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Authors

Bieg-Bourne, Cheyennedra C
Millis, Sherri Z
Piccioni, David E
et al.

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Next-Generation Sequencing in the Clinical Setting Clarifies Patient Characteristics and Potential Actionability

Cheyennedra C. Bieg-Bourne[1,3], **Sherri Z. Millis**[2], **David E. Piccioni**[3], **Paul T. Fanta**[3], **Michael E. Goldberg**[2], **Juliann Chmielecki**[2], **Barbara A. Parker**[3], and **Razelle Kurzrock**[3]

[1]San Diego State University, San Diego, CA

[2]Foundation Medicine, Cambridge, MA

[3]Center for Personalized Cancer Therapy and Division of Hematology and Oncology, UC San Diego Moores Cancer Center, La Jolla, CA

Abstract

Enhancements in clinical-grade next-generation sequencing (NGS) have fueled the advancement of precision medicine in the clinical oncology field. Here we survey the molecular profiles of 1,113 patients with diverse malignancies who successfully underwent clinical-grade NGS (236 to 404 genes) in an academic tertiary cancer center. Among the individual tumors examined, the majority showed at least one detectable alteration (97.2%). Amongst 2,045 molecular aberrations was involvement of 302 distinct genes. The most commonly altered genes were TP53 (47.0%), CDKN2A (18.0%), TERT (17.0%), and KRAS (16.0%), and the majority of patients had tumors that harbored multiple alterations. Tumors displayed a median of four alterations (range, 0–29). Most individuals had at least one potentially actionable alteration (94.7%), with the median number of potentially actionable alterations per patient being 2 (range, 0–13). A total of 94.2% patients exhibited a unique molecular profile, with either genes altered or loci within the gene(s) altered being distinct. Approximately 13% of patients displayed a genomic profile identical to at least one other patient; although genes altered were the same, the affected loci may have differed. Overall, our results underscore the complex heterogeneity of malignancies and argue that customized combination therapies will be essential to optimize cancer treatment regimens.

Keywords

Next-generation sequencing; personalized therapy; cancer genomics; precision medicine; biomarkers

Introduction

The emergence of next-generation sequencing (NGS) and other genomic technologies has fueled the field of precision medicine [1, 2]. Human genome sequencing resources and tools are advancing at a remarkable rate, and identification of genomic aberrations and their

Information for Corresponding Author: Cheyennedra Bieg-Bourne, UC San Diego Moores Cancer Center, 3855 Health Sciences Drive, La Jolla, CA 92093, Phone: 909-286-3928, Fax: 858-822-5380, cbourne09@gmail.com.

potential therapeutic actionability has become an area if great interest in clinical oncology and research. Furthermore, the affordability, reliability, and accessibility of genome sequencing have been major attractions in oncology because physicians now have the ability to potentially match patients to treatments based on their individual molecular profiles. Previous studies have revealed variable efficacy in matching patients to FDA-approved or experimental treatments (precision medicine), and this suggests a fundamental need for continued investigation in this area, especially in the cancer setting [3–6]. Indeed, cancer genome landscapes and precision oncology medicine continue to evolve as a result of the development of therapies that target cancer-specific alterations [1–17].

The complexity of genomic actionability in clinical oncology is rapidly being unveiled. For example, targeted agents for the protein products of *EGFR* mutations have been extensively studied in non-small cell lung cancer with impressive results [7–8]. *ERBB2* (HER2) positivity, frequently seen in invasive breast and gastric cancer, has been targeted effectively with a yield of improved outcomes [9–11, 15, 16]. However, other studies have suggested that some alterations cannot be targeted well, at least with single agents in the refractory metastatic setting. For example, the randomized SHIVA trial, in which the majority of patients received single-agent mTOR inhibitors or hormone modulators matched to PI3K/AKT/mTOR axis alterations or hormone receptor overexpression, respectively, failed to meet its endpoints [18].

Herein, we examined the landscape of molecular alterations from 1,113 patients treated at comprehensive cancer center whose tumors were interrogated by a uniform comprehensive NGS panel in a Clinical Laboratory Improvement Amendments (CLIA) laboratory. Our findings show that the majority of patients' tumors have one or more theoretically actionable alterations, most with multiple alterations, and almost all patients have a distinct genomic profile. The fact that metastatic tumors are both unique and complex speaks to the need for combination therapy regimens that are customized to each individual, and suggests that clinical trials may need redesigning in order to address the reality unveiled by molecular profiling.

Materials and Methods

Patients

Molecular profiles of 1,113 patients' tumors with advanced diverse cancers at the University of California San Diego Moores Cancer Center (La Jolla, CA) beginning in January 2013 were evaluated. Demographic information such as age, gender, and diagnosis were obtained from the physician during the time of requesting genomic testing. This study was performed and consents obtained in accordance with UCSD Institutional Review Board guidelines for de-identified databases. All research was conducted in accordance with the Declaration of Helsinki and Belmont Report guidelines

Next-generation sequencing

Patients underwent clinical-grade CLIA approved next-generation sequencing that interrogates the entire coding sequences of 236 to 404 cancer-related genes (Foundation

Medicine, Cambridge, MA) [Supplemental Table S1, S2, S3, and S4]. Tumors were assessed for genomic aberrations including multiple alterations per gene, rearrangement, deletion, amplification, and short variant. Genomic alterations detected included insertions, base substitutions, copy number alterations, and fusions/rearrangements. The methods for this type of comprehensive genomic profiling have been previously published, and extensive methods can be found elsewhere [19–23].

Molecular Testing

In total, 1,113 patients with metastatic cancer who had NGS were evaluated. Samples were submitted to a CLIA-certified, New York State-accredited, and CAP-accredited laboratory (Foundation Medicine, Cambridge MA) for NGS-based genomic profiling. The pathologic diagnosis of each case was confirmed by review of hematoxylin and eosin (H&E) stained slides and all samples that advanced to nucleic acid extraction contained a minimum of 20% tumor cells. Samples were processed in one of two broad protocols generally defined by solid tumors or hematologic cancers as previously described [20, 21]. For the current analysis, only characterized alterations were included (variants of unknown significance were excluded). For convenience, a brief description of the technology is provided below.

For solid tumors, DNA was extracted from formalin fixed paraffin embedded (FFPE) 10 micron sections. Adaptor-ligated DNA underwent hybrid capture for all coding exons of 236 or 315 cancer-related genes plus select introns from 19 or 28 genes frequently rearranged in cancer [Supplemental Table S1 and S2].

For hematological cancers, DNA and RNA was extracted from either FFPE 10 micron sections of peripheral blood or bone marrow aspirate. Adaptor-ligated DNA underwent hybrid capture for all coding exons of 323 or 404 genes cancer-related genes plus select introns from 24 or 31 genes frequently rearranged in cancer. For some samples, cDNA libraries prepared from RNA underwent hybrid capture for 265 genes known to be rearranged in cancer [Supplemental Table S3 and S4].

Captured libraries were sequenced to a median exon coverage depth of >500x (DNA) or ~3M unique reads (RNA) using Illumina sequencing, and resultant sequences were analyzed for base substitutions, small insertions and deletions (indels), copy number alterations (focal amplifications and homozygous deletions) and gene fusions/rearrangements, as previously described [20, 21]. Frequent germline variants from the 1000 Genomes Project (dbSNP142) were removed. To maximize mutation-detection accuracy (sensitivity and specificity) in impure clinical specimens, the test was previously optimized and validated to detect base substitutions at a ≥5% mutant allele frequency (MAF), indels with a ≥10% MAF with ≥99% accuracy, and fusions occurring within baited introns/exons with >99% sensitivity. Known confirmed somatic alterations deposited in the Catalog of Somatic Mutations in Cancer (COSMIC v62) are called at allele frequencies ≥1% [22, 23].

Statistical Analysis

Patient characteristics including patient frequencies, cancer histology, gender, and age were summarized using descriptive statistics. The sample size was determined by the total available number of patients who underwent genetic testing during the time period of this

study. Medians and ranges of genetic aberrations were calculated by standard techniques. Frequencies of molecular and genomic twins were calculated. All statistical analyses were performed using SAS software version 9.4 (SAS Inc., Cary, NC).

Molecular Profiles and Actionability

Genomic twins were defined as two or more patients with the same altered genes, even if the exact loci altered in the genes differed. In contrast, molecular twins were defined by identical altered genes, with both the loci altered and the type of anomaly being identical. An actionable aberration was defined as an alteration theoretically targetable by at least one FDA approved or investigational drug through the direct target or nearby pathway component.

Results

Patient characteristics

Overall 1,113 of 1,259 patients had adequate tissue for performing NGS. (The tissue inadequate rate for testing was 11.6%). A total of 31 diverse cancer types were observed in 1,113 patients (Table 1 and Figure 1). The most common tumors types were breast (n=142, 12.8%), glioma/glioblastoma (n=122, 11.0%), lung (n=120, 10.8%), and colorectal (CRC) (n=119, 10.7%). The most uncommon tumor types observed in the sample were B-cell lymphoma (n=7) and biliary tract cancers (n=6). The mean age at diagnosis was 54.8 years (95% CI, 53.9–55.7) and 593 patients (52.8%) were women. The majority of the patient population were non-Hispanic Caucasian (n=770, 69.2%), followed by other (n=233, 20.9%) and Asian (n=110, 9.8%).

Molecular test results and actionable aberrations

The median number of characterized alterations per patients was 4 (range, 0 to 29). The tumor types with the highest median number of alterations were skin (median, 9.5, range, 2–16), diffuse large b-cell lymphoma (DLBCL) (median, 6, range, 4–13), and melanoma (median, 6, range, 1–14). The 50 most common genes affected by characterized alterations are shown in Figure 2A [variants of unknown significance (VUSs) were not included]. The four most frequent alterations observed were in the following genes: *TP53* (n=523, 47.0%), *CDKN2A* (n =200, 18.0%), *TERT* (n=189, 17.0%), and *KRAS* (n=178, 16.0%). Amongst the 50 most common genes affected, *ZNF703*, *PIK3R1*, and *CREBBP* were the least frequent to be altered (Figure 2A). As shown in Figure 2B and Figure 2C, the most frequent gene alterations observed varied by tumor types. The most common types of molecular alterations were short variants (single base changes), amplifications, and deletions. Altogether there were 302 distinct genes that had at least one alteration and there was a total of 2,045 different molecular alterations.

For the vast majority of histologies, at least 50% of all individuals amongst each disease grouping had at least one actionable alteration (Tables 1 and 2) [24–44]. In total, 1,054 of 1,113 patients (94.7%) had at least one theoretically actionable alteration. The median number of potentially actionable alterations per patient was 2 (range, 0–13). Of 1,054 patients with at least one potentially actionable aberration (94.6% of all patients), 1,036 had

at least one alteration actionable by an FDA approved drug and 18 had at least one alteration actionable by an experimental drug (Figure 3).

Uniqueness of molecular profiles

Of 1,113 patients who underwent sequencing, only 31 (2.8%) had no detectable genomic alterations. Of 1,082 patients with at least one detectable genomic alteration, 1,048 (96.9%) had a unique molecular profile. Aside from two gastrointestinal stromal tumors, two papillary thyroid tumors, and four central nervous system (CNS) tumors with a single genetic alteration (*PDGFRA* D842V mutation, *BRAF* V600E, and *IDH1* R132H, respectively), no two patients with the same tumor type were molecularly identical. In addition, 26 patients with differing tumor histological types were also molecular "twins."

There were a total of 145 (13.0%) individuals with identical genomic profiles (including the 34 patients mentioned above with at least one molecular "twin"), and 111 additional individuals (10.0%) whose tumors harbored identical aberrant genes, albeit with differing alterations within the genes identified (genomic "twins"). Of the 111 individuals mentioned above, 32 patients (28.8%) had a matched genomic profile amongst patients with the same tumor type, while 79 of the patients (71.2%) had a genomically identical tumor to another patient with a different tumor histology.

Molecular Alterations in Specific Tumor Types

The most common tumor types that underwent genomic testing included breast cancers and gliomas.

Breast Cancer (N = 142 patients)—The most common alterations were in *TP53, PIK3CA, MYC, CCND1,* and *FGF19* genes (49%, 31%, 21%, 20%, and 19% respectively) (Figure 2B). There were 150 genes affected by alterations with 378 distinct molecular alterations. Overall, 99% of patients had at least one alteration; median (range) number of alterations per patients was 5 (0 to 17); 94% of patients had at least one potentially actionable alteration.

Gliomas/Glioblastomas (N = 122 patients)—The most common alterations were in *TP53, ATRX, PTEN, NF1,* and *EGFR* genes (50%, 29%, 23%, 20%, and 14%, respectively) (Figure 2C). There were 100 genes affected by alterations with 310 distinct molecular alterations. Overall, 98% of patients had at least one alteration; median (range) number of alterations per patients was 5 (0 to 13); 96% of patients had at least potentially actionable alteration.

Discussion

The most common tumor types tested paralleled the most common cancers (lung, breast and colorectal) with certain exceptions. For example, gliomas, a relatively rare cancer, were the second most frequent tumor type tested, probably reflecting our physician specialty interest in this area. In contrast, despite the high frequency of prostate cancer, only eight patient tumors underwent NGS, again probably reflecting physician orientation. The vast majority of patients who underwent tissue testing had at least one alteration (1082/1113 (97.2%))

(only 31 patients (2.8%) had no detectable alterations); these rates are similar to those reported in previous studies, albeit with considerably smaller numbers of patients [12, 16, 45]. However, other studies have reported a higher proportion of patients who had no detectable alterations [46, 47]; this could be due to variation in tumor histology types tested, stage of disease, or, most importantly, the interrogation of smaller panels of genes.

About 11.6% of our patients had inadequate tissue for genomic assessment. For patients with inadequate tissue, new technologies such as use of blood-derived cell-free DNA may provide an alternative non-invasive method to evaluate genomic alterations in individuals with cancer. In our patients with adequate tissue, the median number of alterations per tumor was four (range, 0 to 29). The gene most commonly altered was *TP53*, observed in 47% of patients (N = 523), which is a rate similar to that reported in previous studies [12, 13, 45, 48]. *CDKN2A, TERT* and *KRAS* were the next most frequently altered genes (18%, 17%, and 16% of patients, respectively) (Figure 2). These results highlight the importance of utilizing larger panels of genes, as smaller panels have failed to include *CDKN2A* and *TERT* [46]. Moreover, larger panels capture a more comprehensive view of genomic alterations.

The most common tumor types tested were breast and glioma. Among those with breast cancer, the gene most commonly altered was *TP53*, observed in 49% of patients (N=70/142). A similar rate has been observed in previous studies [48, 49]. Furthermore, among those with glioblastomas, the gene most commonly altered was *TP53*, observed in 50% of patients (N = 61/122). Figures 2B and 2C show the most common alterations in these tumor types; the rates are similar to those previously described [48–50].

Importantly, 1,054 of 1,113 patients (94.7%) had at least one theoretically actionable alteration. The median number of potentially actionable alterations per patient was 2 (range, 0–13). Previous studies with smaller cohorts of patients have also documented frequent potential actionability, with over 90% of patients having at least one potentially actionable alteration [16, 45]. In contrast, other researchers found substantially lower percentages of alterations and potentially actionable alterations [51–54], likely due to smaller panel sizes and interrogation of hot-spot regions, rather than the entire exon of the gene being interrogated. For instance, one large study with 2000 patients found that only 39% had at least one potentially actionable mutation. However, that study evaluated hot-spot (rather than NGS) gene panels, and the panel size ranged from 11 to 50 genes [54]. Taken together with our observations, it is apparent that larger gene panels are, unsurprisingly, associated with substantially higher rates of potential actionability.

Molecular and genomic uniqueness was demonstrated in the current study with only 34 of 1,082 patients (3.1%) with ≥ 1 genomic alteration having a molecular profile identical to at least one other patient (both the genes altered and the precise alterations within the genes were the same); only 13.4% (145 patients) had a genomic profile identical to at least one other patient (same aberrant genes but the precise alterations in the genes might differ). In addition, 302 different genes were abnormal in at least one patient and 2,045 distinct molecular alterations were seen across patients. Taken together with the data showing that most tumors harbored multiple aberrations (Table 1), these observations suggest that malignancies are complex, heterogeneous and unique [4, 12, 13, 14]. Since higher numbers

of matches per patient (higher Matching Scores) correlate with better outcome, the implications of this vast heterogeneity are that optimal treatment of patients will require innovative clinical trial designs with N-of-One matching strategies [16, 55].

There are limitations in our study. Several tumor histologies (such as glioblastomas) are disproportionately represented in our sample population due to selection bias, which can reflect the interest of specialty physicians. Importantly, the potential actionability of many of these alterations needs clinical validation. Finally, our previous study of 347 patients suggested that about 25% of our patients receive genomically informed treatment and that the matched patients had improved outcome [17]. The most common reason for failure to match was that the patient died, suggesting that genomic testing may need to be instituted earlier in the disease course. Other important obstacles that have been previously reported include medication and clinical trial access [17]. Analysis of therapy and outcome of the current cohort of patients is ongoing.

In summary, the use of NGS in personalized cancer therapy has produced intriguing findings in the clinical oncology setting. The genetic diversity of patients in oncology has been unveiled with the help of advanced tools such as NGS. Our results show that the majority of patients undergoing cancer treatment had at least one actionable alteration. Further, the hallmark of metastatic tumors is both their complexity and distinctiveness at the genomic level. These results suggest that precise targeting of tumors will require combination treatments that are customized to each cancer [55].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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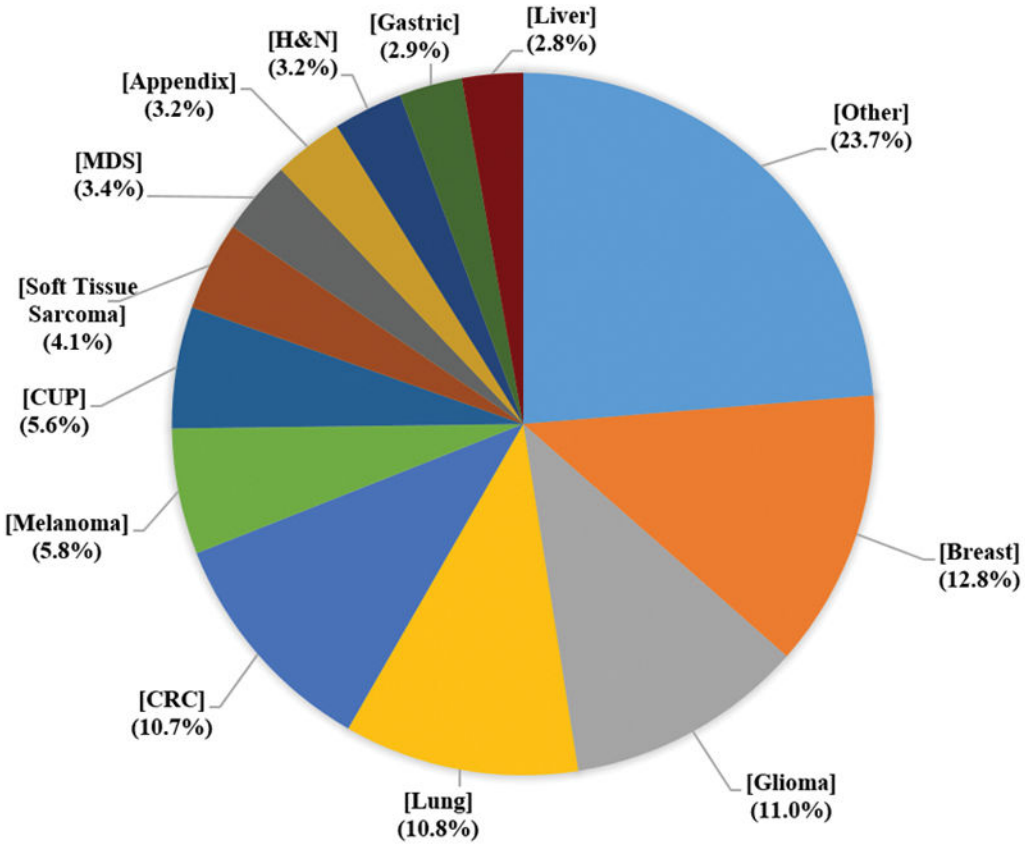

Figure 1. Distribution of tumors (*n* = 1113 patients)

This pie chart displays the frequency and distribution of tumors identified in patients (n=1113 total tumors). Disease groups include breast (n= 142), glioma/glioblastoma (n=122), lung (n=120), colorectal (n=119), melanoma (n=65), carcinoma of unknown primary (n=62), soft tissue sarcoma (n=46), myelodysplastic syndrome (n=38), appendiceal cancer (n=36), head & neck (n=36), gastric (n=32), liver (n=31), and all others (n=264). Other cancers include acute lymphocytic leukemia (n = 10), acute myeloid leukemia (n=20), b-precursor acute lymphoblastic leukemia (n=10), b-cell lymphoma (n=7), biliary (n=6), chronic lymphocytic leukemia (n=16), central nervous system non-glioma (n=9), carcinoma lymphocytic leukemia (n = 16), diffuse large B-cell lymphoma (n=12), lower GI (n=18), multiple myeloma (n=12), neuroendocrine (n=19), ovary (n=24), pancreas (n=25), prostate (n=8), renal (n=8), squamous cell carcinoma (n=12), skin (n=14), thyroid (n=26), and urinary (n=8).

Abbreviations: CUP = carcinoma of unknown primary; CRC = colorectal cancer H&N = head and neck; MDS = myelodysplastic syndrome.

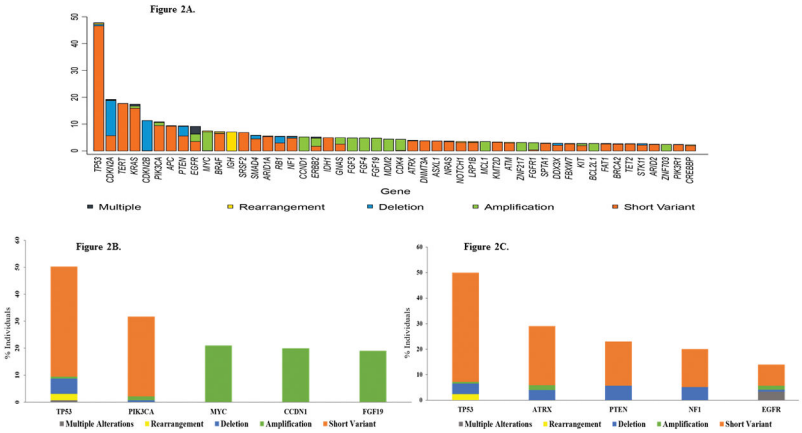

Figure 2.

Figure 2A. The percent of individuals with the 50 most common alterations. This bar graph represents the percent of individuals with the most common molecular alterations. *TP53* alterations were observed in the most number of patients. Types of alterations include multiple distinct alterations: rearrangement, deletion, amplification, and short variant.

Figure 2B. Distributions of alterations among breast cancer. This bar graph represents the 5 most common molecular alterations identified in 142 patients with breast cancer. The y-axis represents the distributions of patients harboring the genetic alteration.

Figure 2C. Distribution of alterations among glioma cancer. This bar graph represents the 5 most common molecular alterations identified in 122 patients with glioma cancer. The y-axis represents the distributions of patients harboring the genetic alteration.

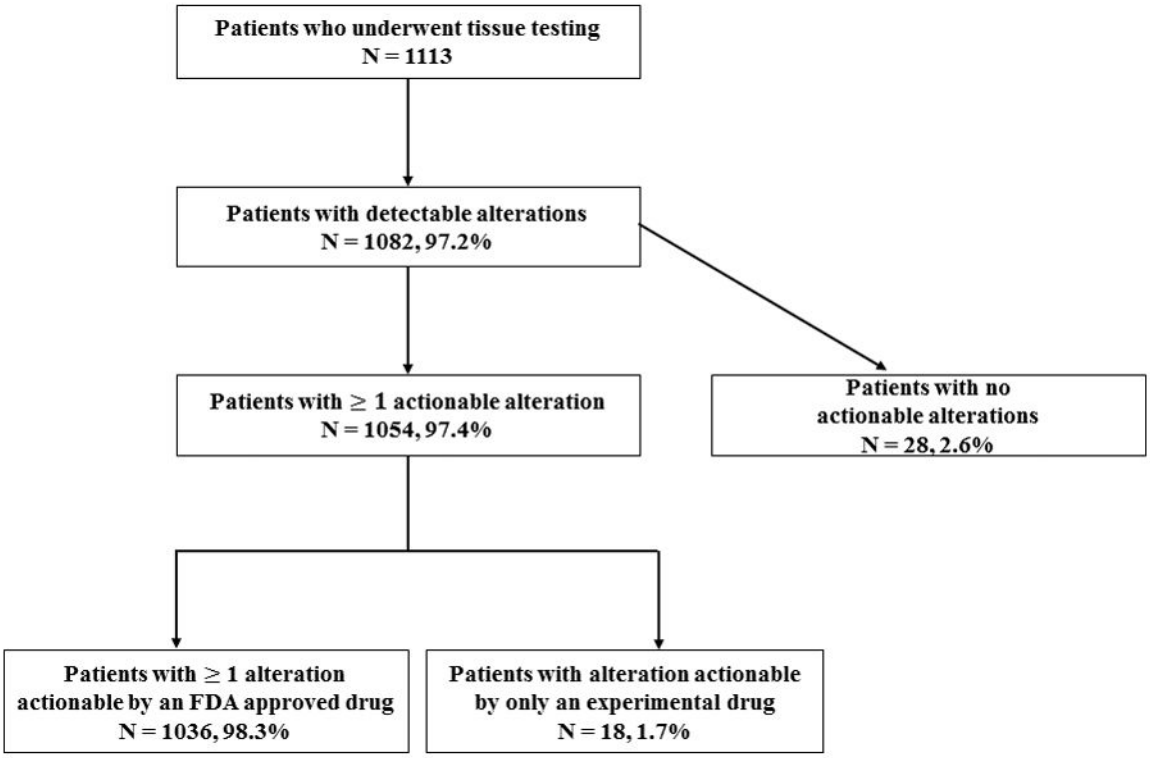

Figure 3. Flowchart of actionability in 1,113 patients who underwent next-generation sequencing (NGS) interrogation

Of all patients harboring at least one actionable alteration (N=1054, 97.4%), 98.3% of them were actionable by an FDA-approved drug and 1.7% were actionable by an experimental drug only.

Table 1

Alterations and actionability in 1,113 patients with 31 diverse cancer types.

Disease Grouping	No. of Individuals	Median No. of Alterations (Range)	Median No. of Actionable Alteration(s) (Range)	No. of Individuals with ≥ 1 Alterations (%)	No. of Individuals with ≥ 1 Actionable Alterations (%)
ALL	10	4 (1–5)	1.5 (1–3)	10 (100.0%)	10 (100.0%)
AML	20	3 (0–10)	1 (0–6)	19 (95.0%)	15 (75.0%)
Appendix	36	3 (0–9)	1 (0–5)	35 (97.2%)	35 (97.2%)
B-ALL	10	4.5 (1–13)	1 (1–4)	10 (100.0%)	10 (100.0%)
B-Cell Lymphoma (NOS)	7	5 (1–9)	1 (0–6)	7 (100.0%)	6 (85.7%)
Biliary	6	4 (1–8)	1.5 (1–4)	6 (100.0%)	6 (100.0%)
Breast	142	5 (0–17)	2 (0–6)	141 (99.3%)	138 (97.2%)
CLL	16	2 (0–11)	1 (0–7)	10 (62.5%)	10 (62.5%)
CNS Non-glioma	9	2 (1–5)	1 (1–2)	9 (100.0%)	9 (100.0%)
CRC	119	5 (1–29)	2 (1–13)	119 (100.0%)	119 (100.0%)
CUP	62	3.5 (0–20)	1 (0–6)	59 (95.2%)	55 (88.7%)
DLBCL	12	6 (4–13)	1.5 (0–7)	12 (100.0%)	11 (91.7%)
Gastric	32	5 (0–14)	1 (0–6)	31 (96.9%)	29 (90.6%)
Glioma	122	5 (0–13)	2 (0–7)	119 (97.5%)	117 (95.9%)
H&N	36	5 (0–16)	1 (0–6)	35 (97.2%)	35 (97.2%)
Liver	31	3 (1–8)	1 (0–4)	31 (100.0%)	28 (90.3%)
Lower GI	18	3.5 (1–15)	2 (1–4)	18 (100.0%)	18 (100.0%)
Lung	120	5 (0–16)	2 (0–7)	119 (99.2%)	116 (96.7%)
MDS	38	3 (0–7)	1 (0–4)	31 (81.6%)	31 (81.6%)
Melanoma	65	6 (1–14)	2 (1–7)	65 (100.0%)	65 (100.0%)
Multiple Myeloma	12	3 (1–8)	1.5 (1–6)	12 (100.0%)	12 (100.0%)
Neuroendocrine	19	3 (0–7)	1 (0–3)	14 (73.7%)	14 (73.7%)
Ovary	24	3 (1–14)	2 (1–8)	24 (100.0%)	24 (100.0%)
Pancreas	25	4 (1–12)	3 (0–5)	25 (100.0%)	24 (96.0%)
Prostate	8	2.5 (1–6)	1 (1–2)	8 (100.0%)	8 (100.0%)
Renal	8	3 (1–8)	1 (1–3)	8 (100.0%)	8 (100.0%)
SCC Other	12	5.5 (2–24)	1.5 (0–10)	12 (100.0%)	11 (91.7%)
Skin	14	9.5 (2–16)	2 (0–7)	14 (100.0%)	12 (85.7%)

Disease Grouping	No. of Individuals	Median No. of Alterations (Range)	Median No. of Actionable Alteration(s) (Range)	No. of Individuals with ≥ 1 Alterations (%)	No. of Individuals with ≥ 1 Actionable Alterations (%)
Soft Tissue Sarcoma	46	2 (0–14)	1 (0–6)	45 (97.8%)	44 (95.7%)
Thyroid	26	2 (1–6)	1 (1–2)	26 (100.0%)	26 (100.0%)
Urinary	8	9 (4–16)	4 (2–10)	8 (100.0%)	8 (100.0%)
All patients	**1113**	**4 (0–29)**	**2 (0–13)**	**1082 (97.2%)**	**1054 (94.7%)**

Abbreviations: ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; B-ALL = b-precursor acute lymphoblastic leukemia; CLL = chronic lymphocytic leukemia; CNS = central nervous system; CRC = colorectal cancer, CUP = carcinoma of unknown primary treatment; DLBCL = diffuse large b-cell lymphoma; H&N = head and neck; GI = gastrointestinal; MDS = myelodysplastic syndrome; No. = number; NOS = non-Hodgkin lymphoma; SCC = squamous cell carcinoma

Table 2

Examples of potentially actionable alterations, pathways impacted and targeted therapies.*

Potentially Actionable Gene	Examples of Pathways	Examples of Potential Targeted Therapies	References
APC	COX-2 inhibitor	celecoxib	[24]
ATM	PARP inhibitor	olaparib	[25]
BCL2L1	BCl2 inhibitor	venetoclax, omacetaxine	[26]
BRAF	MEK or BRAF inhibitor	vemurafenib, cobimetinib, trametinib, dabrafenib	[27, 28]
BRCA2	PARP inhibitor	olaparib	[25]
CCND1	CDK4/6 inhibitor, mTOR inhibitor	palbociclib, everolimus	[29]
CDK2NA	CDK4/6 inhibitor	palbociclib (controversial)	[29]
CDK4	CDK4/6 inhibitor	palbociclib	[29]
DNMT3A	DNA methyltransferase (DNMT) inhibitor (hypomethylating agents)	azacitidine, decitabine	[30]
EGFR	Anti-EGFR therapy	lapatininb, cetuximab, erlotinib, gefitinib, panitumumab, afatinib	[8, 31]
ERBB2	ERBB2 inhibitor	ado-trastuzumab emtansine, lapatinib, pertuzumab, trastuzumab, afatinib	[32]
FBXW7	mTOR inhibitor	temsirolimus, everolimus	[33]
FGF19	FGF receptor kinase inhibitor	Lenvatinib	[34]
FGF3	FGF receptor kinase inhibitor	Lenvatinib	[34]
FGF4	FGF receptor kinase inhibitor	lenvatinib	[34]
FGFR1	FGFR inhibitor	pazopanib, ponatinib, regorafenib	[34]
GNAS	MEK inhibitor	trametinib	[35]
IDH1	IDH1 mutant inhibitor	azacitidine, decitabine; AG-120)[a]	[36]
KIT	KIT inhibitor	nilotinib, pazopanib, everolimus, dasatinib, sunitinib, imatinib, sorafenib, temsirolimus, regorafenib, ponatinib	[37]
KRAS	MEK inhibitor	trametinib, cobimetinib	[28]
MCL1	Mcl-1 downregulation	sorafenib	[38]
MDM2	MDM2 inhibitor	ALRN-6924, SP-141[a]	[39]
MYC	BET inhibitor[a]	barasertib[a]	[40]
NF1	MEK inhibitor	temsirolimus, everolimus, trametinib	[28]
NOTCH1	Gamma-secretase inhibitor	PF-03084014[a]	[41]
NRAS	MEK inhibitor	trametinib	[28]
PIK3CA	mTOR inhibitor	temsirolimus, everolimus	[42]
PIK3R1	mTOR inhibitor	everolimus	[42]
PTEN	mTOR inhibitor	temsirolimus, everolimus	[42]
STK11	mTOR inhibitor	dasatinib, everolimus, temsirolimus, bosutinib	[43]
TET2	DNMT inhibitor	azacitidine, decitabine	[30]
TP53	WEE1 inhibitor[a] and VEGF/VEGFR inhibitor	bevacizumab	[44]

[a] Experimental drugs

* Actionable alterations from the 50 most common genes (Figure 2A).