

Identification of Clonal Hematopoiesis Mutations in Solid Tumor Patients Undergoing Unpaired Next-Generation Sequencing Assays



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Abstract

Purpose: In this era of precision-based medicine, for optimal patient care, results reported from commercial next-generation sequencing (NGS) assays should adequately reflect the burden of somatic mutations in the tumor being sequenced. Here, we sought to determine the prevalence of clonal hematopoiesis leading to possible misattribution of tumor mutation calls on unpaired Foundation Medicine NGS assays.

Experimental Design: This was a retrospective cohort study of individuals undergoing NGS of solid tumors from two large cancer centers. We identified and quantified mutations in genes known to be frequently altered in clonal hematopoiesis (*DNMT3A*, *TET2*, *ASXL1*, *TP53*, *ATM*, *CHEK2*, *SF3B1*, *CBL*, *JAK2*) that were returned to physicians on clinical Foundation Medicine reports. For a subset of patients, we explored the frequency of true clonal hematopoiesis by comparing mutations on Foundation Medicine reports with matched blood sequencing.

Results: Mutations in genes that are frequently altered in clonal hematopoiesis were identified in 65% (1,139/1,757) of patients undergoing NGS. When excluding *TP53*, which is often mutated in solid tumors, these events were still seen in 35% (619/1,757) of patients. Utilizing paired blood specimens, we were able to confirm that 8% (18/226) of mutations reported in these genes were true clonal hematopoiesis events. The majority of *DNMT3A* mutations (64%, 7/11) and minority of *TP53* mutations (4%, 2/50) were clonal hematopoiesis.

Conclusions: Clonal hematopoiesis mutations are commonly reported on unpaired NGS testing. It is important to recognize clonal hematopoiesis as a possible cause of misattribution of mutation origin when applying NGS findings to a patient's care. *Clin Cancer Res*; 24(23); 5918–24. ©2018 AACR.

See related commentary by Pollyea, p. 5790

Introduction

Precision medicine has led to improved prognostication and therapeutic selection in oncology (1); however, an ongoing challenge is uncertainty that clinically relevant tumor-associated mutations are exclusively reported. Because of complexities in variant calling and scarcity of variant-specific clinical data for most cancers, commercial next-generation sequencing (NGS) assays often report discordant interpretations of NGS data, exemplified

by recent articles (2, 3). Furthermore, many NGS platforms sequence tumors without a paired normal sample, which reduces cost and obviates issues associated with reporting germline variants (4), but can exacerbate challenges when adjudicating mutations as somatic or germline. Here, we explore another challenge associated with somatic mutation calling. Given the recent recognition of clonal hematopoiesis in patients with cancer (5), we hypothesized that admixed leukocytes contained within solid

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Translational Relevance

Precision medicine is being increasingly utilized in routine clinical practice for both prognostication and selection of targeted therapies for patients with cancer. To optimize translation of findings to patient care, next-generation sequencing reports should adequately reflect the burden of somatic mutations in the tumor being sequenced. Here, we describe a novel challenge that can arise during interpretation of next-generation sequencing assays—presence of clonal hematopoiesis mutations from admixed leukocytes contained within tumor specimens, leading to potential misattribution of mutation origin. We demonstrate that peripheral blood sequencing can be utilized to confirm clonal hematopoiesis events, and describe gene and allelic frequency patterns of clonal hematopoiesis mutations reported on next-generation sequencing clinical testing. In the absence of paired blood sequencing, clinicians should exert caution when interpreting unfractionated sequencing assays so that accurate therapeutic selection is realized for individual patients.

tumor biopsies may result in identification of clonal hematopoiesis on NGS assays, thus confounding clinical decision-making. We used matched solid tumor and blood sequencing to explore the prevalence of clonal hematopoiesis detection on a commonly utilized commercial NGS assay (Foundation Medicine; FM).

Materials and Methods

Patients

This study was approved by the institutional review boards (IRB) at the University of North Carolina at Chapel Hill (UNC, Chapel Hill, NC) and Moffitt Cancer Center (MCC, Tampa, FL). Research was conducted with in accordance with ethical guidelines as outlined in the Declaration of Helsinki. Individuals eligible for this study included all UNC (February 27, 2013–September 7, 2017) and MCC (February 6, 2013–August 2, 2017) patients with nonhematologic cancers who underwent FM testing as part of routine clinical care. A subset of patients in the UNC cohort were cross-referenced with data generated under the IRB- and Office of Human Ethics–approved protocol, LCCC1108 (NCT01457196), referred to as UNCseq, for whom written informed consent was obtained. Permission to review records of UNC patients who underwent FM testing as part of routine clinical care, in the absence of UNCseq testing, was permitted by the approved IRB protocol. All MCC patients included provided written informed consent to the Total Cancer Care cohort study, an IRB-approved biobanking protocol (MCC14690, MCC13579) in which individuals agree to provide tissue and blood samples for research and to be followed throughout their lifetime (6). Patients were excluded if their primary diagnosis was a hematologic cancer or if the FM sample failed quality control testing.

Identification and classification of clonal hematopoiesis

All mutations reported by FM (excluding amplifications, large indels, and translocations) across protein-coding exons were reviewed. Mutations in genes known to occur as clonal hematopoiesis (*DNMT3A*, *TET2*, *ASXL1*, *TP53*, *ATM*, *CHEK2*, *CBL*, *JAK2*,

and *SF3B1*) in patients with cancer (5) were classified as potential clonal hematopoiesis. Although *PPM1D* is frequently mutated in cancer patients with clonal hematopoiesis (5), it was not included due to absence on FM clinical reports. The variant allele frequency (VAF) and FM classification (variant of known significance or variant of unknown significance) for each reported alteration was tabulated. Violin plots were constructed to examine the VAFs in clonal hematopoiesis genes.

Confirmation of clonal hematopoiesis mutations

For the UNC cohort, patients undergoing FM testing were cross-referenced with patients who underwent UNCseq testing, an NGS assay that utilized paired tumor and blood samples, described previously (7, 8). Alterations reported on FM were compared with UNCseq reads from tumor and blood using Integrative Genomic Viewer (IGV; ref. 9). Clonal hematopoiesis mutations were defined as mutations in which the VAF in blood exceeded that in the tumor; however, if the VAF was 30% or greater in both the tumor and blood, the mutation was considered germline. All other reported mutations were classified as tumor somatic (mutations in tumor tissue only). There was no minimum VAF requirement for inclusion, although at least eight reads were required to support the alternate variant call.

MCC patients with low VAF mutations in clonal hematopoiesis genes (VAF < 20%, except for *TP53* where threshold was <5%) reported on FM testing who had Total Cancer Care–banked blood specimens were selected for targeted NGS sequencing. Matched FM sequencing data and NGS from blood were aligned and visualized in IGV, and VAFs of potential clonal hematopoiesis mutations were recorded.

NGS and bioinformatics of blood samples

Methodology for the UNCseq assay of tumor and matched normal blood NGS has been described previously (7). For MCC patients, genomic DNA was extracted from whole blood samples using Autopure LS Automated DNA Extraction (QIAGEN) and quantified using a Qubit fluorometer (Life Technologies). Targeted NGS was conducted with 200 ng of DNA input using SureSelect^{XTHS} (Agilent) target enrichment with custom capture (all exons from 76 genes were captured, including all 9 clonal hematopoiesis genes of interest) according to the manufacturer's protocol (#G9702-90000). The size and quality of the pre- and postcaptured libraries were evaluated using a 2100 BioAnalyzer (Agilent) or D1000 TapeStation (Agilent), as indicated in the SureSelect^{XTHS} protocol. The enriched library was quantified using a Library Quantification Kit for NGS (KAPA Biosystems), and samples were diluted to a 4 nmol/L concentration and pooled for sequencing. Denaturation was conducted using NaOH; neutralization was conducted with Tris buffer (pH 8.5), and samples were diluted to a concentration of 20 pmol/L in HT1. Samples were diluted to a final concentration between 1.7 and 2.2 pmol/L for sequencing with a v2 Sequencing Reagent Kit and a NextSeq 500 desktop sequencer (Illumina). Paired-end FASTQ files were adapter- and quality-trimmed using TrimGalore v0.4.1/cutadapt v1.14 (10, 11). Quality control was performed with FastQC (12). The trimmed reads were mapped with BWA to GRCh38 (13). Sorting and indexing of resulting BAM files was done with SAMTOOLS (14). Duplicates were marked and base qualities were recalibrated according to GATK best practice (15). Mutations were called by GATK. All suspected clonal hematopoiesis mutations on FM

reports were compared with mutations called from the blood samples in the UNC and MCC cohorts.

Statistical analysis

Descriptive statistics were used to summarize demographic and clinical characteristics of patients included in this study. Means, SDs, and ranges were calculated for continuous variables, and frequencies and percentages were generated for categorical variables. Differences between groups were examined using χ^2 tests (for categorical variables) or Wilcoxon rank sum test (for numerical variables), unless otherwise specified. All tests are two-sided with alpha level 0.05. The analyses were done using SAS 9.4 (SAS Institute). Violin plots were generated using the ggplot2 package in R v. 3.4.3 (16).

Results

Patients

A total of 1,757 FM reports ($n = 768$ and $n = 989$ from UNC and MCC, respectively) from 1,743 patients were included in this study (Supplementary Table S1). The average age of individuals was 58.5 years old, and the cohort included 48% males. The most common tumor types were lung (22%), gastrointestinal and skin (11% each), and sarcoma (10%). There were no statistical differences in demographics between the UNC and MCC cohorts; however, there were differences in the tumor types of the patients included from the two institutions.

Clonal hematopoiesis on FM NGS testing

Of the 1,757 FM reports, 65% (1,139/1,757) had a mutation reported in a gene associated with clonal hematopoiesis. Excluding *TP53*, due to its high mutational frequency across cancer types, 35% (619/1,757) of FM reports had ≥ 1 mutation in a clonal hematopoiesis gene. Presence of mutations in clonal hematopoiesis genes was significantly associated with increasing patient age ($P < 0.001$) and cancer type ($P < 0.001$), when including all clonal hematopoiesis mutations and when excluding mutations in *TP53* (Table 1; Supplementary

Table 1. Characteristics of patients with CH mutations versus those without CH mutations reported on FM testing

Characteristics	CH	No CH	Total	P
Number	1,139	618	1,757	NA
Age (mean)	60.0	55.4	58.4	<0.001
Gender (% male)	537 (47%)	309 (50%)	846 (48%)	0.25
Tumor type				<0.001
Breast	100 (9%)	34 (6%)	134 (8%)	
Gastrointestinal	150 (13%)	40 (6%)	190 (11%)	
Genitourinary	83 (7%)	59 (10%)	142 (8%)	
Gynecologic	101 (9%)	24 (3.9%)	125 (7%)	
Lung	293 (26%)	102 (17%)	395 (22%)	
Sarcoma	77 (7%)	105 (17%)	182 (10%)	
Skin	119 (10%)	65 (11%)	182 (10%)	
Other	216 (19%)	189 (31%)	405 (23%)	

NOTE: Genes include *DNMT3A*, *TET2*, *ASXL1*, *TP53*, *ATM*, *CHEK2*, *CBL*, *JAK2*, and *SF3B1*. Both UNC and MCC patients are included together. The total number of reports is included here because this number represents the total number of FM reports (14 patients had more than one report, from different biopsies taken on different days).

Abbreviation: CH, clonal hematopoiesis.

Table S2). A bimodal distribution in VAFs was observed for several genes (Fig. 1; Supplementary Fig. S1).

Confirmation of clonal hematopoiesis by paired blood sequencing

A subset of UNC patients ($N = 64$) underwent both FM and UNCseq testing. Median coverage for UNCseq samples was $834\times$ for blood and $764\times$ for tumor. Of the 173 potential clonal hematopoiesis mutations identified on FM reports that were compared with UNCseq sequencing, 3.5% of variants (6/173) in 9.4% of patients (6/64) were confirmed to be clonal hematopoiesis events (Table 2); 101 mutations (58.4%) were confirmed to be tumor somatic (Table 3); and 49 variants (28.3%) were unable to be confirmed because the gene of interest reported by FM was not sequenced on UNCseq or the mutation was absent on UNCseq testing. Germline events (mutations in blood and tumor at VAF $\geq 30\%$) were reported by FM on 17 occasions [in 15 patients (23%)], of which 33% were pathogenic or likely

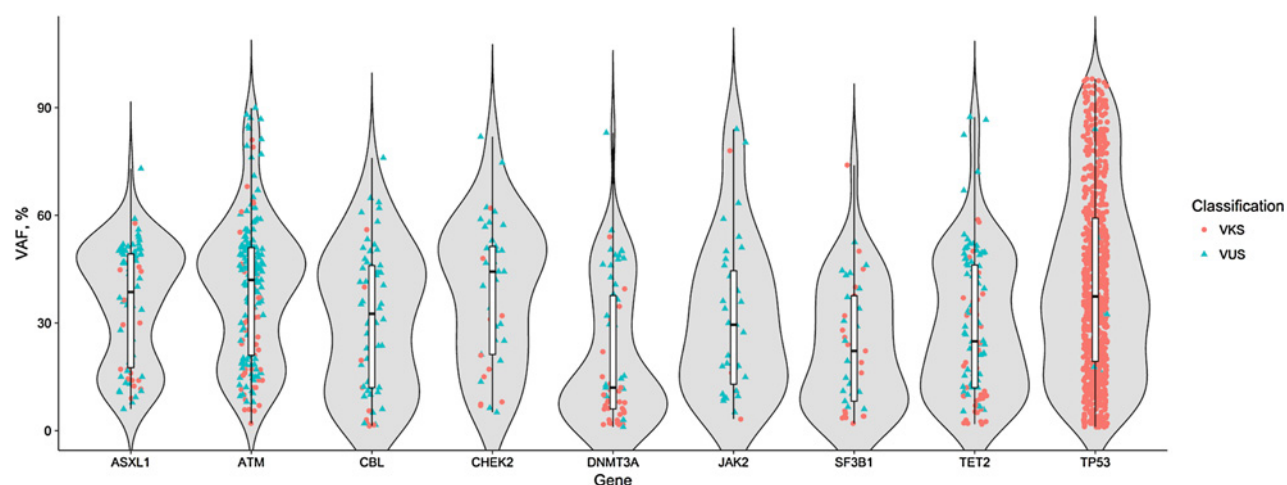


Figure 1.

Violin plots displaying VAFs from FM NGS testing in nine genes recurrently mutated in clonal hematopoiesis. Variants of known significance (VKS) are depicted by red circles, and variants of unknown significance (VUS) are depicted by blue triangles. Boxes represent the 25th and 75th percentiles, with the horizontal line in the middle indicating the median, and the vertical lines representing the 95th percentile.

Table 2. Description of clonal hematopoiesis variants identified on FM testing and confirmed in blood samples

Patient number	Age	Sex	Primary tumor	Testing interval (days)	Gene	Variant	COSMIC H/L count ^a	FM VAF	UNCseq tumor VAF	UNCseq or MCC blood VAF
UNC01	65	F	Ovarian	30	TP53	R280G	8	3	1.8	3.2
UNC02	57	F	Meningioma	1,412	TET2	T940fs*13	1	8.8	1.9	9.7
UNC03	64	F	Ovarian	721	DNMT3A	R882C	457	2	1.9	12
UNC04	71	M	Pancreatic	-24	NRAS	G12D	618	11	5.2	29
UNC05	54	F	Carcinoma NOS	3	DNMT3A	G550R ^b	8	3	2.3	9
UNC06	84	F	Lung	788	PTPN11	V428M ^c	0	0.78	0	1.4
MCC01	68	F	Breast	2,194	DNMT3A	R736C	21	2.1	NA	3
MCC02	49	F	Breast	-60	DNMT3A	R635W	18	2	NA	5
MCC03	58	M	Colorectal	-294	TET2	R1452*	17	2	NA	3
MCC10	84	M	Lung	-195	DNMT3A	W314*	1	15	NA	43
MCC12	64	M	Sarcoma	-46	DNMT3A	I670fs*35	0	6	NA	13
MCC14	75	F	Lung	-57	DNMT3A	R882C	457	1.7	NA	4
MCC15	78	M	Lung	0	SF3B1	K666N	90	5.5	NA	8.1
MCC16	56	M	Brain	12	CBL	L380P	24	1.3	NA	2.5
MCC22	81	F	Lung	-333	TP53	P177R	0	1	NA	2.7
MCC24	78	M	Brain	-38	CBL	C401Y	3	5.5	NA	11.5
MCC24	78	M	Brain	-38	TET2	H1904R	8	17.4	NA	71.7
MCC27	76	M	Lung	-28	SF3B1	Y623C	9	3.6	NA	36.8

NOTE: For testing interval, negative numbers mean that FM testing preceded UNCseq or MCC testing, and positive numbers mean that UNCseq or MCC testing preceded FM. Age is at the time of FM testing.

Abbreviation: NOS, not otherwise specified.

^aRepresents the number of times this amino acid variant was reported in the COSMIC database, in the "haematopoietic and lymphoid" (H/L) category, v85 (released May 8, 2018).

^bListed as a variant of uncertain significance.

^cVariant was reported on FoundationACT testing, which is an assay for circulating tumor DNA.

pathogenic based on published criteria for interpretation of sequence variants (Supplementary Table S3; ref. 17). Several patients were found to have mutations reported by FM, which had multiple sites of origin, such as a patient with a clonal hematopoiesis mutation in *DNMT3A* and a tumor somatic *TET2* mutation (Fig. 2; Supplementary Fig. S2) and a patient with a clonal hematopoiesis mutation in *DNMT3A*, a tumor somatic *TP53* mutation, and a germline mutation in *BRCA2* (Fig. 3; Supplementary Fig. S3).

A subset of MCC patients ($n = 30$) with low VAF mutations in clonal hematopoiesis genes on FM reports who had banked blood available were selected to undergo targeted NGS sequencing. Median target coverage was 820 \times per sample. Of the 53 mutations in clonal hematopoiesis genes reported by FM, 12 (22.6%) were detected at higher VAF in blood from the same individual, thus confirming clonal hematopoiesis in 36.7% (11/30) (Table 2). Overall, the confirmed clonal hematopoiesis events (Table 2) were more likely to have been previously reported in the Catalogue for Somatic Mutations in Cancer (COSMIC) database in the "haematopoietic and lymphoid" category than the tumor somatic events occurring in clonal hematopoiesis genes (Table 3; Supplementary Fig. S4; mean count 97 vs. 2, $P < 0.0001$).

In the UNC cohort, VAFs from true clonal hematopoiesis events were lower than VAFs from tumor somatic events in clonal hematopoiesis genes (median VAF of 3% vs. 27%, respectively, $P < 0.001$). In the UNC cohort, true clonal hematopoiesis events were enriched in patients who had FM testing performed after exposure to chemotherapy or radiotherapy, as opposed to patients who were naïve to chemotherapy and radiotherapy at the time of FM testing [17.7% (6/34) of previously treated patients vs. 0% (0/29) chemotherapy/radiotherapy naïve patients (1 patient excluded due to missing treatment data), $P = 0.027$ by Fisher exact test]. Given that age can be a significant confounder with respect to the presence of clonal hematopoiesis, we analyzed

whether patients who were chemotherapy/radiotherapy naïve at the time of FM testing were younger than the pretreated patients, but there was no statistically significant difference ($P = 0.86$). Because the MCC cohort was selected solely based on the presence of clonal hematopoiesis mutations on FM testing, we did not examine an association for presence of clonal hematopoiesis events with history of prior chemotherapy/radiotherapy among this group, where 57% of patients (17/30) were chemotherapy/radiotherapy naïve at the time of FM testing. When combining both cohorts, we found that 8% of mutations (6/173 UNC + 12/53 MCC) in clonal hematopoiesis genes reported by FM were true clonal hematopoiesis events. Furthermore, the majority of *DNMT3A* mutations (64%, 7/11) were clonal hematopoiesis, whereas the minority of *TP53* mutations (4%, 2/50) were clonal hematopoiesis.

Discussion

Here, we explored the possibility that mutations reported on FM NGS testing of solid tumors were instead clonal hematopoiesis events. Using matched blood sequencing, we found that 8% of mutations reported in clonal hematopoiesis-related genes on FM NGS testing were clonal hematopoiesis. In the UNC cohort, mutations with any VAF were considered potentially clonal hematopoiesis and only 3.5% were confirmed; however, for the MCC cohort, only mutations with low VAFs were considered, and a larger percentage (22.6%) was confirmed. We demonstrated that mutations reported on FM NGS testing with lower VAFs were more likely to be indicative of clonal hematopoiesis; however, the majority of variants could not be confirmed as *bona fide* clonal hematopoiesis. Our results also suggest that mutations in some clonal hematopoiesis-related genes (e.g., *DNMT3A*) are more likely to be clonal hematopoiesis than others (e.g., *TP53*). Among reported mutations in clonal hematopoiesis genes, true clonal

Table 3. Description of variants identified on FM testing that were suspicious for clonal hematopoiesis but were ultimately determined to be true tumor somatic events

Patient number	Age	Sex	Primary tumor	Testing interval (days)	Gene	Variant	COSMIC H/L count ^a	FM VAF	UNCseq tumor VAF	UNCseq or MCC blood VAF
UNC05	54	F	Carcinoma NOS	3	TET2	T1063fs*5	0	19	15	0
UNC07	46	F	Breast	-656	ASXL1	S1115T ^b	0	28	21	0
UNC08	56	M	Melanoma	-6	TET2	S1162F ^b	0	25	23	0
UNC09	61	M	Lung	-274	CHEK2	G151C ^b	0	58	56	0
UNC10	65	F	Uterine	13	CHEK2	L380I ^b	0	5.1	1	0
UNC11	54	F	Colon	-698	CHEK2	H339fs*5	0	7	7	0
UNC12	11	F	Melanoma	-97	SF3B1	K666T	32	40	2	0
UNC13	80	F	Bladder	515	SF3B1	E174Q	0	30.6	8	0
MCC06	59	M	Skin (nonmelanoma)	-936	ASXL1	W898*	2	16	NA	0
MCC07	69	M	Pancreatic	-30	ATM	R3008H	13	8.6	NA	0
MCC08	74	M	Sarcoma	-127	CBL	D390V	0	12	NA	0
MCC13	62	F	Lung	53	CBL	C416Y ^b	4	1.6	NA	0
MCC17	59	F	Lung	-281	ATM	G2891D ^b	4	10.4	NA	0
MCC18	62	F	Skin (nonmelanoma)	-19	ASXL1	Q780*	5	7.8	NA	0
MCC18	62	F	Skin (nonmelanoma)	-19	ASXL1	E332*	0	14.4	NA	0
MCC18	62	F	Skin (nonmelanoma)	-19	ASXL1	P701S ^b	0	17.1	NA	0
MCC18	62	F	Skin (nonmelanoma)	-19	ATM	E647G ^b	0	14.6	NA	0
MCC18	62	F	Skin (nonmelanoma)	-19	DNMT3A	Splice site 1555-1G>A	0	5.6	NA	0
MCC18	62	F	Skin (nonmelanoma)	-19	JAK2	R938Q ^b	0	5.1	NA	0
MCC18	62	F	Skin (nonmelanoma)	-19	JAK2	P429Y ^b	0	8.3	NA	0
MCC18	62	F	Skin (nonmelanoma)	-19	JAK2	L712F ^b	0	9	NA	0
MCC18	62	F	Skin (nonmelanoma)	-19	JAK2	K607* ^b	0	18.7	NA	0
MCC18	62	F	Skin (nonmelanoma)	-19	SF3B1	P813L ^b	0	6.6	NA	0
MCC18	62	F	Skin (nonmelanoma)	-19	SF3B1	M1133I ^b	0	10.3	NA	0
MCC18	62	F	Skin (nonmelanoma)	-19	TET2	H1676Y ^b	0	5.8	NA	0
MCC25	69	M	Brain	-31	TET2	H1881R ^b	4	6.8	NA	0
MCC25	69	M	Brain	-31	TET2	F1733fs*12	0	19.7	NA	5.4
MCC28	59	F	Lung	-32	TET2	Q1529*	2	8.3	NA	0
MCC29	76	F	Skin (nonmelanoma)	-182	ATM	G3019E ^b	0	14.7	NA	0
MCC29	76	F	Skin (nonmelanoma)	-182	ATM	P2512S ^b	0	16	NA	0
MCC29	76	F	Skin (nonmelanoma)	-182	ATM	I1999M ^b	0	18.4	NA	0
MCC29	76	F	Skin (nonmelanoma)	-182	DNMT3A	N717S ^b	5	12.7	NA	0
MCC30	24	M	Brain	-104	DNMT3A	R326H ^b	2	9.6	NA	0
MCC30	24	M	Brain	-104	DNMT3A	I661V ^b	0	13.1	NA	0

NOTE: Analysis was limited to genes commonly reported as clonal hematopoiesis events (*DNMT3A*, *TET2*, *ASXL1*, *ATM*, *CHEK2*, *CBL*, *JAK2*, and *SF3B1*) and excluded *TP53* given that all *TP53* variants, except two (listed in Table 2), were confirmed as tumor somatic.

^aRepresents the number of times this amino acid variant was reported in the COSMIC database, in the "haematopoietic and lymphoid" (H/L) category, v85 (released May 8, 2018).

^bListed as a variant of uncertain significance.

hematopoiesis events were more likely to be reported by COSMIC in the "haematopoietic and lymphoid" category than tumor somatic events occurring in clonal hematopoiesis genes. Although we utilized FM as our comparator, due to this assay being frequently used in clinical practice at our institutions, these results would be applicable for any NGS assay that utilized tumor specimens in the absence of a matched blood comparator.

Oncologic patients with clonal hematopoiesis have inferior outcomes, including increased risk for hematologic malignancies (5, 18, 19) and shorter overall survival (5). The mechanism for shorter survival is poorly understood (5). Studies from non-oncologic populations suggest an increased risk for cardiovascular mortality (20), which is likely more relevant for patients with curable malignancies (21).

Few studies have examined the phenomenon of clonal hematopoiesis mutations being detected during sequencing of solid tumor samples, although recent studies have reported detection of clonal hematopoiesis events in the setting of both germline and cell-free DNA testing (22–25). Paired blood is critical for confir-

mation given the high degree of overlap between clonal hematopoiesis genes (e.g., common: *TP53* and *SF3B1*, less common: *KRAS*, *NRAS*, *IDH1*, *IDH2*; refs. 5, 22) in both hematologic and solid cancers. We have demonstrated that patients with reported mutations in clonal hematopoiesis genes can have true clonal hematopoiesis (Table 2) or tumor somatic events in clonal hematopoiesis genes (Table 3).

Recent studies have outlined the difficulty of differentiating germline mosaic events from acquired somatic events, particularly in *TP53* and *PPM1D*, both recently established as common, therapy-related clonal hematopoiesis events (5, 26). Specifically, *PPM1D* mutations were originally thought to represent mosaic events leading to predisposition to breast and ovarian cancer (27). However, subsequent studies have elucidated that such events are enriched in the peripheral blood of patients with prior chemotherapy and radiotherapy exposure and instead represent acquired somatic events (5, 26, 28).

Limitations of this work include the inability to sort leukocytes from tumor specimens, which would be the "gold-standard" for

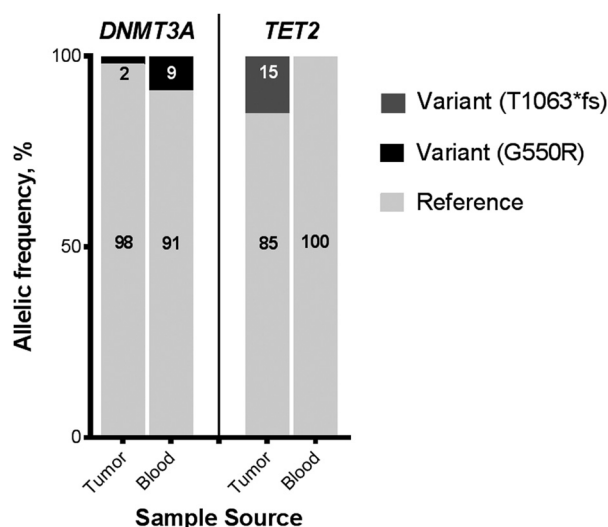


Figure 2.

Sequencing results for a patient with carcinoma not otherwise specified who had a *DNMT3A* mutation (left) in the blood sample and a *TET2* mutation (right) in tumor tissue, both of which were reported on FM NGS testing of the carcinoma biopsy. The *DNMT3A* mutation is seen at a lower level in the tumor sample when compared with blood, indicating clonal hematopoiesis. The *TET2* mutation is only observed in the tumor, confirming tumor somatic origin. VAFs for both tumor and blood specimens were obtained from UNCseq testing. Integrative Genomics Viewer images of these mutations are provided in Supplementary Fig. S1.

determining mutation origin. As a result, it is conceivable that clonal hematopoiesis-type mutations seen in the blood, which are either at lower or absent levels in the tumor tissue, could be due to circulating tumor cells (CTC) with novel mutations not seen in the parent tumor that was sequenced. However, given that the lowest blood VAF observed within this study was 1.4%, this would require an extraordinary amount of CTCs in the peripheral blood (2.8 cells out of 100 circulating cells, in the event of a fully clonal heterozygous mutation) to lead to this possibility. This level of CTCs is rarely if ever seen, as one CTC is estimated for every 1 million circulating leukocytes (29). Next, NGS methodology differed by institution, with MCC patients undergoing a more limited NGS panel for peripheral blood sequencing. As a result, it is possible that the frequency of clonal hematopoiesis mutations reported is an underestimation of the true prevalence, although notably all of the most commonly reported clonal hematopoiesis genes were examined by both institutions (with the exception of *PPM1D* because it was not examined due to the absence on FM reports). Finally, there was heterogeneity in degree of prior cytotoxic therapy that patients had been exposed to at the time of FM sequencing, with a trend toward increased clonal hematopoiesis events being reported in patients with prior chemotherapy/radiotherapy exposure. This finding suggests that clonal hematopoiesis events may more commonly be detected on tumor sequencing from pretreated patients compared with patients who are naïve to both chemotherapy and radiotherapy, consistent with findings from prior clinical and laboratory-based studies (5, 30).

In tumor-only sequencing, incorrect interpretation of results could have unfortunate clinical consequences. For example, a blood-derived RAS mutation [more commonly seen in chronic myelomonocytic leukemia (31) though occasionally seen in

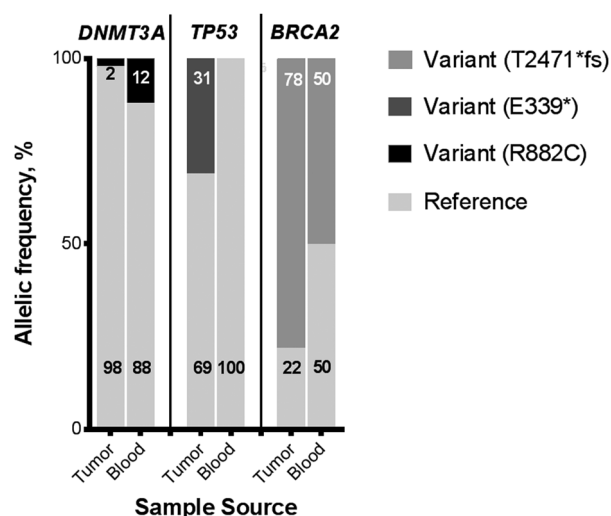


Figure 3.

Sequencing results for a patient with ovarian carcinoma who had *DNMT3A*, *BRCA2*, and *TP53* mutations reported on FM NGS testing of the carcinoma biopsy. The *DNMT3A* mutation (left) is seen at a lower level in the tumor sample when compared with blood, indicating clonal hematopoiesis. The *TP53* mutation (middle) was seen in the tumor tissue but absent in blood sample, consistent with a tumor somatic mutation. The *BRCA2* mutation (right) was seen at a variant frequency of 50% in the blood sample (and 78% in the tumor tissue), representing a germline variant. VAFs for both tumor and blood specimens were obtained from UNCseq testing. Integrative Genomics Viewer images of these mutations are provided in Supplementary Fig. S2.

clonal hematopoiesis (5, 22)] could impact clinical decision-making in colon cancer, resulting in withholding cetuximab or panitumumab in what was thought to be a RAS-mutant tumor. Alternatively, a clinician may extrapolate data from myeloid neoplasms to a solid tumor patient on efficacy of hypomethylating agents in *DNMT3A*-mutant states, when the variant is actually clonal hematopoiesis, such as in multiple patients in our cohort (Figs. 2 and 3; Supplementary Figs. S1 and S2; ref. 32).

Conclusions

Clonal hematopoiesis can be identified in unpaired NGS assays of solid tumor patients, and these suspicious mutations can be confirmed by paired blood sequencing. Improved understanding of NGS limitations allows for accurate application of NGS panels to personalized care of oncologic patients. Ultimately, caution should be exerted in interpretation of these assays so that accurate therapeutic selection is realized for individual patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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