Genome-wide Sequencing of Cell-free DNA Enables Detection of Copy-number Alterations in Patients with Cancer Where Tissue Biopsy is Not Feasible

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ABSTRACT

When tissue biopsy is not medically prudent or tissue is insufficient for molecular testing, alternative methods are needed. Because cell-free DNA (cfDNA) has been shown to provide a representative surrogate for tumor tissue, we sought to evaluate its utility in this clinical scenario. cfDNA was isolated from the plasma of patients and assayed with low-coverage (~0.3×), genome-wide sequencing. Copy-number alterations (CNA) were identified and characterized using analytic methods originally developed for noninvasive prenatal testing (NIPT) and quantified using the genomic instability number (GIN), a metric that reflects the quantity and magnitude of CNAs across the genome. The technical variability of the GIN was first evaluated in an independent cohort comprising genome-wide sequencing results from 27,754 women who consented to have their samples used for research and whose NIPT results yielded no detected CNAs to establish a detection threshold. Subsequently, cfDNA sequencing data from 96 patients with known cancers but for whom a tissue biopsy could not be obtained are presented. An elevated GIN was detected in 35% of patients and detection rates varied by tumor origin. Collectively, CNAs covered 96.6% of all autosomes. Survival was significantly reduced in patients with an elevated GIN relative to those without. Overall, these data provide a proof of concept for the use of low-coverage, genome-wide sequencing of cfDNA from patients with cancer to obtain relevant molecular information in instances where tissue is difficult to access. These data may ultimately serve as an informative complement to other molecular tests.

Introduction

In the era of precision medicine for the treatment of patients with cancer, molecular testing of tumor tissue is a critical component of identifying the optimal treatment pathway for each patient (1). In some cases, however, inaccessibility of the tumor to traditional tissue biopsy results in the lack of molecular information being available. Recently, it was demonstrated that cell-free DNA (cfDNA) circulating in the blood of patients with cancer may provide a source of molecular information when tumor tissue is not available (2–5). Most methods currently utilized and implemented clinically to analyze cfDNA, so-called liquid biopsy assays, apply digital PCR or ultradeep sequencing techniques targeting predefined regions of the genome (4, 6–9). While these methods provide exquisite sensitivity, they will inherently miss any genomic aberration outside of the targeted region(s). We sought to utilize an unbiased approach for the detection of tumor-specific variants using low-coverage, genome-wide sequencing of cfDNA in patients with known cancers, but where tissue biopsy was not medically recommended. Similar methods, originally developed for noninvasive prenatal testing (NIPT), have been utilized in previous studies to enable the detection and characterization of neoplasms in women during pregnancy and in patients with known cancer (10, 11). We built upon these methods to create a quantitative measure of the number and magnitude of copy-number alterations (CNA) across the genome, termed the genome instability number (GIN) and have shown previously that changes in the GIN are linked to therapeutic response (12).

Materials and Methods

Patient information

Patients were selected for study based on their having disease on imaging but that disease being considered as difficult to biopsy because of its location or patient comorbidities. Specifically, patients were considered not biopsiable per the judgment of the physician. The most common reason was that the lesions were small and/or diffuse and located in areas that were difficult to access without considerable risk of morbidity due to the invasive biopsy. This study was performed in accordance with the guidelines of the University of California San Diego Internal Review Board and the Declaration of Helsinki per the PREDICT study (NCT02478931; profile-related evidence determining individualized cancer therapy) and any investigational therapy/procedures for which the patients gave consent. Data were censored effective September 1, 2018; overall survival (OS) was calculated by the method of Kaplan and Meier as the number of days between sample (blood) draw and

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either the date of death or the date of data censoring. *P* value was calculated using the log-rank test in an R programming environment.

Blood collection and processing

Whole blood (~10 mL) was collected in Streck BCT tubes (Streck) and processed to plasma using centrifugation as described previously (12, 13). In patients with more than blood sample available, the first blood draw was used.

DNA extraction

cfDNA from the plasma of each sample (~4 mL) was extracted with a liquid handling platform (Hamilton) using a bead-based method as described previously (13).

Library preparation

Libraries for genome-wide sequencing were created from cfDNA as described previously (14).

Sensitivity mixture model preparation

DNA from four cell lines (SNU-5, NCI-H2170, SNU-16, and HCC827) was obtained from the ATCC and sonicated to resemble cfDNA fragment length profiles (average size of ~170 bp, verified by capillary electrophoresis; Supplementary Fig. S4). Three uniquely barcoded library replicates were generated for each cell line, and libraries were quantified by capillary electrophoresis. Cell line library replicates were each mixed with 12 uniquely barcoded libraries created from the cfDNA of healthy donors so that the cancer cell line library DNA represented 1%, 2%, 3%, 5%, 10%, and 25% of the total mixture.

Genome-wide next-generation sequencing

Normalized libraries were pooled and sequencing was performed using HiSeq2500 (Illumina) instruments as described previously (12, 15, 16). Sequencing generated a mean of 34.1 million sequencing reads for each sample.

Sequencing data analysis

Sequencing data were processed and the GIN was calculated as described previously (12). Briefly, sequencing reads were mapped to the human reference genome (hg19) and subsequently partitioned in to 50 kbp nonoverlapping segments. Regions were selected, and data were normalized as previously described for noninvasive prenatal testing and the resultant-normalized values were used to calculate a

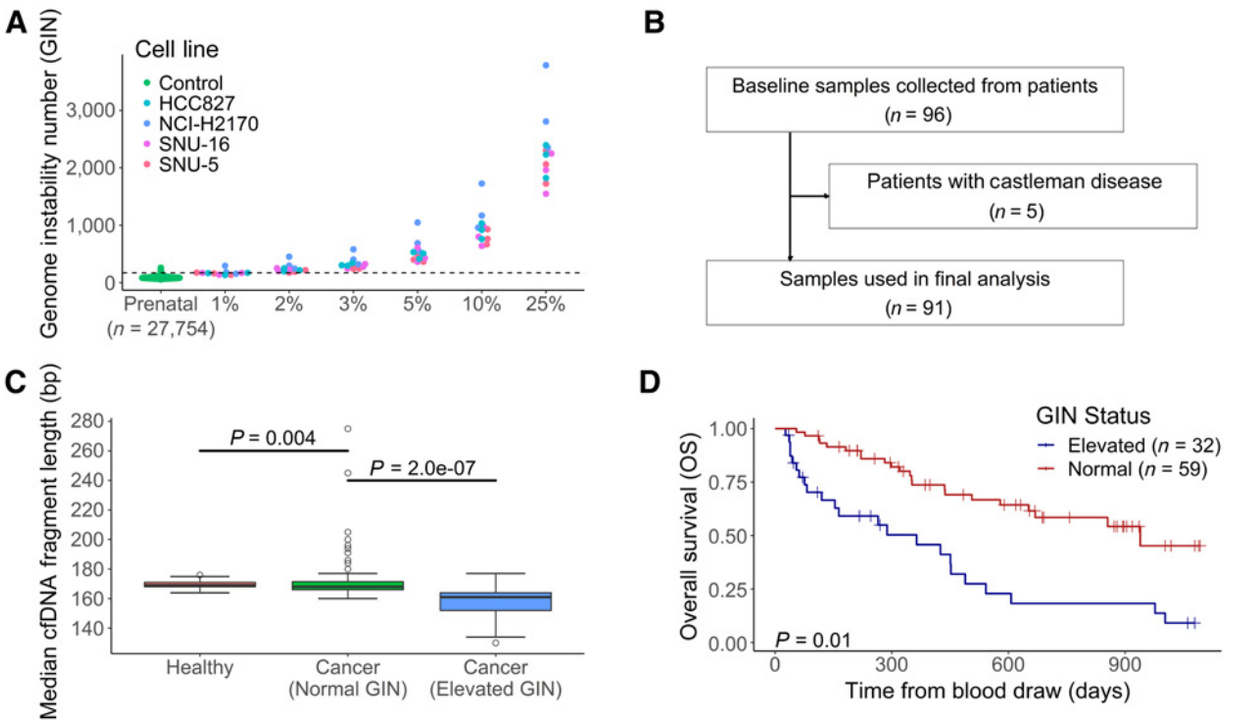

Figure 1.

A, GIN values for training and mixture model samples included in this study. GIN values for 27,742 samples (green) submitted for NIPT for which no CNAs were detected using NIPT algorithms to identify a threshold. Using a threshold of GIN = 170, specificity among the 27,742 samples without known cancer was 99.7%. Using this same threshold, 4/12 (33%) samples at 1% tumor DNA and all samples (60/60) with 2% or greater tumor DNA were detected. **B,** CONSORT diagram for patients enrolled as part of this study. A total of 96 patients were enrolled in this study. Five samples originally enrolled were determined to have Castleman disease, a condition that is not clearly malignant, and were therefore removed from further analysis. All remaining patient samples ($n = 91$) were used in all subsequent analyses. **C,** Relationship between GIN and cfDNA fragment size. cfDNA fragment size was assessed using paired-end sequencing. Across all aliquots from patients with cancer and presumed healthy controls ($n = 168$), a significant difference in cfDNA fragment length was detected between healthy and cancer samples without an elevated GIN ($n = 59$; $P < 0.004$; Wilcoxon rank-sum). In addition, cfDNA fragment length was shorter in patients with an elevated GIN relative to those without ($n = 32$; $P < 2e^{-7}$; Wilcoxon rank-sum). **D,** Relationship between GIN and OS. Kaplan–Meier graph showing the relationship between GIN status (GIN > 170 = Elevated; GIN <170 = Normal) and OS was assessed. OS was calculated from the sample (blood) collection date to either the date of death or the date of data censoring or last follow-up (latter two were both considered censored data). *P* value calculated using log-rank method.

GIN. The GIN is a nonnegative, continuous value calculated as the sum of the absolute deviations of observed normalized sequencing read coverage from expected normalized read coverage across 50,034 autosomal segments. Increasing values of GIN were observed to be indicative of increasing deviation relative to an expected normal genomic profile. Z-scores for each CNA were calculated at 50 kbp resolution as described previously (16).

Results

Low-coverage, genome-wide sequencing of cfDNA samples was performed to obtain unbiased CNA profiles and the GIN was utilized to quantify the level of CNAs present in these patients. Prior to evaluating patient samples, a training cohort was evaluated that expanded on previously reported findings (12). Because the same library preparation, next-generation sequencing (NGS), and data normalization processes were used clinically in our laboratory for NIPT, we utilized deidentified data from 27,742 clinical samples submitted for NIPT and without any detected CNAs to assess the specificity of the assay at the previously established threshold (GIN = 170; ref. 12). At this threshold, 0.3% of the samples (83/27,742) from pregnant women had an elevated GIN (**Fig. 1A**), consistent with an analytic specificity of 99.7%. A model system was then constructed comprising mixtures of fragmented DNA from cancer cell lines that was mixed into cfDNA from healthy donors at six different levels to assess the analytic sensitivity. Three technical replicates of sequencing libraries were prepared from each cancer cell line (SNU-5, NCI-H2170, SNU-16, HCC827) and the resultant GIN was analyzed. At 1% tumor DNA, 3 of the 12 total measurements demonstrated an elevated GIN (**Fig. 1A**). All replicate samples for each cell line showed an elevated GIN when the proportion of tumor DNA was ≥2% (n = 60; **Fig. 1A**; Supplementary Fig. S1). These data suggest an analytic limit of detection of down to 1% tumor DNA, depending on the CNA profile of the tumor (Supplementary Fig. S1).

A total of 96 patients were enrolled in this study. Of these, 5 patients were excluded because of a lack of a clearly malignant condition (Castleman disease), resulting in a final cohort of 91 patients for analysis (**Fig. 1B**). These patients were diagnosed with a total of 42 different tumor types and thus comprised a heterogeneous population of patients with cancer (**Table 1**). The mean age of patients was 55 years (range, 19–84), 51.6% were women, and patients had been previously treated with an average of 1.9 different therapies (range, 0–7).

Because direct comparison of tissue and cfDNA profiles could not be performed in this study, we evaluated alternative methods to demonstrate that the aberrant cfDNA profiles were likely derived from circulating tumor DNA (ctDNA). Data from paired-end sequencing of a cohort of cfDNA samples from 186 individuals without known malignancies were compared with the data from this study (n = 84 with paired-end data available) to determine whether the cfDNA fragment size was different in patients with known tumors (**Fig. 1C**). Consistent with previous reports describing that ctDNA is shorter than cfDNA from nonmalignant cells (17–19), the median fragment length of cfDNA obtained from patients with known cancer, but without an elevated GIN was significantly shorter than cfDNA from healthy donors (P = 0.004; Wilcoxon rank-sum test). In addition, cfDNA from patients with an elevated GIN was significantly shorter than those without an elevated GIN ($P = 2e^{-7}$; Wilcoxon rank-sum test). Because one of the contributors to the GIN is the proportion of ctDNA in the sample, this further supports that ctDNA is shorter than cfDNA from normal cells and that the detected CNAs are likely derived from the tumor.

Table 1. Patient demographics for all (n = 96) patients enrolled in the study.

Characteristics	Results
Total patients, *n* (%)	96
Castleman disease	5 (5.2)
Used in final data analysis	91 (94.8)
Age in years, (range)	55.5 (19–84)
Gender, *n* (%)	
Male	44 (48.4)
Female	47 (51.6)
Previous lines of treatment, mean (range)	1.9 (0–7)
Malignancy, *n* (%)	
Adenoid cystic carcinoma	2 (2.2)
Adrenal carcinoma	2 (2.2)
Ameloblastoma	1 (1.1)
Anal cancer	1 (1.1)
Anaplastic astrocytoma	1 (1.1)
Appendicial cancer	2 (2.2)
Astrocytoma	1 (1.1)
Basal cell carcinoma	1 (1.1)
Brain mass	1 (1.1)
Breast cancer	6 (6.6)
Cholangiocarcinoma	4 (4.4)
Chordoma	1 (1.1)
Colon cancer	1 (1.1)
Colorectal cancer	1 (1.1)
Craniopharyngioma	1 (1.1)
Desmoid tumor	1 (1.1)
Duodenal adenocarcinoma	1 (1.1)
Erdheim-chester disease (ECD)	9 (9.9)
Gastric cancer	2 (2.2)
Gastroesophageal adenocarcinoma	1 (1.1)
Gastroesophageal cancer	1 (1.1)
GIST	1 (1.1)
Glioblastoma	8 (8.8)
Glioma	2 (2.2)
Hepatocellular carcinoma	10 (11)
Kaposi sarcoma	1 (1.1)
Lung cancer	2 (2.2)
Lymphoma	1 (1.1)
Melanoma	1 (1.1)
Meningioma	1 (1.1)
Myoepithelial carcinoma	1 (1.1)
Oligodendroglioma	1 (1.1)
Ovarian cancer	7 (7.7)
Pancreatic cancer	1 (1.1)
Prostate cancer	1 (1.1)
Renal cell carcinoma	1 (1.1)
Sarcoma	5 (5.5)
Sclerosing epithelioid fibrosarcoma	1 (1.1)
Spindle-cell sarcoma	1 (1.1)
Squamous cell carcinoma	2 (2.2)
Thymoma	1 (1.1)
Vulvar cancer	1 (1.1)

Across the entire dataset comprised of 42 different tumor types, an elevated GIN was detected in 35% of samples (32/91; Supplementary Table S1). Because of the diversity of tumor types, samples were grouped based on their physiologic organ system to determine whether general tumor type was associated with detection rate. Indeed, there was a significant association between detection rate and tumor of origin (Supplementary Fig. S2). For example, an elevated GIN was detected in 4 of 6 patients with breast cancer but 0 of 17 patients with

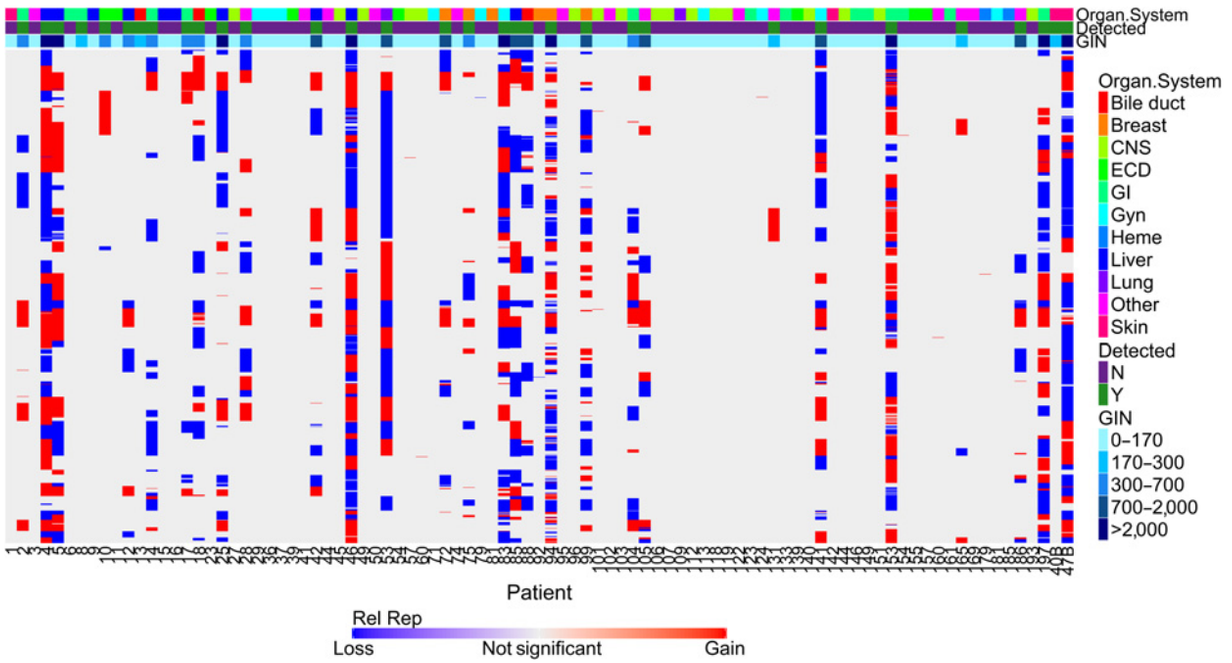

Figure 2.

Relative genome-wide coverage for all patient samples included in this study. Shown are the regions of statistical significance (z-score >10) within each 50,000 bp genomic segments ($n = 50{,}034$) across the genome. Segments without any mapped sequencing reads or known to fail to meet previously determined quality control specifications are not included. Each row is representative of a genomic segment while each column is associated with a specific sample. To minimize the impact of patient gender, only genomic segments located on autosomes are shown. Collectively, detected CNAs covered 96.6% of all autosomes, highlighting the benefit of using genome-wide sequencing. Colored bars are included along the top to indicate the organ system (tissue of origin) for the primary tumor from each patient, whether that cfDNA sample had a GIN exceeding the threshold, and the GIN value from each sample.

tumors of the central nervous system, potentially due to tissue-specific differences in ctDNA levels or the prevalence of CNAs (2). In addition to detection, we tested whether GIN was linked to OS. Despite a wide variety of tumor types, patients with an elevated GIN had a significantly shorter median OS relative to those with a normal GIN (190.5 vs. 484 days; $P = 0.01$, log-rank test; **Fig. 1D**). Larger patient cohorts are needed for future analysis to confirm these results.

The genomic location of genome-wide CNAs was evaluated to provide more insight to the location of CNAs in this cohort with CNAs detected using methods previously described for NIPT (16). Using only CNAs with z-scores >10, CNAs were identified in 60 samples (Supplementary Fig. S3). Collectively, 96.6% (48,324/50,033) of evaluated 50 kbp nonoverlapping autosomal segments were covered by at least one CNA (**Fig. 2**), emphasizing the genome-wide nature of CNAs in this cohort. Select regions showed more frequent CNAs across this patient cohort with the most common CNAs detected being located within a approximately 34 MB region on chromosome 1q and approximately 130 MB region on chromosome 8, suggesting relevance of genes in these regions during carcinogenesis (20).

Discussion

In cases where a physician determines that there is limited feasibility of an invasive tissue biopsy due to an increased risk of potential patient morbidity, alternative methods are needed to provide additional molecular information about their tumors. This study was initiated to determine the feasibility of using low-coverage, genome-wide sequencing of cfDNA from plasma to address this need. An elevated GIN consistent with the presence of circulating tumor DNA was found in 35% of patients and the detection rate varied on the basis of the tumor site, consistent with previous studies that have evaluated multiple tumor types (2, 21). While the detected CNAs may or may not be directly actionable depending on the genomic location and genes affected, the presence of an elevated GIN showed prognostic relevance because those patients with an elevated GIN exhibited significantly worse OS when compared with patients without an elevated GIN. Multiple studies have evaluated the prognostic effect of the presence or abundance of ctDNA as determined by either tumor-associated mutations or CNAs (22, 23); however, there have also been studies that did not see such an effect (12), perhaps due to differences in disease status, tumor type, or a combination thereof. Taken together, these results show promise for the use of ctDNA as a prognostic biomarker in patients with cancer and warrant larger clinical trials to confirm these findings.

While the majority of liquid biopsy studies using cfDNA have been performed using digital PCR or targeted, deep sequencing, there are both strengths and limitations to using a targeted approach. The use of these methods has been shown to enable the identification of variants at 0.1% variant frequency and below within the region of interest, providing potentially clinically relevant markers for therapy selection and residual disease detection; however, these methods are typically focused on single-base hotspots in the case of digital PCR (22, 24) or relatively small panels ranging from hundreds of kilobases to slightly greater than a megabase when using NGS methods (4, 6–9, 25–28). Alternatively, methods that survey the genome for CNAs have been described that may provide a more

comprehensive view (10–12, 29–31). While the method described herein demonstrated a limit of detection of 1%–2% tumor DNA and thus did not demonstrate and thus did not match the analytic sensitivity of targeted methods, it was able to identify CNAs that overlapped with 96.6% of the genome across a patient cohort of 91 patients with a variety of different disease types. In a recent study comparing the overlap of variants detected using both targeted and genome-wide methods, Mohan and colleagues demonstrated that while both targeted and genome-wide aberrations could be detected in the majority of patients using both methods, there were instances where variants were detected using one of these methods and not the other (31). Because of the potential complimentary of these methods and others including cfDNA fragmentation patterns and DNA methylation status, it can be hypothesized that the utilization of multiple methods may be optimal to identify biomarkers linked to patient diagnosis and/or therapy selection, patient prognosis, or response monitoring.

This study was designed to assess the technical feasibility of using low-coverage, genome-wide sequencing as a method to obtain molecular information about a patient's tumor when tissue biopsy was not safely possible, but it has some limitations. First, not all tumors contain CNAs and not all patients will contain a sufficient amount of ctDNA in the plasma; the limit of detection is dependent upon CNA size, magnitude of copy-number change, and tumor DNA fraction. In the analytic assessment of the limit of detection, samples down to 1% ctDNA may be detected using this method, but determining an absolute limit of detection is challenging due to intratumor differences in CNA abundance and magnitude. Efforts are ongoing to reduce the detection limit through further refinement of the algorithm, inclusion of individual CNAs or the incorporation of cfDNA fragment size or other epigenetic factors. In addition, this specific patient cohort has a broad range of tumor types and the clinical utility of this method still needs to be determined. This study was designed as a proof-of-concept study to determine whether cfDNA could potentially be used to ascertain molecular information about the patient's tumor when tumor biopsy was not feasible. Inherently, this study is limited because the direct concordance of the CNA profiles of the tumor and cfDNA cannot be tested; however, the CNA profiles, GIN levels, and the cfDNA fragment length observed in this study are consistent with previous studies and are highly suggestive that they are derived from the tumor itself.

Overall, these data suggest that ctDNA is present in the plasma of patients with cancer in instances where tissue is difficult to access, and provides a proof of concept for the use of low-coverage, genome-wide sequencing of cfDNA from patients with cancer to obtain relevant molecular information. Importantly, an elevated GIN status (compared with normal GIN status), was associated with significantly shorter survival. These data may ultimately serve as an informative complement to other molecular tests.

Authors' Disclosures

T.J. Jensen reports other from Laboratory Corporation of America during the conduct of the study; personal fees from PetDx outside the submitted work; in addition, T.J. Jensen has a patent for US20180032666 pending. A.M. Goodman reports personal fees from Seattle Genetics and EUSA Pharma outside the submitted work. C.K. Ellison reports a patent for US20170342477A1 pending to Sequenom. S. Kato serves as a consultant for Foundation Medicine and CureMatch. S. Kato receives speaker's fee from Roche and is advisory board member with Pfizer. S. Kato has research funding from ACT Genomics, Sysmex, Konica Minolta and OmniSeq. A.R. Mazloom reports a patent for US20200265921A1 issued; and A.R. Mazloom was an employee and stockholder of Sequenom Inc. during the time when the research pertaining to the current article was being performed. G. McLennan reports other from Labcorp during the conduct of the study. R. Kurzrock receives research funding from Boehringer Ingelheim, Debiopharm, Foundation Medicine, Genentech, Grifols, Guardant, Incyte, Konica Minolta, Medimmune, Merck Serono, Omniseq, Pfizer, Sequenom, Takeda, and TopAlliance; as well as consultant and/or speaker fees and/or advisory board for Actuate Therapeutics, Bicara Therapeutics, Inc., Biological Dynamics, Neomed, Pfizer, Roche, TD2/Volastra, Turning Point Therapeutics, X-Biotech; has an equity interest in CureMatch Inc. and IDbyDNA; serves on the Board of CureMatch and CureMetrix, and is a co-founder of CureMatch. No disclosures were reported by the other authors.

Authors' Contributions

T.J. Jensen: Conceptualization, resources, data curation, formal analysis, supervision, writing–original draft, project administration, writing–review and editing. **A.M. Goodman:** Conceptualization, formal analysis, investigation, methodology, writing–original draft, writing–review and editing. **C.K. Ellison:** Conceptualization, data curation, formal analysis, investigation, methodology, writing–original draft, writing–review and editing. **K.A. Holden:** Data curation, investigation. **S. Kato:** Investigation, writing–review and editing. **L. Kim:** Data curation. **G.A. Daniels:** Investigation. **K. Fitzgerald:** Investigation, writing–review and editing. **E. McCarthy:** Investigation, writing–review and editing. **P. Nakashe:** Investigation, writing–review and editing. **A.R. Mazloom:** Data curation, software, formal analysis. **E. Almasri:** Data curation, software, formal analysis. **G. McLennan:** Resources, data curation, investigation, writing–review and editing. **D.S. Grosu:** Conceptualization, writing–review and editing. **M. Eisenberg:** resources, supervision, funding acquisition. **R. Kurzrock:** Conceptualization, resources, formal analysis, writing–original draft, writing–review and editing.

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References

1. Malone ER, Oliva M, Sabatini PJB, Stockley TL, Siu LL. Molecular profiling for precision cancer therapies. Genome Med 2020;12:8.
2. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra24.
3. Newman AM, Bratman SV, To J, Wynne JF, Neville C, Eclov W, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med 2014;20:548–54.
4. Kaseb AO, Sanchez NS, Sen S, Kelley RK, Tan B, Bocobo AG, et al. Molecular profiling of hepatocellular carcinoma using circulating cell-free DNA. Clin Cancer Res 2019;25:6107–18.
5. Ikeda S, Goodman AM, Cohen PR, Jensen TJ, Ellison CK, Frampton G, et al. Metastatic basal cell carcinoma with amplification of PD-L1: exceptional response to anti-PD1 therapy. NPJ Genomic Med 2016;1:16037.
6. Patel H, Okamura R, Fanta P, Patel C, Lanman RB, Raymond VM, et al. Clinical correlates of blood-derived circulating tumor DNA in pancreatic cancer. J Hematol Oncol 2019;12:130.
7. Riviere P, Fanta PT, Ikeda S, Baumgartner J, Heestand GM, Kurzrock R. The mutational landscape of gastrointestinal malignancies as reflected by circulating tumor DNA. Mol Cancer Ther 2018;17:297–305.
8. Ikeda S, Tsigelny IF, Skjevik ÅA, Kono Y, Mendler M, Kuo A, et al. Next-generation sequencing of circulating tumor DNA reveals frequent

alterations in advanced hepatocellular carcinoma. Oncologist 2018;23: 586–93.
9. Ikeda S, Schwaederle M, Mohindra M, Fontes Jardim DL, Kurzrock R. MET alterations detected in blood-derived circulating tumor DNA correlate with bone metastases and poor prognosis. J Hematol Oncol 2018;11:76.
10. Dharajiya NG, Grosu DS, Farkas DH, McCullough RM, Almasri E, Sun Y, et al. Incidental detection of maternal neoplasia in noninvasive prenatal testing. Clin Chem 2018;64:329–35.
11. Bianchi DW, Chudova D, Sehnert AJ, Bhatt S, Murray K, Prosen TL, et al. Noninvasive prenatal testing and incidental detection of occult maternal malignancies. JAMA 2015;314:162–9.
12. Jensen TJ, Goodman AM, Kato S, Ellison CK, Daniels GA, Kim L, et al. Genome-wide sequencing of cell-free DNA identifies copy-number alterations that can be used for monitoring response to immunotherapy in cancer patients. Mol Cancer Ther 2019;18:448–58.
13. Ellison CK, Sun Y, Hogg G, Fox J, Tao H, McCarthy E, et al. Using targeted sequencing of Paralogous sequences for noninvasive detection of selected fetal aneuploidies. Clin Chem 2016;62:1621–9.
14. Tynan JA, Kim SK, Mazloom AR, Zhao C, Mclennan G, Tim R, et al. Application of risk score analysis to low-coverage whole genome sequencing data for the noninvasive detection of trisomy 21, trisomy 18, and trisomy 13. Prenat Diagn 2016;36:56–62.
15. Lefkowitz RB, Tynan JA, Liu T, Wu Y, Mazloom AR, Almasri E, et al. Clinical validation of a noninvasive prenatal test for genomewide detection of fetal copy number variants. Am J Obstet Gynecol 2016;215:227.e1–227.e16.
16. Zhao C, Tynan J, Ehrich M, Hannum G, McCullough R, Saldivar JS, et al. Detection of fetal subchromosomal abnormalities by sequencing circulating cell-free DNA from maternal plasma. Clin Chem 2015;61:608–16.
17. Mouliere F, Chandrananda D, Piskorz AM, Elizabeth K, Morris J, Ahlborn LB, et al. Enhanced detection of circulating tumor DNA by fragment size analysis. Sci Transl Med 2018;10:eaat4921.
18. Underhill HR, Kitzman JO, Hellwig S, Welker NC, Daza R, Baker DN, et al. Fragment length of circulating tumor DNA. PLoS Genet 2016;12:e1006162.
19. Jiang P, Chan CWM, Chan KCA, Cheng SH, Wong J, Wong VWS, et al. Lengthening and shortening of plasma DNA in hepatocellular carcinoma patients. Proc Natl Acad Sci U S A 2015;112:E1317–25.
20. Beroukhim R, Mermel CH, Porter D, Wei G, Donovan J, Barretina J, et al. The landscape of somatic copy-number alteration across human cancers. Nature 2010;463:899–905.
21. Zhang Y, Yao Y, Xu Y, Li L, Gong Y, Zhang K, et al. Pan-cancer circulating tumor DNA detection in over 10,000 Chinese patients. Nat Commun 2021; 12:11.
22. Syeda MM, Wiggins JM, Corless BC, Long GV, Flaherty KT, Schadendorf D, et al. Circulating tumour DNA in patients with advanced melanoma treated with dabrafenib or dabrafenib plus trametinib: a clinical validation study. Lancet Oncol 2021;22:370–80.
23. Li S, Lai H, Liu J, Liu Y, Jin L, Li Y, et al. Circulating tumor DNA predicts the response and prognosis in patients with early breast cancer receiving neoadjuvant chemotherapy. JCO Precis Oncol 2020; 4:PO.19.00292.
24. Milosevic D, Mills JR, Campion MB, Vidal-Folch N, Voss JS, Halling KC, et al. Applying standard clinical chemistry assay validation to droplet digital PCR quantitative liquid biopsy testing. Clin Chem 2018;64: 1732–42.
25. Supplee JG, Milan MSD, Lim LP, Potts KT, Sholl LM, Oxnard GR, et al. Sensitivity of next-generation sequencing assays detecting oncogenic fusions in plasma cell-free DNA. Lung Cancer 2019;134:96–9.
26. Sabari JK, Offin M, Stephens D, Ni A, Lee A, Pavlakis N, et al. A prospective study of circulating tumor DNA to guide matched targeted therapy in lung cancers. J Natl Cancer Inst 2019;111:575–83.
27. Woodhouse R, Li M, Hughes J, Delfosse D, Skoletsky J, Ma P, et al. Clinical and analytical validation of foundation one liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. PLoS One 2020;15:e0237802.
28. Odegaard JI, Vincent JJ, Mortimer S, Vowles JV, Ulrich BC, Banks KC, et al. Validation of a plasma-based comprehensive cancer genotyping assay utilizing orthogonal tissue- and plasma-based methodologies. Clin Cancer Res 2018;24: 3539–49.
29. Hieronymus H, Murali R, Tin A, Yadav K, Abida W, Moller H, et al. Tumor copy number alteration burden is a pan-cancer prognostic factor associated with recurrence and death. Elife 2018;7:e37294.
30. Chen X, Chang CW, Spoerke JM, Yoh KE, Kapoor V, Baudo C, et al. Low-pass whole-genome sequencing of circulating cell-free DNA demonstrates dynamic changes in genomic copy number in a squamous lung cancer clinical cohort. Clin Cancer Res 2019;25:2254–63.
31. Mohan S, Foy V, Ayub M, Leong HS, Schofield P, Sahoo S, et al. Profiling of circulating free DNA using targeted and genome-wide sequencing in patients with SCLC. J Thorac Oncol 2020;15:216–30.