Clinical Chemistry 66:5
697–705 (2020)

Detection of ctDNA from Dried Blood Spots after DNA Size Selection

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BACKGROUND: Recent advances in the study and clinical applications of circulating tumor DNA (ctDNA) are limited by practical considerations of sample collection. Whole-genome sequencing (WGS) is increasingly used for analysis of ctDNA, identifying copy-number alterations and fragmentation patterns. We hypothesized that low-depth/shallow WGS (sWGS) data may be generated from minute amounts of cell-free DNA, and that fragment-size selection may remove contaminating genomic DNA from small blood volumes. Dried blood spots have practical advantages for sample collection, may facilitate serial sampling, and could support novel study designs in humans and animal models.

METHODS: We developed a protocol for the isolation and analysis of cell-free DNA from dried blood spots using filter paper cards and bead-based size selection. DNA extracted and size-selected from dried spots was analyzed using sWGS and polymerase chain reaction (PCR).

RESULTS: Analyzing a 50 μL dried blood spot from frozen whole blood of a patient with melanoma, we identified ctDNA based on the presence of tumor-specific somatic copy-number alterations, and found a fragment-size profile similar to that observed in plasma DNA. We found alterations in different chromosomes in blood spots from 2 patients with high-grade serous ovarian carcinoma. Extending this approach to serial dried blood spots from mouse xenograft models, we detect tumor-derived cell-free DNA and identified ctDNA from the originally grafted ascites.

CONCLUSION: Our data suggest that ctDNA can be detected and monitored in dried blood spots from archived and fresh blood samples, enabling new approaches for sample collection and novel study/trial designs for both patients and in vivo models.

Introduction

Circulating tumor DNA (ctDNA) can be used to sensitively detect and quantify disease burden using a variety of sequencing-based approaches *(1)*. For example, using shallow whole-genome sequencing (sWGS), ctDNA can be detected down to mutant allele fractions of ∼3% through analysis of somatic copy-number alterations (SCNAs) *(2, 3)*. Alternatively, leveraging differences in fragment size between tumor-derived and non-tumor cell-free DNA molecules (cfDNA) can enhance the detection of genomic alterations and the identification of plasma samples from patients with cancer compared to healthy individuals *(4, 5)*. Although sWGS generates data on only a fraction of a single genome (0.3 genome equivalents correspond to ∼1 pg DNA), sequencing libraries for sWGS have traditionally been generated from larger amounts of cfDNA extracted from milliliter volumes of plasma from a venous blood sample *(3)*. Established protocols for collection of plasma for ctDNA analysis require venipuncture, and prompt spinning of EDTA-containing tubes or delayed spinning of tubes containing cell preservatives/fixatives *(6)*. These restrict the possible study designs, requiring samples to be collected by trained staff and limiting the frequency at which serial samples can be collected.

The use of limited blood volumes and dried blood spots for analysis of cfDNA may facilitate new trial designs, widen clinical applications, and enable point-of-care testing

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Received October 4, 2019; accepted February 7, 2020.
DOI: 10.1093/clinchem/hvaa050

and self-collection of samples. Additionally, analysis of minute amounts of blood may facilitate longitudinal ctDNA monitoring from animal models with limited circulating blood volume. In prenatal diagnostics, polymerase chain reaction (PCR) has been used to carry out fetal Rh blood group D antigen (*RHD*) genotyping and HIV detection using maternal dried blood spots (*7*, *8*). In applications to cancer, ctDNA from a limited plasma volume was previously analyzed in a study of a mouse xenograft model, where quantitative PCR was used to measure the human long interspersed nuclear element-1 (*hLINE-1*) as a measure of tumor burden (*9*). In another pilot study in patients with breast cancer, whole-genome amplification was performed on blood obtained from a finger prick (*10*). The study found comparable allelic fractions of somatic mutations between the finger prick sample and matched venous blood (*10*). Sensitive detection of ctDNA from limited volumes or blood spots represents a technical challenge due to the limited total number of mutant molecules. Whole blood samples are considered inferior to carefully collected plasma samples due to the presence of contaminating genomic DNA (gDNA) from lysed white cells (*1*, *11*), which significantly dilutes tumor-derived ctDNA signal. In this study, we present methods for cfDNA extraction from dried blood spots and the subsequent analysis and detection of ctDNA.

Methods

CELL-FREE DNA EXTRACTION FROM DRIED BLOOD SPOTS

The dried blood-spot data in Fig. 1 were generated from a baseline sample from a patient with Stage IV melanoma recruited to the MelResist study (REC 11/NE/0312). MelResist is a translational study of response and resistance mechanisms to systemic therapies of melanoma, including B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) -targeted therapy and immunotherapy. The sample was taken prior to the initiation of anti-*BRAF* targeted therapy and the subsequent clinical timecourse is shown in Wan et al. (*12*), as patient 65. Whole blood samples were collected into 9-mL K2-EDTA tubes. The corresponding plasma sample was derived from a separate K2-EDTA blood tube taken at the same time which underwent double centrifugation at 1600*g* for 10 mins, followed by 18 800*g* for 10 mins in a benchtop microfuge at room temperature. Samples were retrieved after storing at −80°C for 5 years before starting the analysis. Upon thawing, 50 μL of the whole blood were transferred to Whatman FTA™ Classic Cards (Merck) and allowed to air dry for 15 minutes before DNA extraction. For longitudinal xenograft sampling (Fig. 2, C), blood spots were obtained from a tail vein nick, collected with a capillary and spotted onto the Whatman FTA Classic Card. Blood spots obtained from 2 healthy volunteers (analyzed in dPCR experiments) and 2 patients with ovarian cancer (analyzed in sWGS experiments) were derived within 1 hour from blood collected in K2-EDTA tubes, applied to Whatman FTA Classic Cards and stored at room temperature. DNA was extracted from the card using the QIAamp DNA Investigator kit (Qiagen), following the manufacturer's recommended extraction protocol for FTA and Guthrie cards with the following modifications: three 3 mm punches were made from the blood spot, carrier RNA was added to Buffer AL as per the manufacturer's recommendation, and 2 rounds of elution using 25 μL of elution buffer were used.

SIZE SELECTION AND LIBRARY PREPARATION OF BLOOD-SPOT CELL-FREE DNA

We opted to perform size selection to remove contaminating long gDNA fragments that would have prohibited library preparation. Thus, a right-side size selection was performed on DNA eluates using AMPure XP beads (Beckman Coulter). For this purpose, we adapted a protocol for a right-side size selection (*13*). Following optimization of bead:sample ratios for cfDNA fragment sizes, we used a bead:sample ratio of 1:1 to remove contaminating gDNA. The supernatant was retained as part of the right-side size-selection protocol. A second size-selection step used a 7:1 bead:sample ratio to capture remaining fragments, and the size-selected DNA was eluted in 25 μL water. Blood-spot eluates were concentrated using a vacuum concentrator (SpeedVac) to 10 μL volume, which is the maximum recommended volume for downstream library preparation using the ThruPLEX Tag-seq kit (Takara). Sixteen cycles of library amplification were used. Libraries underwent QC using Bioanalyzer 2100 (Agilent) and quantitative PCR (qPCR) with the Illumina/ROX low Library Quantification kit (Roche) on a QuantStudio 6 (Life Technologies). Libraries were submitted for sWGS on a HiSeq4000 (Illumina, paired-end 150 bp).

PLASMA LIBRARY PREPARATION

A plasma cfDNA library was prepared for the matched timepoint where the blood spot was collected. The plasma was obtained as described above. The DNA was extracted using the QIAsymphony (Qiagen) with the DSP Circulating DNA Kit and quantified by digital PCR on a BioMark HD (Fluidigm) using a 65 bp TaqMan assay for the housekeeping gene ribonuclease P/MRP subunit p30 (*RPP30*) (Sigma Aldrich) (*14*) and 55 cycles of amplification (*15*). Using the estimated number of *RPP30* DNA copies per μL of eluate, the cfDNA concentration in the original sample was estimated and 10 ng were used for the library preparation. The ThruPLEX Tag-seq kit (Takara) was used according to the manufacturer's instructions and 7 cycles of

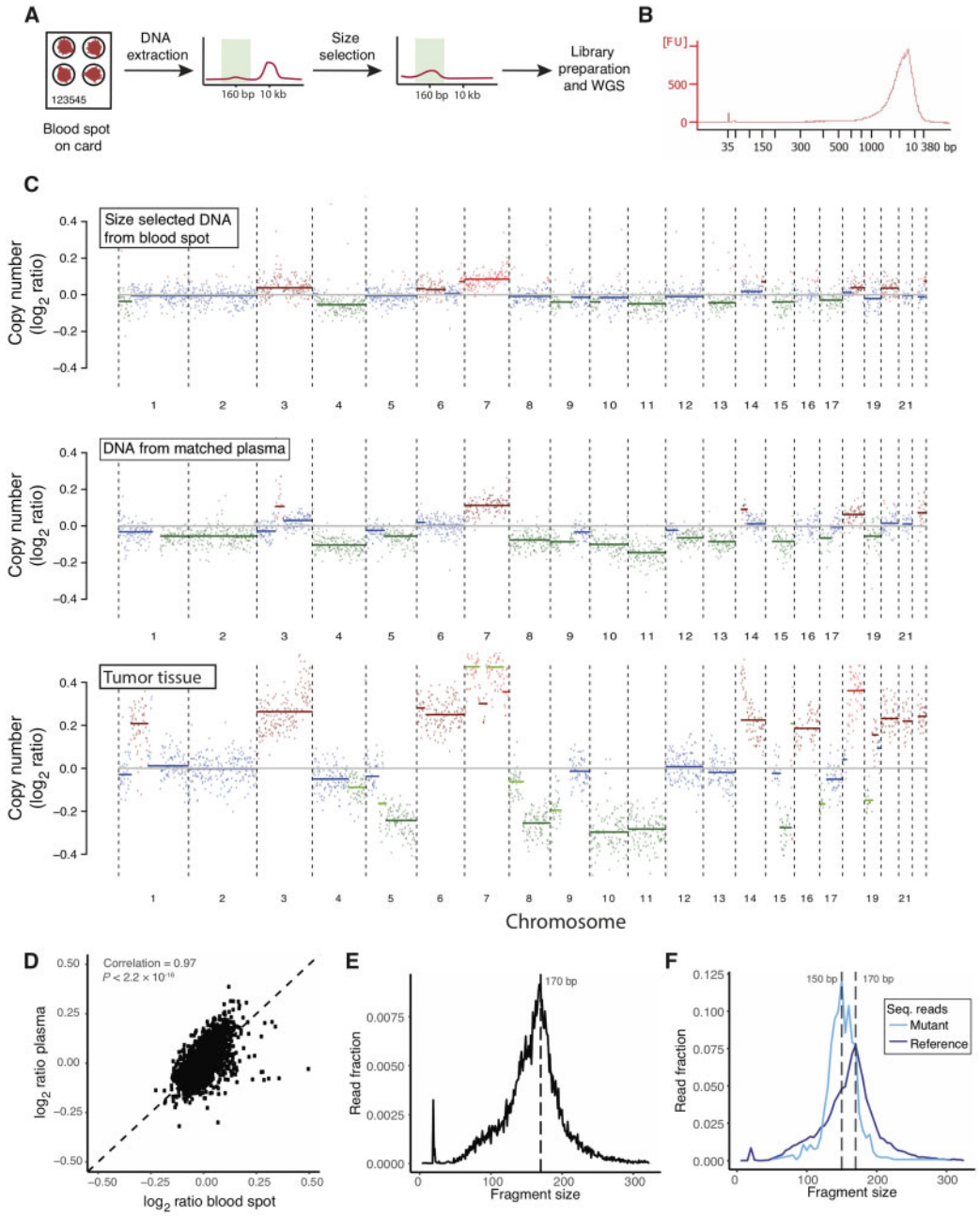

Fig. 1. Detection of ctDNA in a dried blood spot from a cancer patient. A) Overview of the analysis of dried blood spots: DNA extraction, followed by size selection, and low-depth WGS. B) Bioanalyzer trace of DNA extracted from a 50 μL dried blood spot from blood of a patient with advanced melanoma, showing a high level of genomic DNA contamination (>1 kbp) and no clear cfDNA peak (~166 bp). C) Copy-number profiles from sWGS of a sequencing library generated from the same dried blood spot as in (B) after size selection, from a matched plasma sample from the same individual and timepoint and the matched tumor tissue. Segments colored in blue, red, and green indicate regions of copy-number neutrality, gain, and loss, respectively. D) Correlation of $\log_2$ ratios for each copy-number bin using ichorCNA (*2*), comparing bins between matched blood spot and plasma data. The correlation in $\log_2$ ratios for all bins between the two samples was 0.97 (Pearson r, $P < 2.2 \times 10^{-16}$). E) Size profile of the sequencing reads generated from the size selected blood spot DNA library (data shown in panel C). The overall size profile is comparable to that of cfDNA; i.e., with a peak at ~170 bp. F) Length of the sequencing reads (data from panel E) carrying known patient-specific mutations (light blue), and reads carrying reference alleles at the same loci (dark blue).

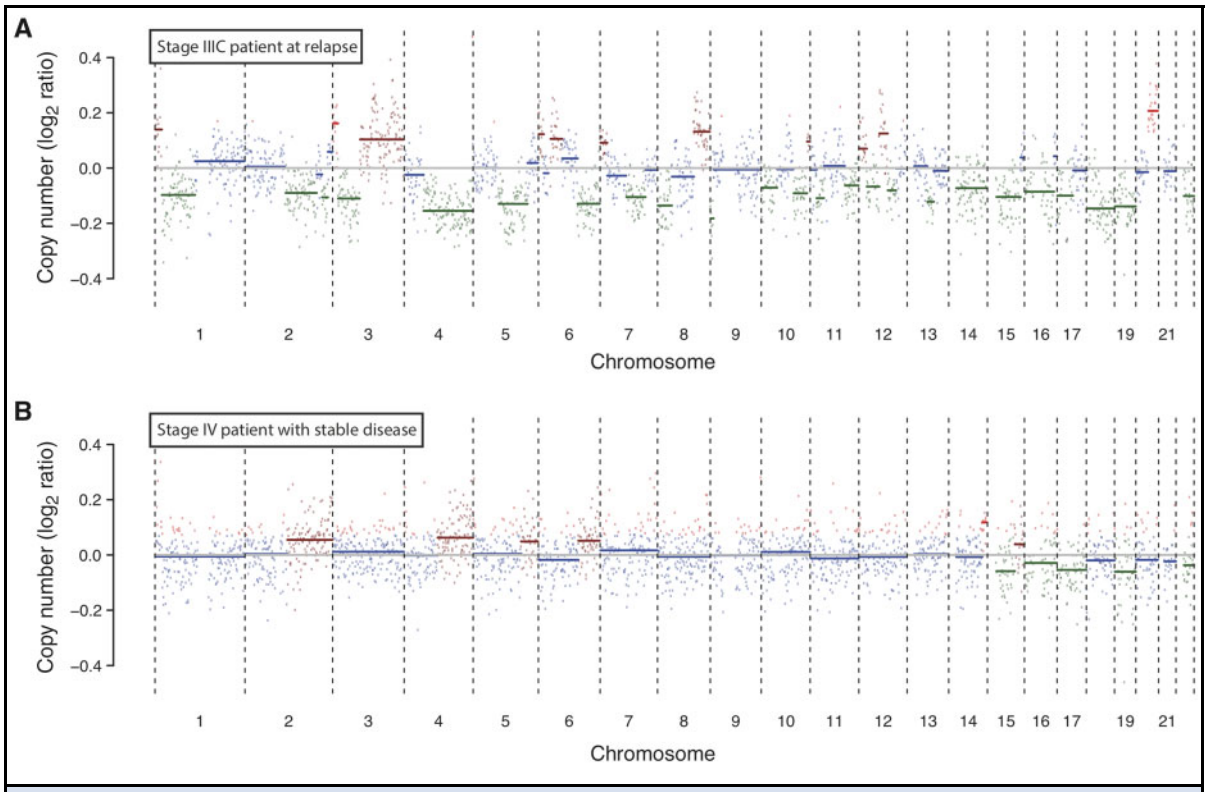

Fig. 2. Copy-number profiles from fresh human dried blood spots. A) Copy-number profile from a fresh blood spot of a Stage IIIC ovarian cancer patient with progressive disease. B) Copy-number profile from a fresh blood spot of a Stage IV ovarian cancer patient with stable disease. Segments colored in blue, red, and green indicate regions of copy-number neutrality, gain, and loss, respectively.

amplification were carried out. After barcoding and sample amplification, the library underwent bead clean-up using recommended conditions, followed by qPCR QC as described in the Size Selection section. The sample was submitted for sequencing on a HiSeq4000 (paired-end 150 bp).

TUMOR LIBRARY PREPARATION

For the blood spot from the patient with melanoma, a tumor sample was collected 6 days after the time-matched plasma and blood spot sample. Tumor DNA was extracted as described by Varela et al. *(16)* and sheared to ∼200 bp fragment length using the Covaris LE220 Focused-ultrasonicator according to the manufacturer's instructions. Fifty nanograms of material were prepared for sWGS using the ThruPLEX Plasma-seq kit (Takara) with 7 cycles of amplification. Bead clean-up and QC were performed as described above. The sample was submitted for WGS on a HiSeq4000 (paired-end 150 bp). Whole exome sequencing and mutation calling were performed as described previously *(16)*. A total of 5073 mutations were identified and used for the analysis of cfDNA and ctDNA fragment length *(12)*.

For the xenograft, material from the engrafted tumor as well as the human ascites sample used for grafting were available for analysis. The samples were extracted using the Qiagen Allprep kit (Qiagen) and the DNA was sheared to 200 bp fragments as described above. Fifty nanograms of DNA were prepared with the ThruPLEX DNA-seq kit (Takara) according to the manufacturer's instructions and followed by the recommended bead clean-up. The samples were quantified using TapeStation (Agilent) and submitted for sequencing on a HiSeq4000 (single-end 50 bp).

SEQUENCING DATA ANALYSIS

FASTQ files were aligned to the UCSC hg19 genome using BWA-mem v.0.7.13 with a seed length of 19, then deduplicated with MarkDuplicates. For sWGS detection of ctDNA, samples were collapsed using CONNOR *(17)* and a family size setting of 1 to optimize the number of included families and the amount

of data retained (Supplemental Fig. 1, D). ichorCNA was run on all samples as described previously *(2)*.

For xenograft sequencing analyses, BAM files underwent alignment to the mouse and human genomes in parallel using Xenomapper *(18)*. Fragment lengths were determined for both resulting files using Picard CollectInsertSizeMetrics *(19)*. Additionally, ichorCNA *(2)* was run on the subset of reads aligning to the human genome to confirm the presence of SCNAs. To visualize the size profile of mutant reads based on a list of patient-specific mutations, we followed previously published methods *(5, 12)*.

LIBRARY DIVERSITY ESTIMATION

To estimate the total number of cfDNA genome copies present in a blood-spot library, we used CONNOR *(17)* to perform deduplication of the blood-spot sequencing library based on endogenous barcodes *(20)* with minimum family sizes ranging between 1 and 5. For each family size setting, the mean deduplicated coverage was calculated using Samtools mPileup. Deduplicated coverage values for each setting were used as input for diversity estimation using a statistical method, SPECIES *(21)*. A minimum family size of 1 was used for the data analysis.

DIGITAL PCR OF DRIED BLOOD SPOTS

Selected blood-spot samples were analyzed using *hLINE-1 (9)* or *RPP30*-based *(14)* digital PCR (dPCR) assays. *RPP30* primers were used with a sequence-specific TaqMan probe while *hLINE-1* primers were used with EvaGreen on a 12.765 or 48.770 Digital Array Chip and run on the BioMark HD (Fluidigm). The (genome copies) count obtained by dPCR were corrected to account for the dead volume of the chip and reported as a concentration of the total sample volume of 50 μL.

Results

We sought to assess the number of cfDNA genome copies that can be sequenced from a single blood drop or dried blood spot. Based on previous reports, the median concentration of cfDNA is approximately 1600 amplifiable copies per mL of blood for patients with advanced cancer *(22, 23)*. This translates to approximately 80 copies of the genome as cfDNA in a blood drop/spot of 50 μL. Assuming a yield in the range of ∼60–80% in DNA extraction and efficiency of ∼15–40% in generating a sequencing library, this is estimated to result in approximately 7–25× representation of the genome in sequencing libraries prepared from cfDNA from a single blood drop. We therefore hypothesized that low-depth WGS of cfDNA can be attainable from a dried blood spot after removal of genomic DNA.

To test this hypothesis, we thawed frozen whole blood from a patient with Stage IV melanoma, and transferred 50 μL to a Whatman FTA filter paper card. After drying the card for 15 minutes, we performed DNA extraction and library preparation from the dried blood spot (Fig. 1, A). Quality control using capillary electrophoresis revealed contaminating gDNA, as indicated by an excess of large DNA fragments for blood spots prepared from frozen whole blood (Fig. 1, B). In order to assess the effect of a freeze-thaw cycle on the fragment-size profile from a dried blood spot, we obtained the size profile of a blood spot generated from fresh whole blood from a healthy volunteer prior to size selection (Supplemental Fig. 1, A), revealing a similar abundance of contaminating genomic DNA compared to whole blood that has undergone a freeze–thaw cycle (Fig. 1, B). cfDNA fragments typically display a characteristic fragmentation profile with a prominent peak at 166 bp *(5, 24)*. This peak was not observed, likely due to the low mass of cfDNA in the blood spot and the larger amounts of gDNA. To remove contaminating gDNA fragments (>500 bp in length), we applied a right-side size selection using AMPure beads. Running another capillary electrophoresis after the bead-based size selection revealed removal of the contaminating genomic DNA to varying degrees (Supplemental Fig. 1, B and C). We generated a sequencing library from the size-selected DNA using the ThruPLEX Tag-seq kit, and obtained a total of 232 107 928 sequencing reads (PE150; Illumina HiSeq4000; Fig. 1, A).

In our data, we achieved a unique sequencing depth of 6× from sWGS following collapsing with a minimum family size of 1 *(17)*. Using a diversity estimator [SPECIES *(21)*], we inferred that up to 10× unique coverage are likely to be achieved from this blood-spot library and may describe the total amount of cfDNA in a blood spot after library preparation (see Methods). Using dPCR, we quantified the DNA of extracted blood spots after bead-based size selection. Using previously described *hLINE-1* primers we observed a high concentration of human DNA (Supplemental Fig. 1, E) *(9)*. We then quantified blood-spot DNA using a less abundant and copy-number neutral gene *(14)*. Across 4 samples we obtained a median of 1322 amplifiable copies in 50 μL, equivalent to roughly 4.4 ng. This is more than 15-fold the expected concentration of 80 copies, which we consider is likely due to varying amounts of remaining contaminating genomic DNA (gDNA) after the bead-based size selection (online Supplemental Fig. 1B and C). Residual gDNA would lead to higher values generated from PCR-based quantification of cfDNA, though their effect on sequencing data would be limited due to their low efficiency of library generation. These findings suggest that the blood-spot material after size selection still contains high molecular weight DNA,

although not enough to inhibit library preparation. Therefore, specific bead ratios for size selection are necessary for effective clean-up of genomic DNA.

Sequencing data obtained from the blood spot were analyzed for SCNAs using ichorCNA (Fig. 1, C) *(2)*. The alterations observed were consistent with those identified in a matched plasma sample from the same patient, isolated by standard plasma DNA-based methods (Fig. 1, C). The extent of SCNAs between the 2 samples was significantly correlated (Pearson $r = 0.75$, $P < 2.2 \times 10^{-16}$, Fig. 1, D) and similar to that found in the initial tumor biopsy copy-number profile (Fig. 1, C). The ichorCNA tumor fraction estimate for the plasma sample was 0.11, compared to 0.07 for the blood spot generated from the matched whole blood sample following size selection. The similarity in ctDNA levels between these samples despite the high level of background in whole blood highlights the efficacy of size selection for isolation of short cfDNA fragments. We also identify a high correlation in $\log_2$ copy-number profile between the plasma sample and the metastatic tumor biopsy taken 6 days later ($r = 0.90$, $P = 2.2 \times 10^{-16}$). Discrepancies in the copy-number profile between these 2 samples likely arise from sampling error in the context of intratumor heterogeneity in metastatic disease, consistent with previous literature showing that subclonal mutations have lower ctDNA allele fractions in plasma *(25, 26)*.

Using sWGS, we show that the overall fragment-size distribution of the human blood-spot cfDNA was comparable to that of cfDNA derived from plasma (Fig. 1, E) *(1, 5, 27)*. We then independently analyzed the size distribution of mutant and wild-type reads, leveraging mutation calls from exome sequencing of matched tumor tissue in order to distinguish mutations from sequencing noise. This confirmed that tumor-derived fragments were shorter in size compared to wild-type fragments, with modal sizes of 150 and 170 bp, respectively (Fig. 1, F), recapitulating size profiles derived from plasma samples of patients with cancer *(1, 5, 27)*.

Next, we assessed the extent of SCNAs in fresh blood spots from whole blood of 2 patients with high-grade serous ovarian cancer (see Methods). The first patient presented with Stage IIIC disease prior to the start of fourth-line chemotherapy, while the second patient had Stage IV disease and was stable on maintenance therapy (Fig. 2). The sample from the patient who presented with a higher disease burden showed a correspondingly greater magnitude of copy-number changes compared to the other patient.

We next considered whether blood-spot analysis may be used for longitudinal ctDNA monitoring in murine patient-derived xenograft (PDX) models. At present, analysis of cfDNA is challenging in small rodents as the volumes of blood required for most traditional ctDNA analysis can only be obtained through terminal bleeding. To assess the feasibility of dried blood-spot analysis in animal models, we sampled 50 μL of whole blood onto a dried blood-spot card from an orthotopically implanted ovarian tumor PDX model. DNA was extracted and sequenced (see Methods). Following alignment of sequencing reads, both human genome (tumor-derived) and mouse genome (wild-type) reads were observed, showing characteristic fragmentation patterns of mutant and wild-type cfDNA *(5)* (Fig. 3, A). SCNAs were observed in the human sequencing reads, which mirrored the profile of both the original patient ascites sample and the matched PDX tumor in the mouse (Fig. 3, B). Finally, we quantified the amount of human material in longitudinal blood spots ($n = 3$) from a xenograft model using dPCR with primers against *hLINE-1 (9)*. The concentration of *hLINE-1* tracked the tumor volume in the mouse over a course of 29 days (Fig. 3, C). Both of these applications demonstrate that blood spots can be used to monitor disease burden in animal models.

Discussion

In this study, we have demonstrated a new method to detect ctDNA in drops/spots of whole blood from both human and PDX samples. Our analysis mirrors observations previously made in cfDNA plasma analysis. This approach relies on the use of size selection to remove genomic DNA, combined with ctDNA measurement approaches such as sWGS that leverage signal from across the entire genome. Because of the small amount of material collected in this way, the analysis of any individual locus would have limited sensitivity due to the small number of genome copies of cfDNA that may be obtained from a single blood spot (in the order of 80 copies). This method is therefore most appropriate for analysis by highly multiplexed approaches leveraging signal from multiple loci. In this article, we demonstrated analysis by low-depth WGS and by PCR-based analysis of repetitive elements. Other approaches can include analysis of multiple mutated loci *(12)* or epigenetic analysis *(28)*.

We analyzed a dried blood spot from a patient with melanoma and observed a good correlation in the copy-number profiles obtained from the blood spot and a time-matched plasma and tumor sample. We obtained similar cfDNA and ctDNA size profiles as observed from standard plasma DNA-based methods. We further analyzed fresh blood spots from patients with ovarian cancer showing varying degrees of copy-number changes, which reflected the patients' disease burden. Further work on larger cohorts with fresh finger prick blood is warranted before progressing toward broader use of blood spots for ctDNA monitoring. This work

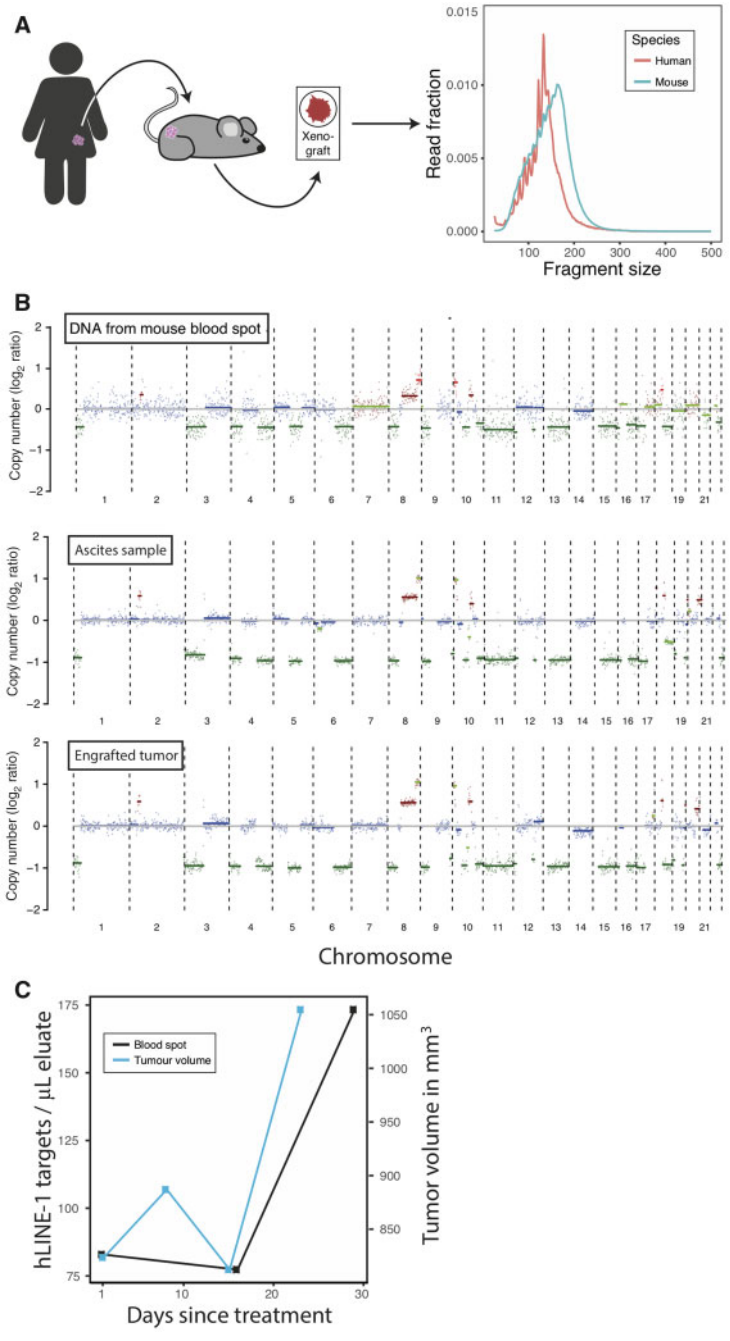

Fig. 3. ctDNA detection from a dried blood spot in a xenograft model. A) sWGS analysis of whole blood taken from a mouse xenograft model of ovarian cancer (illustrated in the left panel). The fragment lengths of reads aligning to the human genome (red, representing tumor ctDNA) were shorter than those aligning to the mouse genome (blue, representing non-tumor cfDNA). B) Copy-number profiles were successfully generated from a dried blood spot from the mouse ovarian xenograft model (Methods). The copy-number profiles of the original human ascites sample and the engrafted tumor are also shown. Segments colored in blue, red, and green indicate regions of copy-number neutrality, gain, and loss, respectively. C) Human sequences were quantified using a previously validated *hLINE-1* assay *(9)* by dPCR. ctDNA levels are reported as *hLINE-1* counts per µL eluate for longitudinal blood-spot samples of a xenograft. Corresponding tumor volume measured in mm^3 is also shown for the same timeframe.

should assess the amounts of ctDNA in blood spots and compare the ctDNA fractions between blood spots and matched plasma, assessing the extent to which they are influenced by contaminating gDNA. Additionally, the sensitivity limit for ctDNA analysis in blood spots should be determined with both WGS and targeted sequencing approaches. In future, the potential application of personalized sequencing panels *(12)* to sequencing data could facilitate highly sensitive monitoring of disease even from small volumes.

In addition, we have demonstrated the value of this approach in animal models, allowing the detection of SCNAs, human specific *hLINE-1* sequences, and the characteristic ctDNA fragmentation pattern from dried blood spots of PDX models. In the monitoring of ctDNA in small animal models, overcoming low circulating blood volumes is a major challenge. Although tail vein blood sampling in rodents has already been used for longitudinal cancer monitoring from small blood samples, analysis was limited to high copy-number markers such as *hLINE-1* repeat sequences *(9)*. Here, we highlight the possibility of next generation sequencing of blood-spot cfDNA, enabling shallow and up to 6× WGS. Collecting samples as dried blood spots would further simplify the logistical burden associated with serial sampling of plasma by removing the need for prompt centrifugation.

From a practical standpoint, the application of dried blood spots could enable high-frequency ctDNA monitoring of patients and animal models. Methods relying on copy-number alterations have detected ctDNA down to a mutant allele fraction of approximately 1–3% *(2, 5)*, limiting their application to patients with advanced disease. However, even within the advanced disease space, we envisage that this methodology may be useful for patients for longitudinal cancer monitoring, as an adjunct to conventional sampling from peripheral blood tubes, and potentially enabling self-sampling for cancer monitoring. Sampling and preanalytical processing can be further simplified, potentially supporting new study designs incorporating wider populations and more frequent collection of smaller sample volumes. We hope that detection of ctDNA from limited blood volumes will enable novel approaches for cancer monitoring, such as self-collection of samples at home followed by shipping and centralized analysis, or novel point-of-care devices. Although a qualitative and quantitative comparison of ctDNA in both finger-prick and peripheral venous blood samples was not performed in this study, such analyses represent a logical next step for taking small-volume liquid biopsy analyses into the clinic.

In addition to applications in oncology, sequencing of cfDNA from dried blood spots may have utility in other areas of medical diagnostics including noninvasive prenatal testing, monitoring transplant rejection, and analysis of the cellular origins of cfDNA to identify physiological or pathological changes such as identification of tissue-specific cell death *(28)*. Given that nucleic acids can remain stable on card for years *(29)*, Guthrie cards might also be a useful medium for storage and shipping of nonblood samples such as saliva. The approach we demonstrate here, whereby interfering genomic DNA can be removed or excluded as part of molecular analysis, may also prove useful for analysis of biobanked or archived samples that may have been collected by protocols that do not effectively remove genomic DNA.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Human Genes: *RHD*, Rh blood group D antigen; *hLINE-1*, human long interspersed nuclear element-1; *BRAF*, B-Raf proto-oncogene, serine/threonine kinase; *RPP30*, ribonuclease P/MRP subunit p30.

Author Declaration: A version of this paper was previously posted as a preprint on bioRxiv as https://www.biorxiv.org/content/10.1101/759365v1.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

K. Heider, J.C.M. Wan, and N. Rosenfeld wrote the manuscript. K. Heider, J.C.M. Wan, and J. Belic carried out the experiments and data analysis. J. Hall and S. Boyle generated and prepared the animal model. I. Hudecova, W.N. Cooper and D. Gale helped in designing the study. P.G. Corrie, N. Rosenfeld, and D. Gale led and coordinated work on the MelResist study from which the human blood spot was used. C.G. Smith, J.D. Brenton, and N. Rosenfeld supervised the project.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: N. Rosenfeld, J.D. Brenton, and D. Gale, Inivata Ltd, a cancer genomics company that commercializes ctDNA analysis. Inivata had no role in the conceptualization, study design, data collection and analysis, decision to publish or preparation of the manuscript.
Consultant or Advisory Role: N. Rosenfeld, J.D. Brenton, and D. Gale, Inivata Ltd.
Stock Ownership: N. Rosenfeld, J.D. Brenton, and D. Gale, Inivata Ltd.
Honoraria: None declared.
Research Funding: The University of Cambridge and Cancer Research UK (grant numbers A20240 and A29580). The research leading to these results has received funding from the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013)/ERC Grant Agreement n.337905.

Healthy volunteer samples were provided by the Cambridge Blood and Stem Cell Biobank, which is supported by the Cambridge NIHR Biomedical Research Centre, Wellcome Trust - MRC Stem Cell Institute and the Cambridge Experimental Cancer Medicine Centre, UK.

Expert Testimony: None declared.

Patents: Cancer Research UK has filed patent applications protecting methods described in this manuscript. K. Heider, J.C.M. Wan, C.G. Smith and N. Rosenfeld, are listed as inventors on this patent.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

Acknowledgments: We thank Catherine Thorbinson, Emily Barker and Alex Azevedo from the MelResist study group and the Cambridge Cancer Trials Centre, Addenbrookes Hospital, Cambridge. We also thank Carolin Sauer for acquisition of mouse samples and preparation of sequencing data.

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