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Precision Medicine and Imaging

Clinical Cancer Research

False-Positive Plasma Genotyping Due to Clonal Hematopoiesis

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Abstract

Purpose: Plasma cell-free DNA (cfDNA) genotyping is increasingly used in cancer care, but assay accuracy has been debated. Because most cfDNA is derived from peripheral blood cells (PBC), we hypothesized that nonmalignant mutations harbored by hematopoietic cells (clonal hematopoiesis, CH) could be a cause of false-positive plasma genotyping.

Experimental Design: We identified patients with advanced non–small cell lung cancer (NSCLC) with *KRAS*, *JAK2*, or *TP53* mutations identified in cfDNA. With consent, PBC DNA was tested using droplet digital PCR (ddPCR) or next-generation sequencing (NGS) to test for CH-derived mutations.

Results: We first studied plasma ddPCR results from 58 patients with *EGFR*-mutant NSCLC. Two had *KRAS* G12X detected in cfDNA, and both were present in PBC, including one where the *KRAS* mutation was detected serially for 20 months. We then studied 143 plasma NGS results from 122 patients with NSCLC and identified 5 *JAK2* V617F mutations derived from PBC. In addition, 108 *TP53* mutations were detected in cfDNA; for 33 of the *TP53* mutations, PBC and tumor NGS were available for comparison, and 5 were present in PBC but absent in tumor, consistent with CH.

Conclusions: We find that most *JAK2* mutations, some *TP53* mutations, and rare *KRAS* mutations detected in cfDNA are derived from CH not tumor. Clinicians ordering plasma genotyping must be prepared for the possibility that mutations detected in plasma, particularly in genes mutated in CH, may not represent true tumor genotype. Efforts to use plasma genotyping for cancer detection may need paired PBC genotyping so that CH-derived mutations are not misdiagnosed as occult malignancy. *Clin Cancer Res; 24(18); 4437–43. ©2018 AACR.*

See related commentary by Bauml and Levy, p. 4352

Introduction

Genomic analysis of plasma cell-free DNA (cfDNA) is increasingly clinically adopted as a method for noninvasive genotyping of advanced cancers. Such "liquid biopsy" approaches potentially allow cancer genotyping in the absence of adequate tumor tissue, resulting in the FDA approval of the cobas EGFR mutation test v2 for plasma genotyping of non–small cell lung cancer (NSCLC) when tumor tissue is unavailable (1). More recent advances in the development of highly sensitive plasma next-generation sequencing (NGS) assays have motivated ambitious plans to expand the clinical use of plasma genotyping (2), to develop assays that can facilitate early detection of residual disease following curative therapy (3), and to eventually develop cancer screening tests.

Unfortunately, discordance between genotyping of tumor tissue and plasma cfDNA has hampered the use and interpretation of these new assays (4). One recognized cause of tumor/plasma discordance is variable shed of tumor DNA into the plasma, causing false-negative plasma genotyping (5). A second well-documented cause of tumor/plasma discordance is tumor heterogeneity, particularly in the setting of drug resistance (6). However, variable DNA shed and tumor heterogeneity fail to fully account for the recurring reports of inaccurate plasma genotyping reported in the literature (7, 8).

Because a large proportion of cfDNA is derived from peripheral blood cells (PBC; ref. 9), we hypothesized that somatic mutations within nonmalignant hematopoietic cells, known as clonal hematopoiesis (CH; ref. 10), might be a recurring source of discordance between tumor genotyping and plasma cfDNA genotyping. Our specific aim was to identify false positives due to CH, that is, instances where plasma genotyping, used with the intent of identifying NSCLC genotype, instead identified mutations derived from CH and not the tumor.

Materials and Methods

Six large publications studying CH were reviewed to identify genes of interest (11–16). Per patient prevalence estimates of mutations in relevant CH genes were calculated by review of publicly available data (Fig. 1). The genes most commonly mutated (*DNMT3A*, *ASXL1*, and *TET2*; refs. 11, 12) are not covered by most clinically available plasma genotyping assays. However, several other genes with potential importance to cancer biology have been reported as occasionally mutated in CH: *JAK2*, *TP53*, *GNAS*, *IDH2*, and *KRAS*. For this analysis, we focused on mutations in three cancer-associated genes that could be

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Despite increasing use of plasma cell-free DNA (cfDNA) genotyping for cancer care, this biospecimen remains poorly understood. Here, we report recurrent mutations in *JAK2*, *TP53*, and *KRAS* detected in cfDNA on validated plasma genotyping assays that are in fact derived from white blood cells (i.e., clonal hematopoiesis, CH) rather than from tumor. Our approach highlights that clinicians must be aware that mutations detected in cfDNA, especially in CH-related genes, may not always reflect tumor genotype. Furthermore, future efforts to develop plasma cfDNA genotyping as a tool for cancer detection may require paired genotyping of peripheral blood cells so that CH-derived mutations are not misdiagnosed as occult malignancy.

genotyped using available highly sensitive assays: *JAK2*, *TP53*, and *KRAS*.

Patient cohort

Patients with advanced NSCLC who had undergone plasma cfDNA genotyping as part of ongoing research or routine clinical care were identified. Plasma cfDNA genotyping was performed either using a validated droplet digital PCR (ddPCR) assay (performed in a research lab and described previously; ref. 5) or using a commercially available plasma NGS assay (Guardant Health). Under an institutional review board–approved protocol, patients were consented for blood collection so that PBC, which are usually discarded after spinning the plasma, could be saved for further genomic analysis.

ddPCR

DNA was extracted from PBC using the DNeasy blood and tissue kit (QIAGEN) per the manufacturer's recommendation. DNA was tested for recurring point mutations (e.g., *KRAS* G12X and *JAK2* V617F) using ddPCR. Primer/probes for the *JAK2* V617F assay were custom made by Life Technologies.

- Forward primer sequence: 5′-AAGCTTTCTCACAAGCATTTGGTTT-3′.
- Reverse primer sequence: 5′-GAAAGCCTGTAGTTTTACTTACTCTCGT-3′.
- Probe sequences: 5′-VIC-CTCCACAGACACATACT-MGB-NFQ-3′, 5′-FAM-CTCCACAGAAACATACT-MGB-NFQ-3′.
- Cycling conditions: 10 minutes at 95°C followed by 40 cycles of a two-step thermal profile of 15 seconds at 94°C denaturation and 60 seconds at 58°C.

Highly sensitive NGS

NGS of PBC DNA was performed using a custom QIAseq V3 amplicon-based panel containing PCR amplicons spanning the entire exome of *TP53* and *KRAS*, and sequenced on an Illumina NextSeq at an average mean read depth of 1,200. DNA libraries were prepared using a custom QIAseq V3 targeted DNA kit principally, as per the manufacturer's instructions. Note that 15 ng of isolated DNA was enzymatically fragmented and end repaired in a 25-μL reaction. The reaction conditions were as follows: 4°C for 1 minute, 32°C for 14 minutes, and 65°C for 30 minutes. Immediately after, adapter ligation reagents were directly added to the 25 μL of DNA: 10 μL 5X ligation buffer, 5 μL DNA ligase, 2.8 μL IL-N7## barcoded adapters, and DNAse-free water were added to a total of 50 μL. The reaction continued at 20°C for an additional 15 minutes, after which the reaction was purified using 90 μL of QIAseq beads and eluted into 12 μL of nuclease-free water. 9.4 μL of DNA was mixed with 5 μL of the QIAseq targeted panel, 4 μL of 5X TEPCR buffer, 0.8 μL of IL-forward primer, and 0.8 μL of HotStarTaq DNA polymerase. The PCR enrichment condition was 95°C for 13 minutes, 98°C for 2 minutes, six cycles of 98°C for 15 seconds and 65°C for 15 minutes, and 72°C for 5 minutes. Each reaction was cleaned once using QIAseq beads to remove unused primers. Enriched DNA was combined with 400 nmol/L of IL-universal primer, 400 nmol/L of IL-index primer, 1X of UPCR buffer, and 1 μL of HotStarTaq DNA polymerase in a volume of 20 μL. The universal PCR condition was 95°C for 13 minutes, 98°C for 2 minutes, 20 cycles of 98°C for 15 seconds and 60°C for 2 minutes, and 72°C for 5 minutes. The DNA library was purified and sequenced on Illumina NextSeq

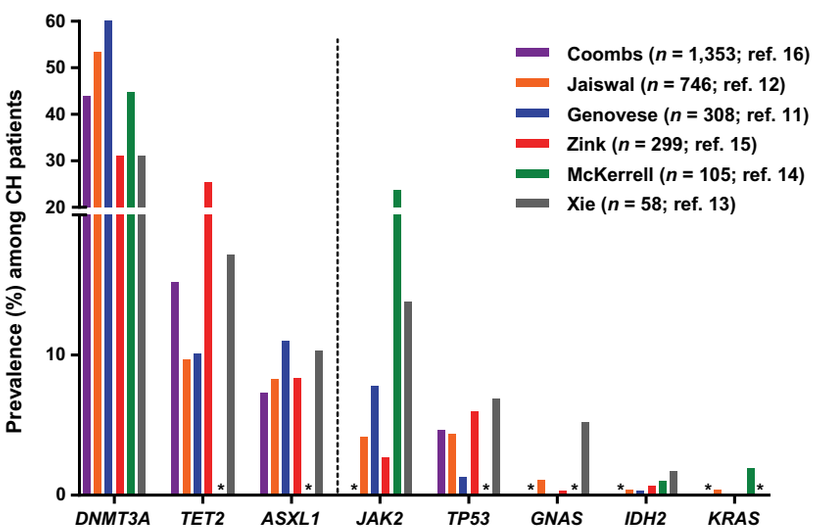

Figure 1.
Genes commonly mutated in CH. Review of six publications (11–16) studying CH reveals three commonly mutated genes: *DNMT3A*, *TET2*, and *ASXL1* (left). In addition, several cancer-related genes (right) are less commonly mutated in CH but are covered by many commercial plasma genotyping assays. Genes not reported in a given publication are marked with an asterisk.

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(paired-end, 2 × 151 bp) following the manufacturer's user manual (Illumina).

Tumor genotyping

Tumor NGS was performed at the Center for Advanced Molecular Diagnostics of Brigham and Women's Hospital using a hybrid-capture–based NGS platform spanning 275 to 447 genes. An institutional database of NSCLC tumor NGS results was used for comparisons (17).

Results

False-positive plasma ddPCR due to CH

We first queried our multiyear institutional experience performing plasma genotyping of key *EGFR* and *KRAS* mutations in patients with advanced NSCLC using a recently validated ddPCR platform with a negligible false-positive rate (5). Reviewing a cohort of 221 patients with advanced NSCLC undergoing plasma ddPCR for *EGFR* and *KRAS* mutations, 58 were known to harbor an *EGFR* mutation based on tumor genotyping (Fig. 2A). Two of these cases (3%) had plasma ddPCR that was positive for a *KRAS* codon 12 mutation. PBC DNA was collected and tested and determined to be the source of the *KRAS* mutation in both cases.

The first case was a 78-year-old female with newly diagnosed advanced *EGFR*-mutant NSCLC whose initial plasma ddPCR revealed both an *EGFR* exon 19 deletion (2.2% allelic fraction, AF) and a *KRAS* G12D (2.0% AF) mutation. Treatment with erlotinib resulted in a durable response on imaging and clearance of the plasma *EGFR* mutation; however, the *KRAS* mutation persisted in cfDNA over the course of 20 months on therapy (Fig. 2B and C). Occult malignancy was suspected, but imaging with abdominal ultrasound and CT colonography revealed no occult malignancy. Noting that *KRAS* mutations have been reported in rare patients with CH (12, 14), the *KRAS* G12D mutation was retested in paired cfDNA and PBC and detected in plasma at 1.2% AF and PBC at 0.5% AF, whereas the *EGFR* exon 19 deletion was undetectable in PBC (Supplementary Fig. S1). To pinpoint the origin of this *KRAS* mutation, PBC were sorted via fluorescence-activated cell sorting (Fig. 2D); ddPCR detected the *KRAS* mutation only in lymphocytes (1.7% AF) and across four lymphoid subpopulations. NGS confirmed the presence of the *KRAS* G12D mutation, as detected with ddPCR, but the absence of any other mutations in 8 cancer-associated genes (Supplementary Fig. S1). These data suggest that a common lymphoid progenitor acquired a *KRAS* G12D mutation and underwent clonal expansion, consistent with CH (5).

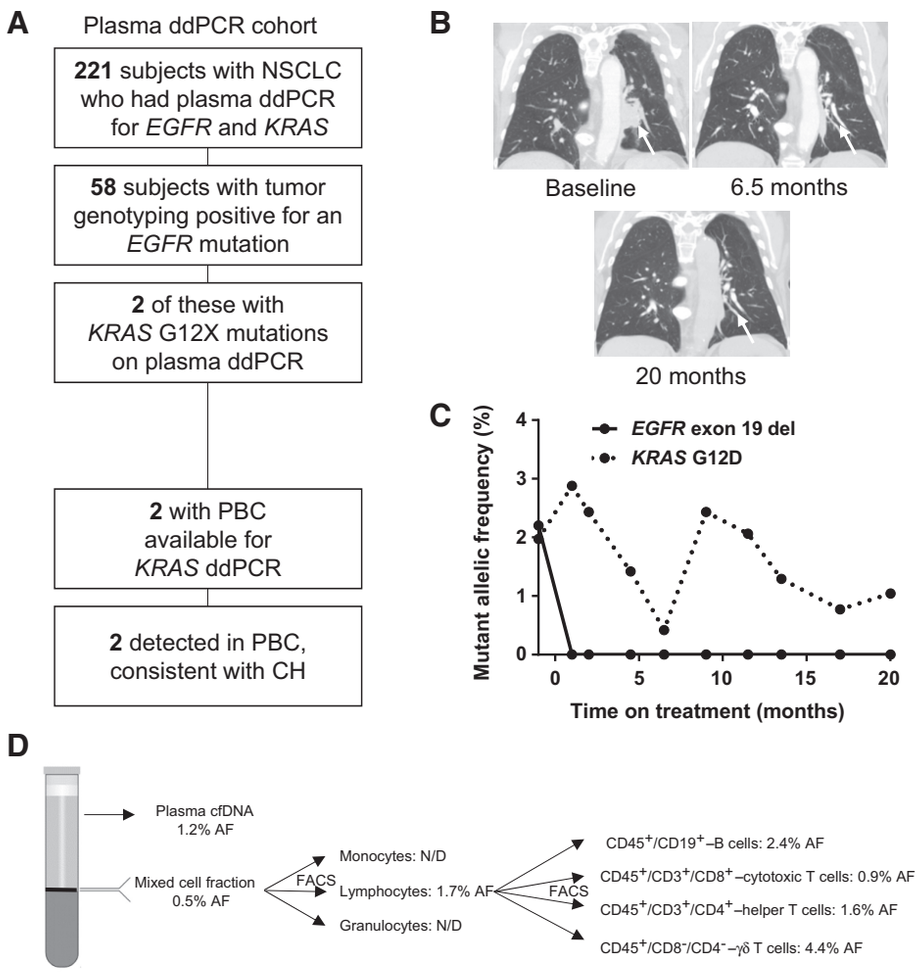

Figure 2.
Testing for CH using ddPCR of PBC from patients with advanced NSCLC. **A,** 221 patients completed plasma ddPCR for *EGFR* and *KRAS* driver mutations. Two patients with *EGFR* mutations on tumor NGS had *KRAS* G12X mutations detected in plasma. PBC for both were positive for the *KRAS* mutation. **B,** One of these patients had advanced *EGFR*-mutant lung cancer with a durable response to erlotinib therapy (arrow). **C,** Serial ddPCR of plasma cfDNA reveals clearance of the *EGFR* exon 19 deletion but sustained low levels of a *KRAS* G12D mutation over 20 months on therapy. **D,** Genotyping of DNA from the PBC identifies the presence of the *KRAS* G12D mutation, and further fluorescence-activated cell sorting (FACS) determined the mutation to be present in lymphocyte subpopulations, but not in monocytes or granulocytes. AF, allelic fraction; N/D, not detected.

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The second case was a 61-year-old female with advanced *EGFR*-mutant NSCLC being treated with erlotinib. Prior tumor NGS of a resection specimen had detected an *EGFR* L858R mutation but no *KRAS* mutation. Upon acquired resistance to erlotinib, commercial plasma NGS did not detect the *EGFR* L858R mutation but did detect a *KRAS* G12S mutation; the *KRAS* G12 mutation was then confirmed at our institution using ddPCR at 0.38% AF. We performed ddPCR of DNA from PBC, which revealed a *KRAS* G12 mutation (0.41% AF) and no detectable *EGFR* L858R. NGS of a tumor biopsy performed following erlotinib resistance detected the *EGFR* L858R mutation and a new T790M mutation but no *KRAS* mutation.

False-positive plasma NGS due to CH

We then studied 143 commercial plasma NGS results from 122 patients with advanced NSCLC treated at our center (Fig. 3A). Fourteen mutations were detected in three genes associated with CH (*JAK2*, *GNAS*, *IDH2*), including 6 cases (4.9%) positive for a *JAK2* V617F mutation, which is rarely seen on tumor genotyping of NSCLC (0.26%, $P = 0.0001$). PBC were available for analysis in 5 cases, and ddPCR detected the *JAK2* V617F mutation in each (AF 0.13%–4.66%, Table 1; Supplementary Fig. S2); 3 had tumor NGS available, and all were *JAK2* wild type.

Finally, we studied *TP53* mutations found on plasma NGS, as these are common in NSCLC but also can be seen in CH. Reviewing the 143 plasma NGS results, 108 *TP53* mutations were detected. Interestingly, the number of *TP53* mutations per specimen was higher than expected, with 7 specimens having >2 different *TP53* mutations, which is rare on tumor NGS and suggests a polyclonal process (Fig. 3B). For 33 of the *TP53* mutations detected on plasma NGS, PBC and tumor NGS were available for comparison. Fourteen *TP53* mutations were detected on tumor NGS, but not in PBC, consistent with tumor-derived variants, whereas the majority of the *TP53* mutations were not detected on tumor NGS (Table 1). For five mutations, NGS of PBC detected the *TP53* mutation, but tumor NGS did not, consistent with CH; in some of these cases, *TP53* mutations from the NSCLC and CH were both found concurrently in plasma (Fig. 3C). Fourteen *TP53* mutations were detected on neither tumor NGS nor PBC NGS, such that their source could not be determined; 13 of these had an AF ≤0.5% plasma NGS, near

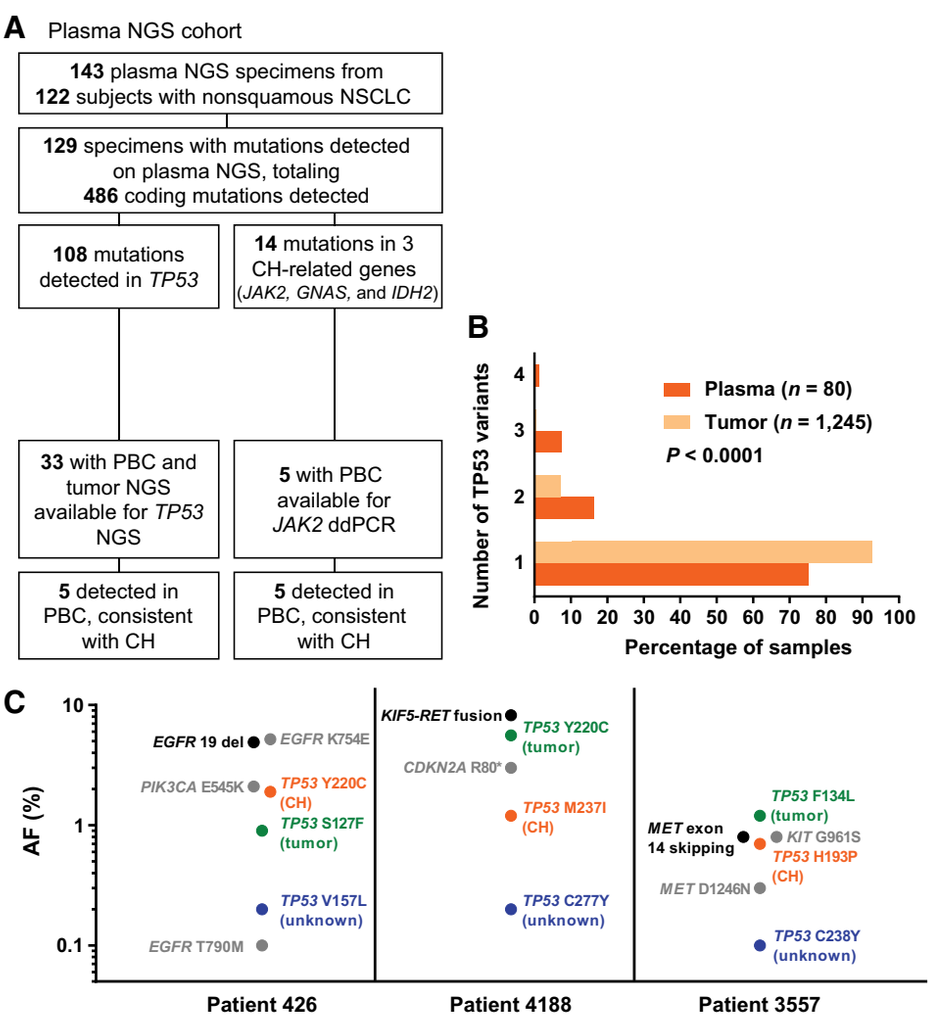

Figure 3.
Testing for CH through NGS of PBC from patients with advanced NSCLC. **A,** One hundred twenty-two patients had plasma NGS, revealing 14 mutations in CH-related genes and 108 mutations in *TP53*. PBC were available for analysis for 5 *JAK2* mutations and 33 *TP53* mutations (see Table 1). **B,** Comparing the number of coding *TP53* mutations across 80 plasma specimens and 1,245 tumor specimens with a *TP53* mutation detected using NGS, more *TP53* variants were detected per sample in plasma as compared with tumor ($P < 0.0001$, χ^2 test), with 0.5% of tumor samples showing >2 mutations compared with 8.8% plasma samples. **C,** Three cases had multiple *TP53* mutations found on plasma NGS, shown in relationship to the known oncogenic driver mutations (black), where some *TP53* mutations were derived from tumor (green) and some were derived from CH (orange).

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Table 1. Genotyping of peripheral blood cells identifies clonal hematopoiesis in patients with plasma genotyping positive for *JAK2* mutations (top) or *TP53* mutations (bottom)

ID	Age	Plasma NGS result (AF %)	PBC testing method	Tumor NGS result	PBC testing result (AF %)	Mutation source
JAK2 analysis						
4433	58	*JAK2* V617F (19.5)	ddPCR	Not detected	Detected (4.66)	CH
4379	63	*JAK2* V617F (2.5)	ddPCR	Not available	Detected (0.19)	CH
4014	59	*JAK2* V617F (0.5)	ddPCR	Not detected	Detected (0.68)	CH
349	84	*JAK2* V617F (0.3)	ddPCR	Not detected	Detected (0.14)	CH
4447	87	*JAK2* V617F (0.2)	ddPCR	Not available	Detected (0.13)	CH
TP53 analysis						
426	64	*TP53* Y220C (1.9)	NGS	Not detected	Detected (1.72)	CH
4188	73	*TP53* M237I (1.2)	NGS	Not detected	Detected (2.66)	CH
3557	70	*TP53* H193P (0.7)	NGS	Not detected	Detected (1.1)	CH
3717	73	*TP53* V272M (0.5)	NGS	Not detected	Detected (1.29)	CH
423	67	*TP53* R110L (0.3)	NGS	Not detected	Detected (0.56)	CH
427	47	*TP53* G244C (11.2)	NGS	Detected	Not detected	Tumor
424	49	*TP53* V157F (8.5)	NGS	Detected	Not detected	Tumor
3856	43	*TP53* R280I (5.9)	NGS	Detected	Not detected	Tumor
4188	73	*TP53* Y220C (5.6)	NGS	Detected	Not detected	Tumor
4250	59	*TP53* R248Q (5.1)	NGS	Detected	Not detected	Tumor
3983	47	*TP53* R175H (4.6)	NGS	Detected	Not detected	Tumor
4096	59	*TP53* H179R (4.4)	NGS	Detected	Not detected	Tumor
4321	50	*TP53* Y220C (2.5)	NGS	Detected	Not detected	Tumor
4254	68	*TP53* E271* (1.7)	NGS	Detected	Not detected	Tumor
3557	70	*TP53* F134L (1.2)	NGS	Detected	Not detected	Tumor
426	64	*TP53* S127F (0.9)	NGS	Detected	Not detected	Tumor
3847	50	*TP53* V172F (0.8)	NGS	Detected	Not detected	Tumor
3717	73	*TP53* E204* (0.3)	NGS	Detected	Not detected	Tumor
3706	59	*TP53* R337L *(0.2)*	NGS	Detected	Not detected	Tumor
4014	59	*TP53* H193Y (13.2)	NGS	Not detected	Not detected	Uncertain
1258	81	*TP53* Y220C (0.5)	NGS	Not detected	Not detected	Uncertain
4488	68	*TP53* R282W (0.3)	NGS	Not detected	Not detected	Uncertain
4096	68	*TP53* E258* (0.3)	NGS	Not detected	Not detected	Uncertain
4055	48	*TP53* R249S (0.3)	NGS	Not detected	Not detected	Uncertain
4188	73	*TP53* C277Y (0.2)	NGS	Not detected	Not detected	Uncertain
3856	43	*TP53* R213* (0.2)	NGS	Not detected	Not detected	Uncertain
1258	81	*TP53* I162F (0.2)	NGS	Not detected	Not detected	Uncertain
426	64	*TP53* V157L (0.2)	NGS	Not detected	Not detected	Uncertain
424	49	*TP53* E298* (0.2)	NGS	Not detected	Not detected	Uncertain
3856	43	*TP53* G199E (0.1)	NGS	Not detected	Not detected	Uncertain
3557	70	*TP53* C238Y (0.1)	NGS	Not detected	Not detected	Uncertain
3553	64	*TP53* Y236C (0.1)	NGS	Not detected	Not detected	Uncertain
2966	57	*TP53* Y220C (0.1)	NGS	Not detected	Not detected	Uncertain

the limit of detection (LOD) of most plasma genotyping assays and making validation using an orthogonal assay extremely difficult.

Discussion

The enormous enthusiasm behind the potential clinical applications of plasma cfDNA analysis has begun to outpace our biological understanding of this new biospecimen. A range of data indicate that plasma cfDNA is a complex mixture of DNA from many sources, including germline, fetal, infectious, and malignant (2, 18). For example, it is clear that mutations detected on plasma genotyping can be of somatic or germline origin (18), and can be truncal tumor mutations or heterogeneous resistance mutations (6). Here, we demonstrate an added element of complexity—that mutations in the cfDNA can be of hematopoietic origin yet risk being mistaken as tumor-derived mutations. These CH-derived mutations can be seen at a range of AF in cfDNA, with a median AF of 0.9% across the 10 CH-derived mutations we detected.

Our findings affect the use of plasma genotyping for tumor profiling in search of targeted therapy options. We and others have advocated for the use of plasma genotyping in patients with advanced NSCLC where tumor tissue is unavailable for genomic analysis, with the caveat that a positive result can be trusted while a negative result should reflex to tumor genotyping. The finding that *KRAS* and *TP53* mutations detected in plasma may not represent tumor genotype adds complexity to this approach. Importantly, targetable mutations in genes like *EGFR*, *BRAF*, and *MET* have not been described in CH; if these are detected in plasma with a rigorously validated assay, they can likely be trusted and used for initiation of targeted therapy. However, the same may not be true for *KRAS* or *TP53* mutation—a clinician might mistakenly assume that these mutations in cfDNA are tumor derived and indicate the absence of a targetable genotyping. Our finding of CH-derived mutations in *KRAS* and *TP53* supports the established paradigm that treatment can be initiated based upon a targetable mutation detected in cfDNA, whereas the absence of a targetable mutation in cfDNA should be followed by genotyping of a tumor biopsy specimen (6).

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Second, our findings affect the development of plasma genomics as a tool for detecting minimal residual disease (MRD) or as a cancer screening assay. For example, in a patient who has completed curative therapy for cancer, detection in plasma of mutations in cancer-associated genes like *TP53*, *KRAS*, or *JAK2* could be interpreted as indicating the presence of residual cancer (3), whereas these mutations might in fact be derived from CH. It has been reported that CH is seen in 25% of patients with nonhematologic cancers (16); indeed, *TP53* mutations may be even more common in PBC of patients previously treated with chemotherapy as part of curative therapy (19), increasing the chance of finding such mutations at a time point when MRD is being evaluated. The clearest way to overcome this challenge would be paired genotyping of both plasma cfDNA and PBC DNA, which could allow an assay to screen out hematopoietic-derived mutations. Such a step would certainly increase the complexity and cost of cfDNA analysis, and would require the saving of PBC, which are routinely discarded when spinning plasma. Mutations detected in cfDNA and not in PBC could then be considered potentially tumor derived and indicative of risk. Of course, it is then possible that the incidental detection of *JAK2* mutations in plasma or in PBC could lead to the overdiagnosis of hematologic malignancies that are clinically nonsignificant. This is merely one symptom of a broader challenge, which is that there remains no established management strategy for patients incidentally diagnosed with CH (10).

Further prospective study is needed to better capture the prevalence of CH-related false positives and their potential impact on the positive predictive value (PPV) of these assays. In our analysis, the prevalence of CH-derived *KRAS* mutations was 3%, which, given *KRAS* mutations are common in many cancer types, is unlikely to impact the PPV of KRAS plasma genotyping. In contrast, *JAK2* mutations are rare in solid tumors, so the PPV of *JAK2* plasma genotyping may be low—most of these mutations in cfDNA are likely to be CH derived. We note that others have found rare *JAK2* mutations in plasma NGS from a large cohort of patients with colorectal cancer (20); however, this study did not perform PBC and tumor sequencing to confirm the hematopoietic etiology of these mutations. In another case report, an *IDH2* mutation found in the cfDNA of a patient with metastatic colon cancer was determined to be CH derived after sequencing of a bone marrow biopsy specimen (21); our data highlight that bone marrow biopsy is not needed for detection of CH-derived mutations, as these can be easily detected in PBC. Our most surprising result is the finding that many *TP53* mutations in cfDNA may be CH derived—only 14 of 33 *TP53* mutations could be confirmed to be tumor derived, though many low AF *TP53* mutations could not be found in PBC as well. Thus, the PPV of TP53 plasma genotyping remains unclear but could be lower than previously believed. Some might disagree with whether mutations in cfDNA due to CH should in fact be considered false positives as these are not technical errors but rather real variants derived from nontumor tissue. However, because plasma cfDNA genotyping is intended to offer a liquid biopsy of the tumor, the clinical impact of such CH-derived mutations is a false-positive result that potentially misdiagnoses a cancer patient with an incorrect genotype. Because CH-derived mutations cause a misleading diagnostic result, they should be acknowledged as false positives, much like a false-positive d-dimer in a patient with cancer undergoing work-up for thromboembolism (22) or a false-positive CT scan in a patient undergoing lung cancer screening who is found to have benign nodules (23).

Our approach was technically limited in its leveraging of ongoing commercial plasma NGS efforts, which increases the clinical relevance of our findings, but means we were unable to perform plasma and PBC sequencing with identical assays. Prospective efforts that include paired NGS of cfDNA and PBC DNA using identical assays will be needed. It should be acknowledged that available estimates of CH prevalence [5%–13% of persons over 70 years old (11, 13) and 25% of cancer patients (16)] are based upon moderate-depth sequencing efforts with an LOD around 2% AF. With the highly sensitive NGS approaches being offered by commercial plasma NGS laboratories (LOD ~0.5% AF or lower), CH may in fact be much more common, and thus a condition we will need to be ready to recognize as a part of our routine cancer care and a potentially common finding in plasma genotyping results.

Disclosure of Potential Conflicts of Interest

P.A. Jänne reports receiving commercial research grants from Astellas, AstraZeneca, Daiichi Sankyo, Eli Lilly, and PUMA, holds ownership interest (including patents) in Gatekeeper Pharmaceuticals, and is a consultant/advisory board member for AstraZeneca, Boehringer Ingelheim, Chugai Pharmaceuticals, Eli Lilly, Genentech/Roche, Ignyta, LOXO Oncology, and Pfizer. C.P. Paweletz reports receiving speakers bureau honoraria from AstraZeneca and BioRad, and is a consultant/advisory board member for DropWorks. G.R. Oxnard is a consultant/advisory board member for AstraZeneca, Boehringer Ingelheim, DropWorks, Genentech, GRAIL, Inivata, LOXO, Sysmex, and Takeda/Ariad. No potential conflicts of interest were disclosed by the other authors.

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Study supervision: C.P. Paweletz, G.R. Oxnard

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