9 patients, we selected a total of 36 chromosomal junctions of interest, corresponding to three to five personalized junctions per specimen. Of these, 20 were intrachromosomal and 16 were interchromosomal junctions (Supplementary Table 1). Chromosome 4 was involved most frequently (in 7 unique junctions and in 11 as a partner) followed by chromosome 3 (in 10 junctions) (Fig. 2).

Following the selection of DNA junctions, PCR primers were designed for each junction flanking the breakpoints. All patients provided baseline plasma samples and four patients provided at least one post-treatment plasma sample. Thirty of the 36 junctions with sufficient sensitivity and specificity on qPCR in tumor specimens and controls were selected for ctDNA testing. Out of nine patients with baseline draws, seven had detectable ctDNA in plasma (78%) with 25 of the 30 junctions (83%) detected (Supplementary Table 4). Subsequent plasma was collected in four out of these seven patients. Three patients had detectable ctDNA junctions on follow-up, corresponding with persistent disease. Case MesoMon001 with epithelioid peritoneal mesothelioma had three detectable inter-chromosomal junctions, all involving chromosomes 2 and 4 (2::4) and one intra-chromosomal junction in chromosome 4 (4::4). Of these, one junction was rejected due to contamination, and the other three were followed in plasma. Following treatment with preoperative therapy with carboplatin, pemetrexed, and durvalumab and surgery, none of the junctions could be detected by qPCR,

Table 1. Patient Characteristics

Characteristic	Value
Sex, n (%)	
Female	4 (44)
Male	5 (56)
Age (median, range)	66 y (47-76)
Histology, n (%)	
Epithelioid	5 (56)
Biphasic	3 (33)
Sarcomatoid	1 (11)
Site, n (%)	
Pleural	5 (56)
Peritoneal	4 (44)

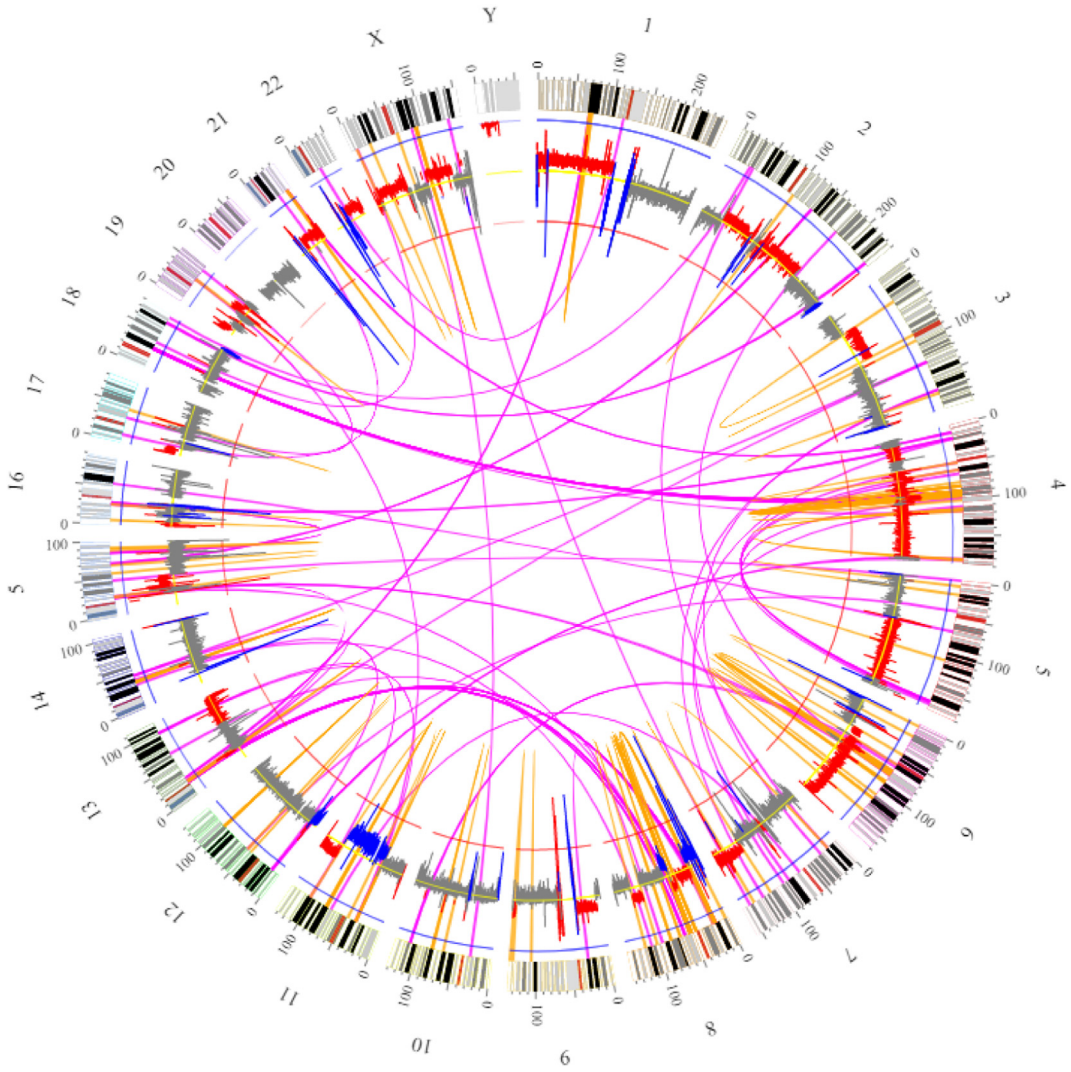

Figure 4. Representative Circos plot (Mesomon002). In this figure chromosome coverage is colored according to their bioinformatically determined level; with grey, blue, and red dots indicating normal diploid, gains and losses, respectively, and junctions represented as pink lines linking inter-chromosomal regions and orange lines for intra-chromosomal regions. This specimen demonstrates multiple intra- and inter-chromosomal rearrangements with gains and losses in multiple chromosomes.

suggesting treatment response (Figs. 3 and 5). At the last follow-up, this patient had no visible disease on imaging. In case MesoMon002 with biphasic pleural mesothelioma, 4 chromosomal junctions were selected from tumor sequencing for ctDNA detection (Supplementary Table 1): two intra-chromosomal (8::8 and 4::4) and two inter-chromosomal (1::22 and 8::13). The 8::8 junction was omitted due to lack of confirmation on tumor tissue PCR. The other three junctions passed quality control and were selected for monitoring. This patient had a mixed response to treatment with improvement in pleural disease and development of osseous metastases at first follow-up after treatment with ipilimumab and nivolumab, which correlated with persistent ctDNA detection of all three selected junctions on follow-up (Figs. 3 and 5).

We selected four intrachromosomal junctions of interest in case MesoMon003 (3::3, 4::4, 15::15, and 3::3), and one junction was discarded due to negative detection in tumor tissue by PCR at baseline (4::4). At first follow-up, nine weeks after initiating treatment with pembrolizumab, this patient had a favorable response to treatment and decreased ctDNA detection for all three junctions. At the second follow-up, nine weeks later, ctDNA detection of junctions by qPCR increased, suggesting increased tumor burden. Disease progression was confirmed with computed tomography (CT) imaging in this patient (Figs. 3 and 5). Similarly, for case MesoMon006 with sarcomatoid peritoneal mesothelioma, we selected five junctions of interest (4 intrachromosomal, 5::5, 9::9, 17::17 and 17::17; and 1 interchromosomal 8::15). Following initial treatment with ipilimumab and nivolumab, this patient continued to have persistent disease on imaging, which correlated with persistence of all five junctions in the follow-up plasma specimen (Figs. 3 and 5).

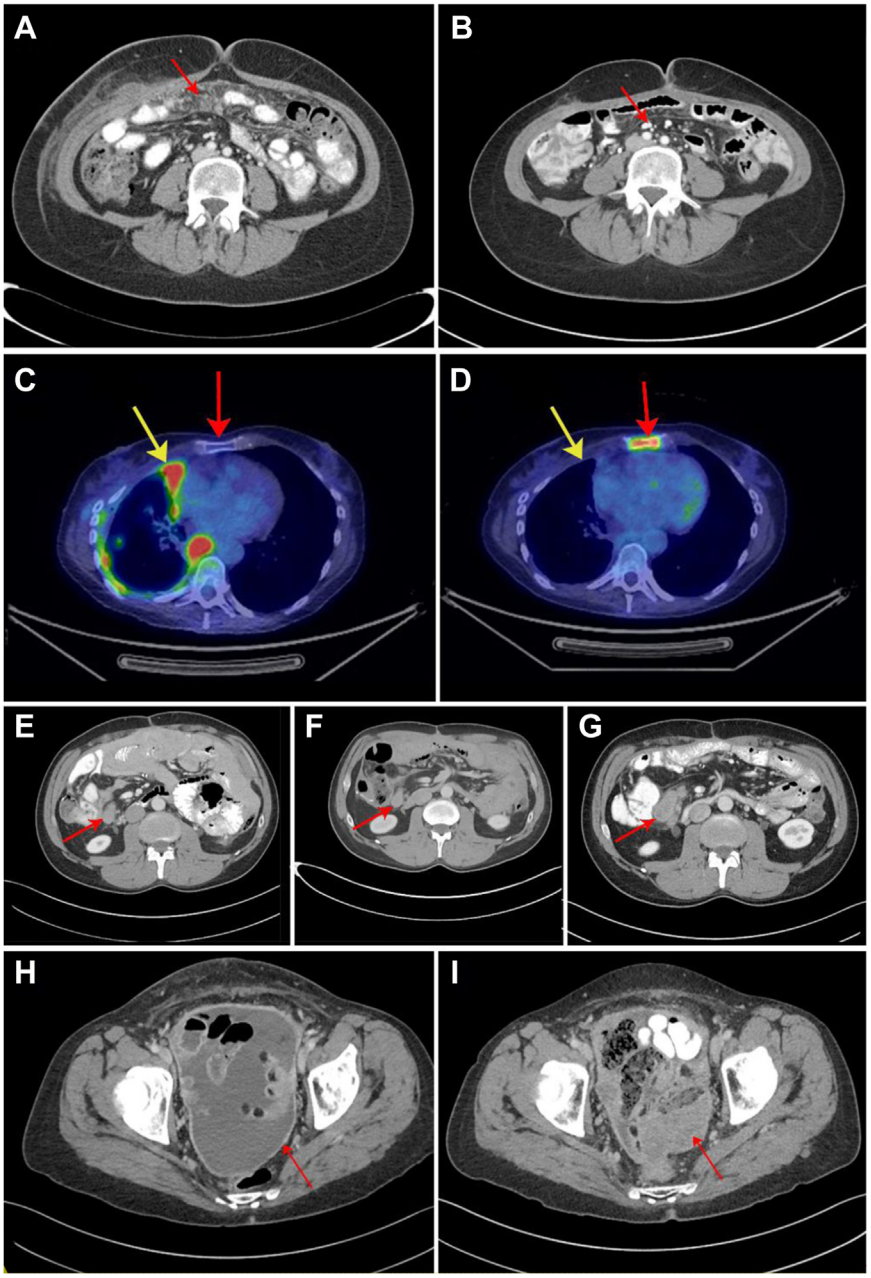

Figure 5. Representative images and response to treatment. Pre- and post-treatment scans of patients. *(A)* MesoMon001, showing initial peritoneal mesothelioma in the left panel, and *(B)* post-resection NED. *(C)* MesoMon002, showing initial FDG avid sites of pleural mesothelioma, and *(D)* mixed response with new sternal metastasis in the right panel. *(E)* MesoMon003, showing peritoneal nodule that *(F)* responded initially to treatment, appearing smaller, and later *(G)* progressed in the right panel. *(H)* MesoMon006, showing ascites initially, and *(I)* disease progression after treatment. FDG, fluorodeoxyglucose; NED, no evidence of disease.

Discussion

In this pilot study, we established the feasibility of creating personalized ctDNA panels of DNA junctions that result from chromosomal rearrangements in mesothelioma. We also showed that the dynamics of ctDNA detection were associated with clinical responses on imaging in the patients who provided serial specimens. Individualized ctDNA based on chromosomal rearrangements could potentially improve upon cfDNA monitoring based on prior approaches using non-synonymous mutations which are infrequent in mesothelioma.

We detected junctions of interest in patients with epithelioid, sarcomatoid, and biphasic histologies, attesting to the generalizability of our assay across histologic variants of mesothelioma. Chromosomes 1 through 4 were more often involved with inter- and

intra-chromosomal rearrangements, as can be expected owing to the longer lengths of these chromosomes. This personalized, tumor-informed junction assay approach detected ctDNA in the plasma of 78% of patients (7/9) at baseline. Contrarily, prior studies have identified tumor-specific genetic variants in cfDNA in 30% of cases.[7] We also showed that detection or loss of these junctions in the peripheral blood of four patients correlated with persistent or responsive disease, respectively. In the cases where we were not able to detect ctDNA, one case had a very low tumor burden that was visualized with video-assisted thoracoscopic surgery and not clearly seen on CT-based imaging. The second patient whom we did not identify ctDNA had widespread biphasic pleural mesothelioma. These cases demonstrate that a low tumor burden may limit detection of ctDNA and that other uncertain technical, clinical, or biologic features may also limit ctDNA detection, such as low sensitivity of whole genome sequencing and low shedding of tumors despite significant disease burden. Mesothelioma also tends to have low rates of apoptosis and tumor necrosis,[19,20] which may explain low tumor DNA shedding in the blood. Regardless, this pilot study demonstrates an improvement in the baseline detection of ctDNA using junctions instead of single nucleotide variants. It is also the first study to demonstrate concordance between the dynamics of individualized ctDNA detection and disease status in mesothelioma.

While cfDNA technologies were initially developed as tools for prenatal screening for common aneuploidies, their uses have expanded and are now a cornerstone of diagnostic evaluations in multiple cancers.[21] This is most apparent in the case of NSCLC, where ctDNA detection is a validated and often, necessary tool for therapy selection in patients with metastatic disease. In fact, the addition of plasma next-generation sequencing (NGS) analysis to tissue-based NGS at diagnosis of NSCLC was shown to markedly increase detection rates of therapeutically actionable targets.[22] Current guidelines recommend ctDNA assessment in tandem or before tissue sequencing given its relatively shorter turnaround time and non-invasive approach.[23] ctDNA concentration has also been correlated with higher tumor burden, shorter duration of response, and increased likelihood of progression. For instance, in the phase III ALEX trial, ctDNA concentration correlated with the number, size, and total volume of tumors in patients with metastatic ALK fusion lung cancer.[24] Further, liquid biopsies are increasingly emerging as a tool for early detection and diagnosis of solid organ malignancies.[25,26]

While molecular residual disease (MRD) detection in the blood has been the standard of care for hematologic malignancies, ongoing MRD studies in solid tumors could impact clinical practice in the near future. There have been significant efforts to evaluate MRD in breast cancer, colorectal cancer, and lung cancers.[27,28] Clearance of ctDNA after concurrent chemoradiation therapy for localized NSCLC strongly predicted superior outcomes, regardless of subsequent durvalumab therapy.[29] In stage II colon cancer, adjuvant chemotherapy based on the detection of ctDNA post-surgery led to reduced adjuvant chemotherapy utilization while maintaining disease-free survival rates.[30] In the BR.36 randomized phase II trial, molecular response defined as maximal molecular allele fraction clearance at the third cycle of pembrolizumab in patients with metastatic NSCLC, was associated with Response Evaluation Criteria in Solid Tumors-based objective response, longer progression-free survival, and longer overall survival.[31] In the I-SPY 2 trial, ctDNA clearance after neoadjuvant therapy was associated with improved rates of pathologic complete response and overall survival in early-stage breast cancer whereas lack of ctDNA clearance after neoadjuvant therapy was predictive of poor response and metastatic disease recurrence.[32] In a prospective phase II trial, bespoke tumor-informed ctDNA assays using tumor whole exome sequencing demonstrated that ctDNA dynamics during treatment with pembrolizumab correlated strongly with progression-free survival, overall survival, clinical response, and clinical benefit in five solid malignancies.[33]

Response assessment to treatment is particularly challenging for mesothelioma due to a variety of reasons such as (1) lack of obvious and measurable pleural or pulmonary tumors, (2) presence of pleural effusion limiting tumor visibility, (3) fibrosis and inflammation from pleurodesis and surgery, and (4) variable fluorodeoxyglucose activity on Positron Emission Tomography–CT. Further, mesotheliomas tend to have a low tumor mutational burden, which limits longitudinal monitoring using standard liquid biopsy NGS approaches.[3,4] This creates an opportunity for a personalized assay such as the one we present here for disease monitoring. Tumor-informed ctDNA monitoring could potentially complement traditional imaging-based response assessment, particularly in challenging cases. Our previous work exhibiting the high prevalence of complex chromosomal rearrangements in mesothelioma provides a rationale for a platform for personalized junction-based ctDNA monitoring. Our proof-of-concept prospective study demonstrates the feasibility of detecting chromosomal rearrangements in tumors of all enrolled patients and now highlights the possibility of monitoring these structural variants in the plasma as a reflection of tumor burden as well as a marker for response to treatment.

Our study has several limitations. First, our sample size was relatively small due to the rarity of the mesothelioma and the coronavirus disease 2019 pandemic,

which limited patient enrollment and tissue and plasma acquisition. Three patients who enrolled in this study did not return for subsequent plasma sample collection during the pandemic and received treatment locally. Research-based tissue biopsies were paused during the height of the pandemic, and the original diagnostic materials did not yield sufficient DNA for whole genome sequencing and quality control in other patients. Second, we also had a short follow-up time with only limited on-treatment specimens, limiting our attempts to serially monitor disease status with blood. Third, while we were able to identify junctions of interest in all tumor specimens, we did not detect the junctions in the plasma of two out of nine patients. Although one of these patients had a very low tumor volume, the other patient had widespread sarcomatoid peritoneal mesothelioma. It is possible that we did not detect ctDNA in the plasma of these patients because of the lack of shedding of tumor DNA into circulation, our junction probe design strategy, blood processing, or other uncharacterized biological variables.

In conclusion, our pilot study provides proof-of-concept of a blood-based, personalized serial monitoring tool for mesothelioma. Further improvement in junction selection, specimen handling, and validation in larger studies can potentially provide a minimally invasive, tumor-informed disease monitoring tool for mesothelioma, especially in cases with difficult-to-interpret imaging findings.

CRediT Authorship Contribution Statement

Aaron S. Mansfield: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Visualization, Writing - original draft, Writing - review and editing.

George Vasmatzis: Conceptualization, Data curation, Investigation, Methodology, Resources, Supervision, Writing - review and editing.

Kaushal Parikh: Data curation, Formal analysis, Investigation, Visualization, Roles/Writing - original draft, Writing - review and editing.

Faye R. Harris: Data curation, Investigation, Methodology, Validation, Writing - original draft Writing - review and editing.

Giannoula Karagouga: Data curation, Investigation, Methodology, Validation, Writing - original draft, Writing - review and editing.

Sarah H. Johnson: Data curation, Investigation, Validation, Visualization, Writing - review and editing.

Jay Mandrekar: Formal analysis, Investigation, Writing - review and editing.

Amy Schrandt: Investigation, Writing - review and editing.

Alexa McCune: Investigation, Writing - review and editing.

Dorsay Sadeghian: Investigation, Writing - review and editing.

Roy Debarshi: Investigation, Writing - review and editing.

Katarzyna Polonis: Investigation, Writing - review and editing.

Mitesh J. Borad: Investigation, Writing - review and editing.

Tobias Peikert: Investigation, Writing - review and editing.

Farhad Kosari: Investigation, Writing - review and editing.

John Cheville: Investigation, Methodology, Writing - review and editing.

Janet Schaefer Klein: Project administration, Writing - review and editing.

Aaron O. Bungum: Investigation, Resources, Writing - review and editing.

Eric S. Edell: Investigation, Resources, Writing - review and editing.

Athanasios Gaitatzes: Investigation, Software, Validation, Visualization, Writing - review and editing.

Disclosure

Dr. George Vasmatzis is the owner of WholeGenome LLC; Dr. Aaron S. Mansfield reports consulting activities with AbbVie, AstraZeneca, BMS, Genentech, Janssen, Takeda Oncology, Sanofi Genzyme, Gilead, Johnson & Johnson Global Services, Chugai Pharmaceutical Co., Ltd. (Roche), TRIPTYCH Health Partners, Ideology Health LLC, Intellisphere LLC, Answers in CME, Immunocore; study funding and subsequent publication processing fees from Bristol Myers Squibb; travel support from Roche; and is a non-remunerated director of the Mesothelioma Applied Research Foundation and member of the Friends of Patan Hospital Board of Directors. The remaining authors declare no conflict of interest.

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Supplementary Data

To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at [https://doi.org/10.1016/j.jtocrr.2024.100692].

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