Cancer Diagnostics and Molecular Pathology

Clinical Actionability of Comprehensive Genomic Profiling for Management of Rare or Refractory Cancers

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Disclosures of potential conflicts of interest may be found at the end of this article.

Abstract

Background. The frequency with which targeted tumor sequencing results will lead to implemented change in care is unclear. Prospective assessment of the feasibility and limitations of using genomic sequencing is critically important.

Methods. A prospective clinical study was conducted on 100 patients with diverse-histology, rare, or poor-prognosis cancers to evaluate the clinical actionability of a Clinical Laboratory Improvement Amendments (CLIA)-certified, comprehensive genomic profiling assay (FoundationOne), using formalin-fixed, paraffin-embedded tumors. The primary objectives were to assess utility, feasibility, and limitations of genomic sequencing for genomically guided therapy or other clinical purpose in the setting of a multidisciplinary molecular tumor board.

Results. Of the tumors from the 92 patients with sufficient tissue, 88 (96%) had at least one genomic alteration (average 3.6, range 0–10). Commonly altered pathways included p53 (46%), RAS/RAF/MAPK (rat sarcoma; rapidly accelerated fibrosarcoma; mitogen-activated protein kinase) (45%), receptor tyrosine kinases/ligand (44%), PI3K/AKT/mTOR (phosphatidylinositol-4,5-bisphosphate 3-kinase; protein kinase B; mammalian target of rapamycin) (35%), transcription factors/regulators (31%), and cell cycle regulators (30%). Many low frequency but potentially actionable alterations were identified in diverse histologies. Use of comprehensive profiling led to implementable clinical action in 35% of tumors with genomic alterations, including genomically guided therapy, diagnostic modification, and trigger for germline genetic testing.

Conclusion. Use of targeted next-generation sequencing in the setting of an institutional molecular tumor board led to implementable clinical action in more than one third of patients with rare and poor-prognosis cancers. Major barriers to implementation of genomically guided therapy were clinical status of the patient and drug access. Early and serial sequencing in the clinical course and expanded access to genomically guided early-phase clinical trials and targeted agents may increase actionability. *The Oncologist* 2016;21:1–11

Implications for Practice: Identification of key factors that facilitate use of genomic tumor testing results and implementation of genomically guided therapy may lead to enhanced benefit for patients with rare or difficult to treat cancers. Clinical use of a targeted next-generation sequencing assay in the setting of an institutional molecular tumor board led to implementable clinical action in over one third of patients with rare and poor prognosis cancers. The major barriers to implementation of genomically guided therapy were clinical status of the patient and drug access both on trial and off label. Approaches to increase actionability include early and serial sequencing in the clinical course and expanded access to genomically guided early phase clinical trials and targeted agents.

INTRODUCTION

Significant advances in sequencing technologies, "synthetic lethal" strategies to identify targets for loss of function in tumor suppressors, and pharmacologic innovations have increased both our understanding of the spectrum of alterations present in human cancers and development of therapeutics [1–5]. Although an ever-growing spectrum of genomic alterations is now therapeutically targetable, the clinical actionability of genomic testing is not known.

Widespread sequencing efforts demonstrate that most targetable genomic alterations are not malignancy specific, cannot be predicted by histology alone, are observed in "target-silent" tumors, and are useful as predictive or prognostic biomarkers [2, 6–8]. Furthermore, there is a need to identify mechanisms and alternative therapeutic strategies after development of drug resistance [9–13]. Genomic profiling may help circumvent these inherent clinical challenges by identifying drivers responsible for survival advantage and drug resistance. It also offers an attractive approach for rare tumors, given that insufficient numbers of patients are available to complete clinical trials, and establishment of guidelines for best clinical practice remains elusive.

Despite these advantages, the benefits and limitations of genomic sequencing in clinical practice requires prospective assessment. Several studies demonstrate that it is possible to obtain high-quality sequencing data from formalin-fixed, paraffin-embedded (FFPE) tissues [14, 15]. However, it remains unclear how often such genome characterization will actually lead to implemented, useful clinical interventions.

To understand the feasibility of clinical-grade genomic sequencing, we implemented a prospective, comprehensive genomic profiling protocol with an institutional, multidisciplinary molecular tumor board. Clinical action based on genomic profiling results was possible in 31% of patients in this study. Results suggest that this approach is feasible, has clinical actionability, and defines potential barriers to broader implementation.

METHODS

Study Participants

One hundred patients with rare or refractory tumors, evaluated at the Rutgers Cancer Institute of New Jersey, provided informed consent for a prospective trial for point-of-care tumor genomic profiling from April 2013 to December 2013 (supplemental online Fig. 1). Relevant clinical history, pathology, and genomic profiling results were reviewed at a formal molecular tumor board (MTB). Profiling results were discussed in the context of clinical course, tumor type, mutational frequency, role in cancer biology/behavior, preplanned therapy, and considerations for genomically guided therapy. Based on consensus recommendations, those with actionable alterations were referred for clinical trials, US Food and Drug Administration (FDA)-approved therapies (on- or off-label), and genetic counseling, if appropriate, and were followed for clinical course. The MTB did not provide recommendations for nontargeted therapies, and the therapeutic approach was ultimately the treating physician's choice. The protocol was approved by the Institutional Review Board of Rutgers Robert Wood Johnson Medical School.

Comprehensive Genomic Profiling

FFPE tissue was sent to Foundation Medicine (Cambridge, MA, https://www.foundationmedicine.com/) for next-generation sequencing using the Clinical Laboratory Improvement Amendments (CLIA)-certified FoundationOne platform [15]. Targeted sequencing of the entire coding sequence was done for 236 genes and 47 introns of 19 genes involved in fusions at a depth of ×500 to ×1,000. Alterations included point mutations, deletions, amplifications, duplications, insertions, rearrangements, and splice variants that were identified as known or likely pathogenic changes, as reported by the FoundationOne assay. Variants of unknown significance (VUS) and synonymous single-nucleotide polymorphisms (SNPs) are not included in tables depicting alterations in tumors. Calculations for "per tumor alterations" excluded VUS and SNPs.

Genomic Profile Clustering

For unsupervised clustering, each of the 92 samples had at least one detected genomic alteration representing only 118 of the possible 236 genes evaluated. A bidirectional, agglomerative hierarchical clustering with Jaccard's distance measure was performed. Choice of distance measure was motivated by the sparseness of the data but also allowed analysis to ignore a type and a number of genomic alterations within a gene or a pathway for a given sample. Data were treated as binary (0 = no alteration, 1 = alteration). The clustering and visualization were done with a modified version of "heatmap.2" from the R package "gplots" (R Foundation for Statistical Computing, Vienna, Austria, https://www.r-project.org/foundation/) [16]; the corresponding dissimilarity structure was produced by "dist" with binary distance measure.

RESULTS

Genomic Spectrum Reflects Both Intertumoral and Tumor Type–Specific Commonalities

Four pediatric and 96 adult patients with rare histology or refractory cancers underwent tumor genomic profiling for a varied spectrum of epithelial and mesenchymal origin tumors (supplemental online Figs. 1, 2; supplemental online Table 1). At enrollment, 91% of patients were stage IV and had received a mean of 2.5 prior therapies. Sixty-five percent of patients were alive with disease at a year or more after testing, with a mean overall survival of 129 days after enrollment. Of the 100 patients enrolled, eight did not have genomic profiling because of inadequate tissue amount, poor tissue/DNA quality, or the patient came off study. Ninety-two patients had successful genomic analysis, with 88 (96%) having at least one genomic alteration; the average number of alterations per tumor was 3.6 (range 0–10) (Fig. 1A), with urothelial and endometrial cancers displaying the highest mutational burden (supplemental online Fig. 2).

The most commonly altered genes included *TP53* (41%), *CDKN2A/B* (cyclin-dependent kinase inhibitor 2A/B) (22%), *KRAS* (16%), *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit a) (15%), *PTEN* (phosphatase and tensin homolog) (14%), and *BRAF* (7%). There was also a large set of low-frequency but actionable changes in tyrosine kinase genes (*ALK* [anaplastic lymphoma kinase], *KIT* [stem cell growth factor receptor], *ERBB2* [erb-B2 receptor tyrosine kinase 2], *FGFR4* [fibroblast growth factor receptor 4], *KDR* [vascular endothelial growth factor receptor 2], *MET* [hepatocyte growth factor receptor], *NTRK1* [neurotrophic tyrosine kinase receptor 1], *PDGFR* [platelet derived growth factor receptor]) and tumor suppressor genes (*BRCA1*, *BRCA2* [breast cancer type 1, breast cancer type 2]). The panel of gene alterations was sorted into functional pathways (supplemental online Tables
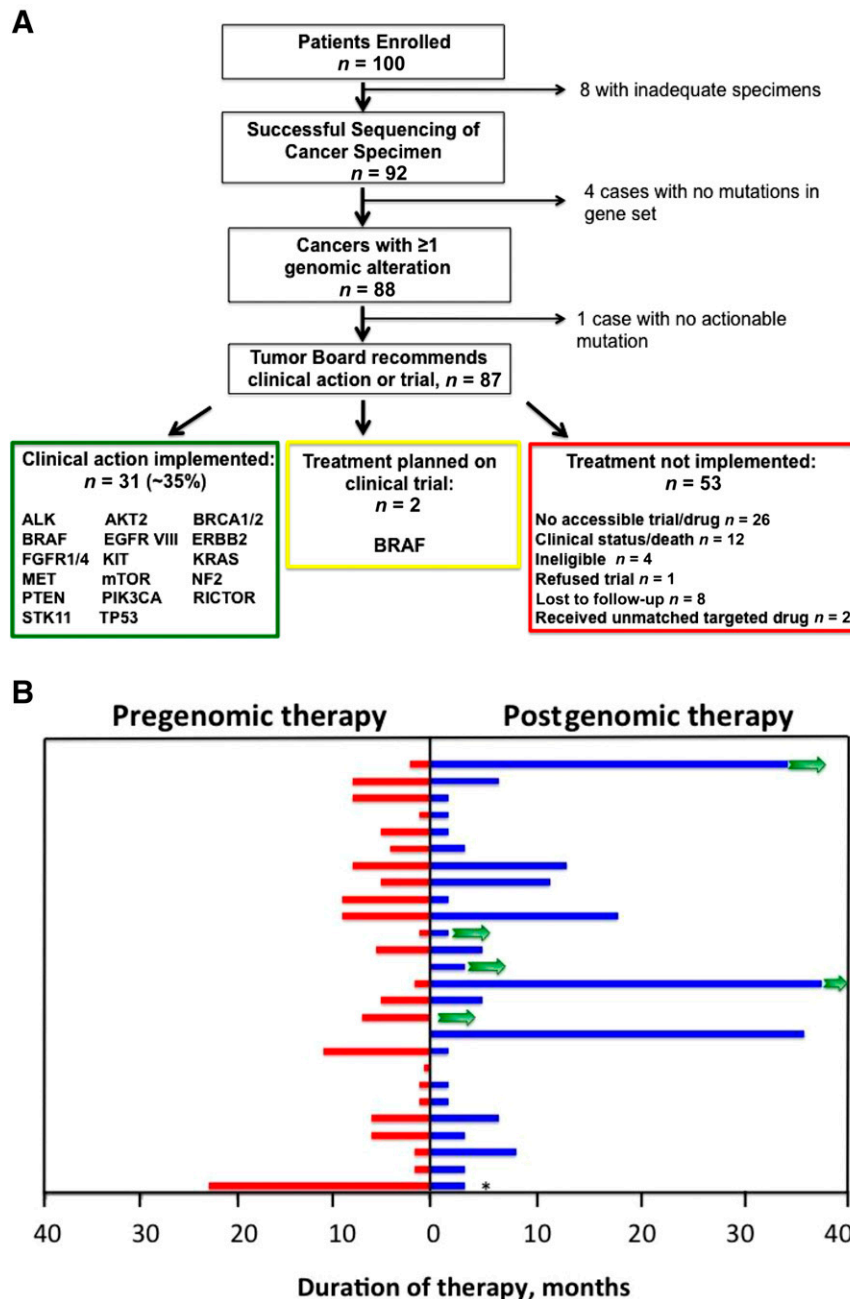
A:

Patients Enrolled
n = 100

→ 8 with inadequate specimens

Successful Sequencing of
Cancer Specimen
n = 92

→ 4 cases with no mutations in
gene set

Cancers with ≥1
genomic alteration
n = 88

→ 1 case with no actionable
mutation

Tumor Board recommends
clinical action or trial, n = 87

Clinical action implemented:
n = 31 (~35%)

ALK	AKT2	BRCA1/2
BRAF	EGFR VIII	ERBB2
FGFR1/4	KIT	KRAS
MET	mTOR	NF2
PTEN	PIK3CA	RICTOR
STK11	TP53	

Treatment planned on
clinical trial:
n = 2

BRAF

Treatment not implemented:
n = 53

No accessible trial/drug n = 26
Clinical status/death n = 12
Ineligible n = 4
Refused trial n = 1
Lost to follow-up n = 8
Received unmatched targeted drug n = 2

B:

Pregenomic therapy | Postgenomic therapy

Duration of therapy, months

Figure 1. Tumor genomic profiling consort diagram and patient outcomes. **(A):** Comprehensive genomic profiling of tumors from 100 patients with rare or refractory cancers. Time from genomic testing to generation of formal recommendations averaged 3–4 weeks. Clinical action included enrollment in a therapeutic clinical trial, FDA-approved therapy, off-label use of approved therapy, discontinuation of ineffective targeted therapy, germline mutation testing, and diagnostic reclassification. Genes with alterations for which clinical action was implemented are listed in the respective categories. Two patients are being screened for participation in clinical trial with targeted therapy. Two patients with action implemented had targeted therapy prescribed but were then lost to follow-up. **(B):** Duration of therapy in individual patients before genomic profiling and with targeted therapy (not significant). Comparisons are for nontargeted therapy before genomic profiling (red) and duration of targeted therapy after genomic profiling (blue). Green arrows indicate patients with ongoing therapy. Last patient was on combined therapy with targeted agent, which was discontinued (∗) at 5 months for side effects. Patient has ongoing response with monotherapy.
Abbreviation: FDA, U.S. Food and Drug Administration.

2, 3), including the most common: p53 (46%), RAS/RAF/MAPK (45%), PI3K/AKT/mTOR (35%), transcription factors (31%), cell cycle (30%), and receptor tyrosine kinases (RTKs) (27%; excluded fibroblast growth factor [FGF] pathway alterations) (Fig. 2A). Functional pathways, such as p53, RAS/RAF/MAPK, PI3K/AKT/mTOR, cell cycle, FGFR/FGF, Wnt (wingless-related integration site)/β-catenin, and DNA repair have profiles in which one or more

altered genes are enriched (Fig. 2B). In contrast, transcription factors and other RTKs have a wider spectrum of affected genes but may reflect tissue specificity based on tumor type. Furthermore, some tumor subtypes correlated with a higher probability of harboring specific alterations (e.g., *BRAF*-altered papillary thyroid cancers).

As tumor subtype may reflect mutational landscape and this study profiled diverse tumor types, unsupervised clustering of
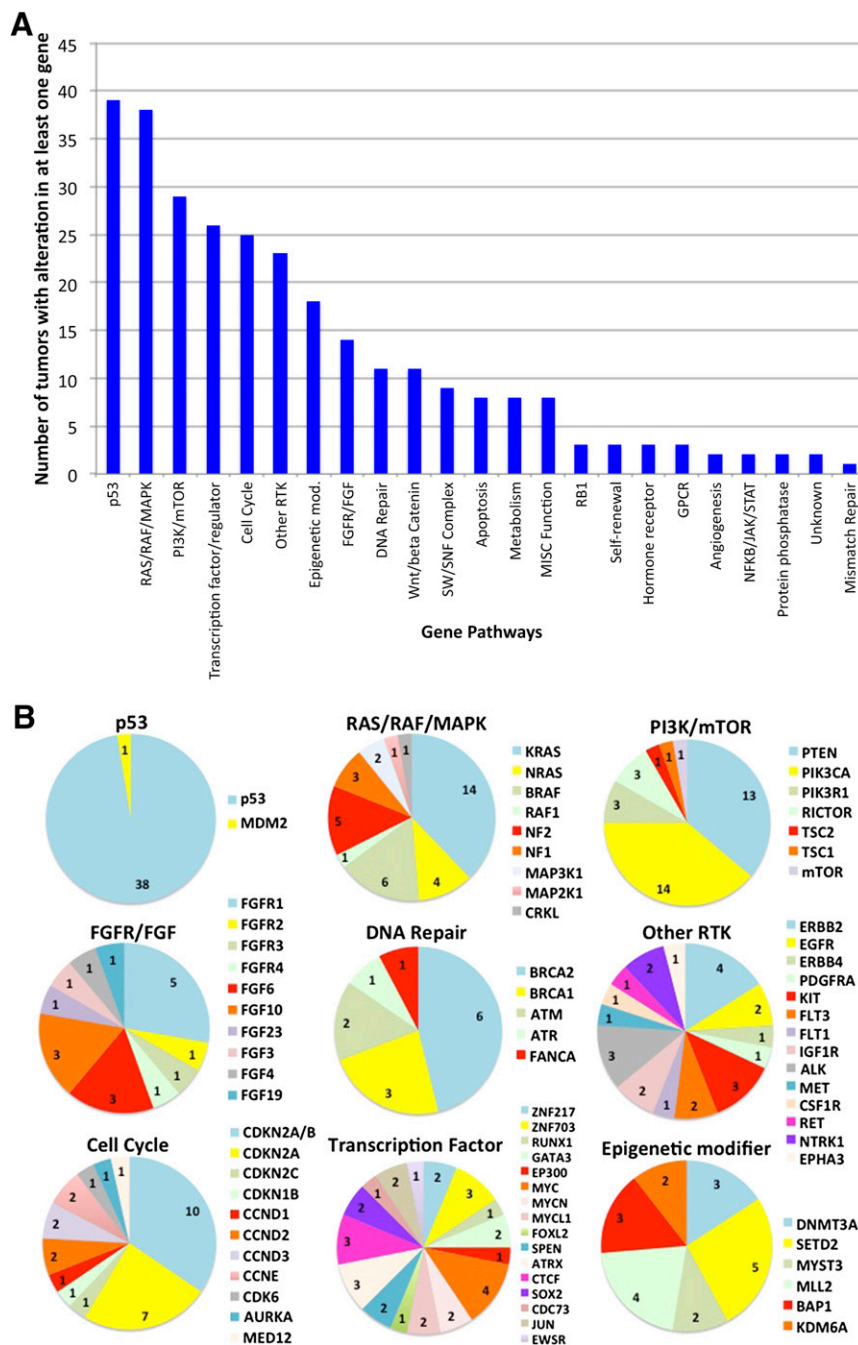
Figure 2. Mutational landscape identified in 92 different tumors based on functional pathways. **(A):** Types of alterations and number of alterations were reflective of tumor subtypes profiled. **(B):** For 9 of the 10 most frequent pathways, the frequency of genes with alterations within those pathways occurring is depicted. Wnt/β-catenin is not shown but had only two genes represented: *APC* (adenomatous polyposis coli) ($n = 8$) and *MSH6* (MutS Homolog 6) ($n = 1$). Numbers represent the frequency of alterations affecting that gene.

tumors with at least one genomic alteration was performed based on gene (Fig. 3A) and pathway (Fig. 3B) alterations. Notably, some tumor subtypes clustered more closely together than tumors of differing subtypes. Serial specimens clustered more closely to prior specimens than to similar tumors from other patients, suggesting preservation of specific patterns of mutations. Triple-negative breast cancers clustered closely with ovarian cancers (primarily high-grade serous), confirming prior data that these tumors may have similar underlying biology. Pathway clustering demonstrated that, despite diversity in specific mutations, certain pathways are commonly altered in diverse tumors (e.g., MAPK, PI3K), and these

paired pathway anomalies are relevant for future design of multidrug approaches.

Genomic Profiling Alters Clinical Management

The majority of tumors had alterations for which the MTB had recommended action (Fig. 1A). Of the 88 cases with identified alterations, 87 had clinically relevant alterations. The critical question was, how many of these patients with potentially actionable genomic alterations identified actually had a change in clinical management based on sequencing results? Thirty-one patients had implementation of genomically guided therapy. Another two

Figure 3. Hierarchical clustering of tumors with at least one genomic alteration by gene **(A)** and functional pathway **(B)**. Tumor subtypes are represented in colored text below heat map and with corresponding boxes above heat map. In **(A)**, box color (rows) within heat map depicts alteration in gene by type of genomic alteration; in **(B)**, it depicts alteration in genes in functional pathway by type of genomic alteration. Alteration key: red, amplification; green, mutation; blue, deletion; purple, rearrangement; black, splice; orange, fusion; white, no alteration. The remaining colors are multiple alteration subtypes. Numbers in parentheses represent specimen number, and a, b, and c represent serial specimens.

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6   Utility of Tumor Molecular Profiling

Figure 3. Continued.

patients are under screening for genomically guided clinical trials. Thus, 35% of patients with actionable alterations, and ~31% of study patients overall, received genomically guided therapy.

Management decisions based on genomic results (Table 1) included referral to clinical trials, use of FDA-approved drugs, and off-label use of targeted therapy when clinical trials were unavailable (e.g., vemurafenib for *BRAF* V600E-mutated papillary thyroid cancer, lapatinib for *ERBB2*-mutated urothelial cancer, platinum for *BRCA1/2* mutations). Examples of clinical responses included an intestinal carcinoid with *FGFR4* mutation with resolution of bulky mediastinal lymphadenopathy and stability of retroperitoneal lymph nodes with sorafenib for 23 months; and an ongoing, 21-month stable response to everolimus in a heavily pretreated male breast cancer with 8p11 amplification. A patient with metastatic papillary renal cell carcinoma with *MET* H1094L had

objective clinical improvement within 2 months of initiating crizotinib and is described elsewhere [17].

For patients receiving genomically guided therapy, the mean number of treatments implemented postsequencing was 1.5, whereas patients who did not receive targeted therapy despite target identification had a mean of only 0.3 treatments ($p = .0001$). Of patients who received targeted therapy, duration on targeted therapy was 7.6 months (Fig. 1B) compared with 4.2 months for patients not receiving any targeted therapy (unpublished data, $p = .07$). However, this includes patients who did not receive any therapy postsequencing. For those patients who were able to receive any nontargeted therapy, duration on nontargeted therapy was 6.1 months. This was not statistically different from the duration for those who received targeted therapy. One patient was on combined therapy including a targeted agent, but the latter had to be discontinued at 5 months because of significant side effects

Table 1. Genomic alterations for which clinical action was implemented.

Tumor type	Genomic Alteration	Clinical action
Gallbladder	*BRCA2* Q373*	Germline testing
		Continued therapy, platinum salt
		Recommendation for enrollment in PARP inhibitor trial
Colon	*ALK* R214H	Crizotinib
	FGFR1 amplification	Regorafenib
Iodine-refractory recurrent papillary thyroid	*BRAF* V600E	Vemurafenib: documented response, clinical improvement lasting several months
Renal cell, papillary	*MET* H1094L	MET inhibitor crizotinib with clinical improvement within 2 months and duration of response 5 months
Urothelial	*ERBB2* S310F	Lapatinib with transient clinical response
Platinum-resistant ductal carcinoma breast, TNBC	*BRCA1* E23fs*17	Etoposide
Krukenberg tumor, likely upper GI source	*EGFR* VIII (epidermal growth factor receptor variant)	Afatinib
Carcinosarcoma (had initial histologic diagnosis of GIST)	Lacked *KIT* or *PDGFR* mutation	Discontinued imatinib
	BRCA2 T3033fs*12	Patient died suddenly before starting platinum-based therapy
Pancreatic	*PIK3CA* P104R; *FBXW7* R479Q	Dual PI3K and mTOR inhibitor
GIST	*BRAF* V600E, Lacked *KIT* or *PDGFR* mutation	Discontinued imatinib; started regorafenib on recurrence
Colon	*PIK3CA* E545A; *PTEN* D24fs*17	Dual PI3K and mTOR inhibitor
Endometrial, endometrioid	*PIK3CA* H1047R; *PTEN* Y336*	Everolimus plus exemestane
GIST	*KIT* V559A	Regorafenib, sorafenib (prior imatinib, sunitinib)
Carcinoid	*FGFR4* D425N	Sorafenib
Colon	*ERBB2* amplification	Regorafenib (trastuzumab denied by insurance)
Phyllodes	*FGFR1* N546K	Pazopanib
Breast cancer	*FGFR1* amplification	Everolimus
Cervical cancer (HPV-associated)[a]	*PIK3CA* E545K; *RET* (rearranged during transfection) V706M	AKT inhibitor
Ductal carcinoma breast, TNBC[a]	*BRCA2* N863fs*11, S1982fs*22	PARP inhibitor
Ovarian serous carcinoma[a]	*BRCA1* Q172fs*62; *BRCA2* truncation exon 11	PARP inhibitor
Ovarian serous carcinoma[a]	*BRCA1* Q1240*	PARP inhibitor; cisplatin
GE junction adenocarcinoma	*ERBB2* amplification	Capecitabine plus lapatinib
Merkel cell	*MTOR* amplification	Temsirolimus
Colon	*KRAS* Q61H	Regorafenib
GIST	*KIT* L576_P577insPYDL	Imatinib
Mesothelioma	*NF2* (neurofibromin 2) Q456fs*39	Pemetrexed plus temsirolimus
Anaplastic oligodendroglioma	*PIK3CA* H1047R	Temozolomide plus everolimus
Colon	*KRAS* G12V	Regorafenib
Pancreatic	*STK11* (liver kinase B1) W308fs*8	Pemetrexed
Renal cell, solid tubular/papillary type	*NF2* splice site 1574+2T > A	AKT inhibitor
Fallopian tube	*TP53* K132Q	Carboplatin
Approved for recommended therapy but died before administration		
Melanoma	*CCDC91* (coiled-coil domain-containing protein 91)-*BRAF* fusion	Trametinib
Urothelial, micropapillary	*AKT2* amplification, *FGFR1* amplification, *RICTOR* (rapamycin-insensitive companion of mammalian target of rapamycin) amplification	Temsirolimus

[a]Initiated targeted therapy on genotype-agnostic trial before profiling results of genomic profiling.
Abbreviations: GE, gastroesophageal; GI, gastrointestinal; GIST, gastrointestinal stromal tumor; HPV, human papilloma virus; mTOR, mammalian target of rapamycin; PARP, poly(ADP-ribose) polymerase; PI3K, phosphatidylinositol 3-kinase; TNBC, triple-negative breast cancer.
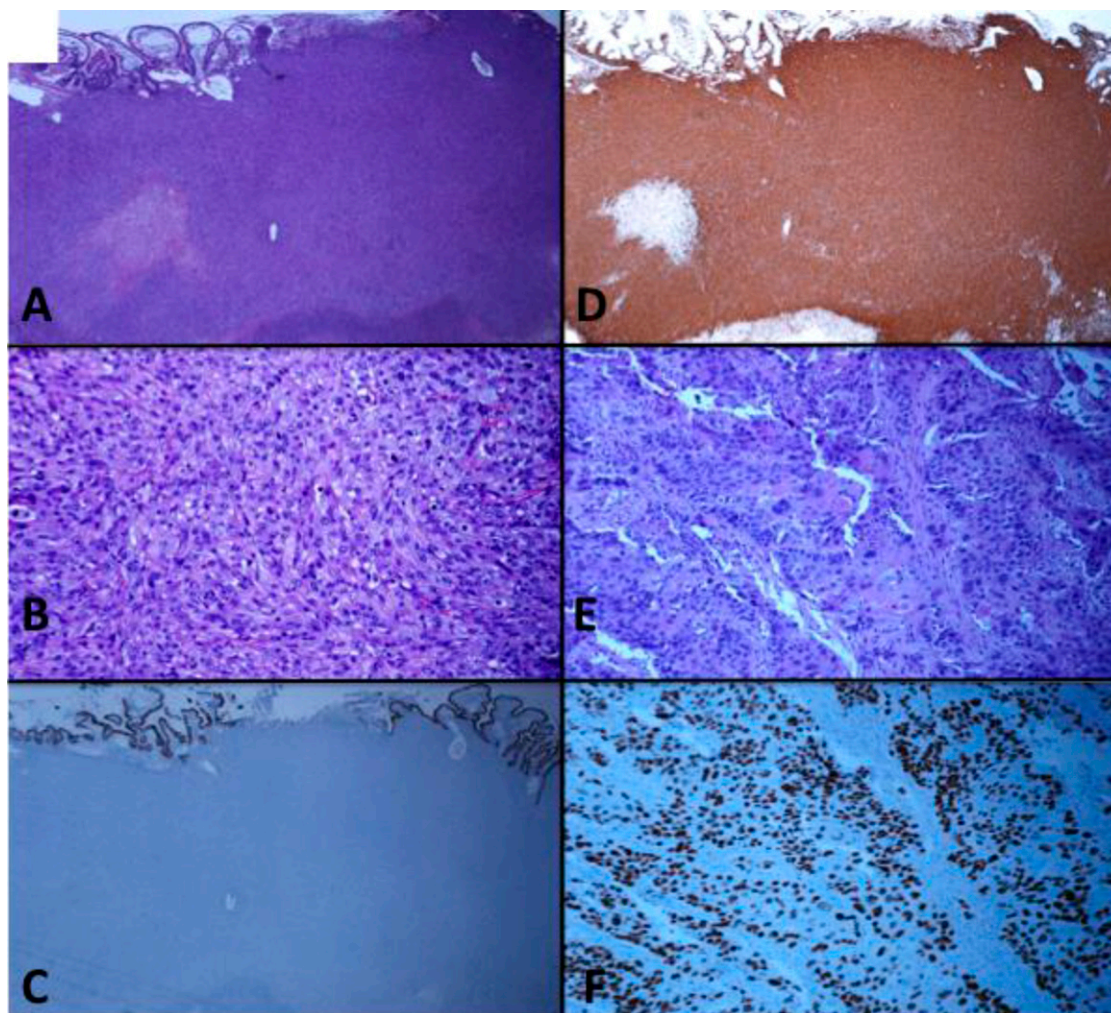
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Figure 4. Genomic profiling aids in the diagnostic analysis of two anatomically distinct tumors from a single patient and ultimately suggests that tumors arose from the same precursor. Abdominal mass **(A–D)** shows a high-grade, vimentin-positive sarcoma with predominant epithelioid appearance and focal necrosis. **(A, B):** hematoxylin and eosin, low and high power, respectively; **(C, D):** low power, pancytokeratin and vimentin, respectively. The ovarian mass **(E, F)** shows a poorly differentiated, p40-positive squamous cell carcinoma. **(E):** Hematoxylin and eosin, high power. **(F):** p40, low power. CD117 expression was absent (data not shown).

(Fig. 1B). The patient has ongoing response with nontargeted monotherapy. Within the group receiving postgenomic, nontargeted therapy, two patients had a 24-month duration of this therapy, both for the treatment of breast cancer. Of these, one had a triple-negative breast cancer with a *BRCA2* variant, for which she received a platinum-based regimen on clinical trial. The second patient had a hormonally responsive breast cancer and was receiving a combination of anastrozole plus fulvestrant as second-line therapy in the metastatic setting. Inclusion of these patients substantially increased the duration on nontargeted therapy, from 4.8 months to 6.1 months. Therefore, the ability to document increased duration of postgenomic therapy compared with pregenomic therapy is highly dependent on tumor type as well as availability of effective standard and targeted therapies for specific tumor types. As there are currently five patients with ongoing treatment with targeted therapy, the mean duration on genomically guided therapy would be expected to increase with continued follow-up.

Fifty-three patients with potentially actionable genomic changes did not receive genomically guided therapy primarily because of lack of availability of a clinical trial or access to an FDA-approved drug ($n = 26$). An additional 12 patients did not receive treatment because of deteriorating clinical status. The remaining patients were ineligible for clinical trials because of comorbid conditions ($n = 4$), refused trial participation ($n = 1$), received nonmatched targeted drugs ($n = 2$), or were lost to follow-up ($n = 8$).

Tumor Genomic Profiling Augments the Cancer Diagnostic Armamentarium

Several patients with synchronous, histology-discordant tumors at different sites had genomic analysis of all sites. A diagnostically challenging referral of a synchronous, small bowel tumor and pelvic mass with unique histologic appearance initially led to diagnosis of atypical gastrointestinal stromal tumor and ovarian squamous cell carcinoma, respectively, despite noticeably absent CD117 expression (Fig. 4). The former represented a high-grade sarcoma (Figs. 4A–4D), whereas the ovarian mass showed squamous cell carcinoma (Figs. 4E, 4F). Genomic profiling at both sites revealed identical alterations in six genes (supplemental online Table 2). Of six alterations private to the pelvic mass, four occurred within an amplicon containing the putative driver gene, *CCND1* (cyclin D1). The spectrum of alterations was highly suggestive of both arising from the same Müllerian precursor,

displaying both sarcomatous and carcinomatous components indicative of carcinosarcoma.

An additional patient initially presented with a pancreatic adenocarcinoma and a synchronous mucinous lung cancer that was believed to be a second unrelated primary. Sequencing of both tumor specimens showed identical mutations in *KRAS* and *TP53*, again suggesting that these arose from pancreatic adenocarcinoma as a common precursor. Had this information been known at diagnosis, the patient may have been spared surgical resection.

Serial Genomic Sequencing Demonstrates Molecular Evolution

Nine patients had analysis of serial samples at different time points, with a mean concordance rate of 59% (range, 0%–100%) (supplemental online Table 2). For example, a patient with metastatic, triple-negative breast cancer had analysis of three specimens: primary site and brain metastasis, both of which responded to platinum-based chemotherapy, and recurrent, platinum-resistant cancer that progressed locally (supplemental online Fig. 3). All specimens from this patient had a core set of mutations (i.e., *BRCA1*, known germline), *TP53*, *PIK3CA*, and *RUNX1-ATLN* (runt-related transcription factor 1; atlastin GTPase 1) fusion. Both the brain metastasis and recurrent tumor had additional private mutations, suggesting evolution from a common precursor. Concordance was higher between the two breast sites (80%) than with the brain specimen (50%). Notably, the brain metastasis carried an *ERBB2* amplification not observed in either breast specimen. No reversion mutation/internal deletion was noted in *BRCA1*, despite this known mechanism of resistance for poly(ADP-ribose) polymerase and platinum agents [18–20]. A complex genomic event cannot be ruled out. Thus, serial analysis of specimens lends insight into tumor evolution and heterogeneity. This finding also underscores the importance of analyzing recurrent metastatic disease that progressed after initial response to targeted agents, as potentially actionable targets may be gained or lost as a result of tumor drift or molecular evolution, particularly under selection pressure.

Tumor Sequencing Can Lead to Germline Testing

Genomic sequencing may raise the possibility of germline mutation testing and is disclosed to participants during consent. At the MTB, allele frequency and tumor purity are routinely analyzed when the possibility of germline variants is raised. In this cohort, previously unknown alterations in *BRCA1/2* were suggested by tumor sequencing in four patients. In two of these patients with *BRCA2* mutations, further testing showed no evidence of a germline *BRCA2* mutation. Two other patients were confirmed to have germline *BRCA1/2* mutations and received genetic counseling.

Known alterations in *BRCA1* or *BRCA2* were confirmed in four additional cases without evidence of reversion mutations. One patient with an activating *MET* mutation in a papillary renal cell cancer was also informed of the possibility of germline mutation. Overall, 10% of tumors from this cohort exhibited alterations in known cancer susceptibility genes. Germline testing was recommended based on tumor genomic profiling and resulted in identifying three individuals with no prior knowledge of their carrier status.

Tumor genomic profiling without matched normal tissue has the potential to mistakenly identify germline variants as somatic tumor mutations. Recent reports have suggested that paired normal/tumor tissue analysis is necessary to eliminate false-positive calls of germline variants [21, 22]. In this study, bioinformatics analysis using allele frequency, estimated tumor purity, and ploidy was used to flag potential germline variants [23]. Of 317 variants identified as pathogenic or likely pathogenic, 29 were flagged as potentially germline (supplemental online Table 4). Of these, nine variants (e.g., in *BRCA1/2*) were clearly identified as likely germline at the MTB discussion and referred to genetic counseling. Several variants (in *RB* and *TP53*) were eliminated as germline variants on further manual analysis. The remaining potential germline variants result in a potential false-positive rate of 5.7% (18 of 317). Analysis of measured allele frequency, tumor purity, and read depth data by our MTB allows identification of many of these potential germline variants and is now routinely done. As such, our MTB recommends genetic testing for identified potential germline variants when placed in the context of tumor type, personal history, and family history.

DISCUSSION

Advances in sequencing technologies have led to the ability to perform clinical-grade, high-depth sequencing of cancer gene panels to identify clinically relevant tumor genomic alterations. To determine its clinical actionability, we implemented a comprehensive genomic profiling protocol with an integrated institutional, multidisciplinary MTB. We successfully performed genomic profiling in 92% of rare or poor-prognosis cancers from patients who had exhausted standard therapies, and this testing led to actual change in clinical management in 31% of patients. Importantly, our study also identified recurrent and unsuspected targetable genomic alterations, consistent with findings by other groups [24–33]. Some differences between studies may reflect the spectrum of frequency of tumor types as well as methodology and gene coverage in sequencing panels.

The main limitation for genomic profiling not leading to clinical action was lack of drug access/availability of clinical trials, similar to a recent report [31]. In some cases, clinical trials were available but included only a specific tumor subtype, suggesting a need to significantly increase access through eligibility. However, mutational profiles should be placed in context of the biology of cell/tissue origin, as exemplified by differences in response to vemurafenib for *BRAF* V600E mutant melanoma versus *BRAF* V600E mutant colorectal cancer. *BRAF* V600E remains a genomic target and driver in colon cancer; however, to effectively target it in colon cancer, feedback pathways mediating activation of EGFR and rapid resistance have to be addressed [26, 34–36]. Multigene predictors of response for targeted therapy may need to be considered [37].

Clinical deterioration in 12% of patients precluded enrollment on clinical trials, which strongly supports tumor sequencing earlier in the disease course to maximize the window of benefit from genomically guided therapy. Our data show that repeat biopsies upon tumor progression, especially after therapies that may induce significant selection pressure, are critical to identify tumor evolution and mechanisms of resistance [38–44]. Thus, there is a need to do genomic analysis "early and often" in the course of treatment.

Although many validated drivers commonly found in diverse tumor types do not have targeted therapy available even in early-phase trials, much ongoing drug development may identify approaches for these alterations that could reach the clinic in the future and increase eligibility for genomically

guided therapy. During the course of this study, clinical trials of IDH inhibitors became available, rendering *IDH1/2* (isocitrate dehydrogenase 1/2) alterations actionable [45]. Moreover, characterization of novel gene alterations found in individual tumors is vital, given that specific alterations may confer differential protein functionality or drug sensitivity, similar to our novel *BRAF* fusion identified in a melanoma that may be resistant to currently available BRAF inhibitors [46].

Target actionability reflects drug availability in current clinical trials [31, 32]. A recent report demonstrated that use of an 11- to 50-gene hotspot mutation panel led to an 11% rate of enrollment onto genotype-guided trials, the majority of which were driven by alterations in *PIK3CA/AKT1/PTEN/BRAF* [31]. Our study used a panel targeting a larger range of genomic events and was integrated with an MTB. This may have led to the higher actionability rate, although similar targets were identified. Our study confirms that clinical-grade genomic analysis is feasible and can guide clinical decision-making in a subset of patients. Similar obstacles to implementation of genomically guided therapy were identified, including lack of trials, poor clinical status, and lack of drug access. There is a tradeoff between breadth and depth of sequencing by current assays [47], and it is not yet clear what the optimal approach and platform is for tumor genomic analysis. Expectations are that with further advances, it will be possible to perform more comprehensive sequencing in greater depth. Improvements in target-drug matching and efficacy of newer drugs may lead to superior outcomes on targeted therapy.

A critical component of this protocol was implementation of an integrated, institutional MTB. The MTB brings together a multidisciplinary group of professionals to discuss optimal, genomically guided clinical options for each patient, a vital point as the landscape of specific alterations as predictive markers continues to evolve. For example, *PIK3CA* and *PTEN* alterations were initially thought to be potential markers for response to mTOR inhibitors, but were recently shown not to be the case in some settings [48]. MTB affords clinicians an avenue for better understanding of assay strengths/limitations, result interpretation/applicability, and clinical implementation, which may then translate into greater uptake of genomic testing and improved physician-patient communication [49, 50]. The systematic multispecialty overview at the MTB also enhances successful drug acquisition for off-label use of the rapidly evolving spectrum of targeted agents, especially important for rare tumors lacking established standards of care.

Although this study highlights the potential of point-of-care tumor sequencing, the ultimate question is whether it will lead to improved survival and/or quality of life for cancer patients. This approach can only lead to improved patient outcome if partnered with development of novel targeted agents, expanded trial portfolios, and access to clinical trials. Further advances in directed use of single or combinatorial targeted therapy will be needed to translate promise of tumor genomic characterization into improved patient outcomes.

AUTHOR CONTRIBUTIONS

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