original report

Integrative Molecular Analysis of Patients With Advanced and Metastatic Cancer

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abstract

PURPOSE We developed a precision medicine program for patients with advanced cancer using integrative whole-exome sequencing and transcriptome analysis.

PATIENTS AND METHODS Five hundred fifteen patients with locally advanced/metastatic solid tumors were prospectively enrolled, and paired tumor/normal sequencing was performed. Seven hundred fifty-nine tumors from 515 patients were evaluated.

RESULTS Most frequent tumor types were prostate (19.4%), brain (16.5%), bladder (15.4%), and kidney cancer (9.2%). Most frequently altered genes were *TP53* (33%), *CDKN2A* (11%), *APC* (10%), *KTM2D* (8%), *PTEN* (8%), and *BRCA2* (8%). Pathogenic germline alterations were present in 10.7% of patients, most frequently *CHEK2* (1.9%), *BRCA1* (1.5%), *BRCA2* (1.5%), and *MSH6* (1.4%). Novel gene fusions were identified, including a *RBM47-CDK12* fusion in a metastatic prostate cancer sample. The rate of clinically relevant alterations was 39% by whole-exome sequencing, which was improved by 16% by adding RNA sequencing. In patients with more than one sequenced tumor sample (n = 146), 84.62% of actionable mutations were concordant.

CONCLUSION Integrative analysis may uncover informative alterations for an advanced pan-cancer patient population. These alterations are consistent in spatially and temporally heterogeneous samples.

INTRODUCTION

Genomic profiling is widely used in cancer care to identify actionable alterations for individual patients within the context of precision medicine (PM).[1] However, only 2% to 11% of those patients with sequencing performed receive a genomically matched therapy, which may be a result of the availability and accessibility of clinical trials,[2-6] patient factors and comorbidities, disease state or alternative options, or patient preference.[5]

Despite these drawbacks, PM studies continue to provide insights into the molecular underpinnings of cancer. We and others have shown that performing whole-exome sequencing (WES) and RNA sequencing is feasible in a clinical setting and may provide relevant information beyond targeted gene panels in certain settings.[3,7,8] Sequencing matched tumor and normal (germline) DNA has the additional benefit of uncovering unforeseen hereditary conditions, including cancer risk mutations. In particular, germline DNA repair defects (DRDs) are more common than previously anticipated across adult advanced cancer populations.[9-11] Robinson et al[12] published their MET500 cohort with WES and RNA sequencing data from 500 patients with metastatic disease of varied tumor primary and biopsy sites. Our study, obtained from our own cohort of 759 samples from 515 patients with advanced and metastatic cancer, complements this data set as it adds WES data from approximately the same number of patients. Of importance, our data add needed information about tumor evolution, clonality, and tumor heterogeneity by analyzing multiple samples obtained from individual patients.

PATIENTS AND METHODS

Patients with locally advanced and metastatic cancer were prospectively enrolled in an institutional review board (IRB)–approved PM Trial (IRB No. 1305013903) with written informed consent. The consent process included explaining the risk and potential consequences of tumor biopsy, somatic and germline sequencing, as well as offering the opportunity to participate in a rapid

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key objective

We assessed whether developing a multidimensional precision oncology program is feasible and informative for patients with cancer with advanced disease.

Knowledge generated

We established a comprehensive clinical genomics program for this patient group. The rate of clinically relevant alterations across 515 patients with advanced solid tumors was 39% by whole-exome sequencing, which was improved by 16% by adding RNA sequencing. Multisample analysis of individual patients revealed a concordance rate of clinically significant alterations of 84.62%.

Relevance

Multidimensional genomic analysis is feasible and informative in a clinical setting and can improve clinical care for some patients.

autopsy program.[13] Tumor DNA for WES was obtained from fresh frozen or formalin-fixed, paraffin-embedded tissue. Seventy image-guided biopsies from prostate cancer bone metastases using an optimized bone biopsy protocol[14] were performed. Frozen or formalin-fixed, paraffin-embedded tissue slides were evaluated by study pathologists for diagnosis and tumor cell content. Germline DNA was obtained from blood samples (circulating mononuclear cells), buccal swabs, or benign tissue as described previously.[3] Part of the data presented in this manuscript have already been published.[3,13,15]

WES was performed on each patient's tumor/matched germline DNA pair using previously described protocols.[3] We used a clinical-grade WES test—Exome Cancer Test Version 1—approved by New York State Department of Health (ID# 43032) and described in detail in Rennert et al.[16] This approach allows for assessment of more than 21,000 genes through the development and implementation of computational approaches for tumor mutational burden and neoepitope analysis, as well as integration with other data, including RNA sequencing, to improve the identification of clinically relevant and actionable alterations. RNA sequencing was performed on a subset of cases with sufficient fresh frozen tissue available. For details of RNA sequencing data analysis, see the Data Supplement. To evaluate the concordance of tier 1 and tier 2 alterations between multiple samples from the same patient and to gain high-fidelity results, a cutoff of 20% for variant allele frequency was used. In addition, we developed patient-derived organoids from fresh tissues using previously described protocols,[4,17] and we used cell lines to functionally validate outlier targetable alterations.

WES alterations were categorized on the basis of on their actionability and clinical or biologic relevance.[3] Alterations in 49 actionable or clinically significant genes were reported within Category 1, alterations in 508 known cancer-associated genes within Category 2, and somatic alterations of unknown significance within Category 3.[3] We developed an open-access, dynamic, Web-based PM knowledge base as an interactive online tool where variants are carefully interpreted in the context of tumor type.[18]

WES results were conveyed to the referring physician in the form of an Exome Cancer Test Version 1 report.[3] Selected cases are presented at a regular, continuing medical education–accredited PM tumor board, which discusses sequencing results in the context of a patient's history, available literature, and treatment options, including active clinical trials.

Pathogenic germline findings were reported to the referring physician if they occurred in any of the genes deemed reportable by the American College of Medical Genetics and Genomics (ACMG),[19] and these patients were referred for genetic counseling and results were confirmed by targeted testing in a Clinical Laboratory Improvement Amendments–/Clinical Laboratory Evaluation Program–certified laboratory.

Study cohort demographic data were obtained through electronic health record search. Ethnicity was inferred through a computational analysis (EthSEQ; https://cran.r-project.org/package=EthSEQ) of germline single-nucleotide polymorphisms.[20]

RESULTS

Patient Characteristics

Between February 2013 and December 2016, 515 patients were prospectively enrolled (Fig 1 and Data Supplement). The majority of patients presented with metastatic (n = 319; 62%) or recurrent (n = 46; 8.9%) disease. Of patients, 149 (28.9%) had primary tumors available for study analysis. Disease status was unknown for one patient (0.2%). Median number of prior systemic therapies before tissue evaluation was three (range, zero to 14). The majority of patients were of European (n = 231; 44.9%) or Ashkenazi (n = 165; 32.1%) heritage (EthSEQ data; Data Supplement), compatible with the location of the Englander Institute for Precision Medicine (Upper East Side of Manhattan, New York, NY).[21]

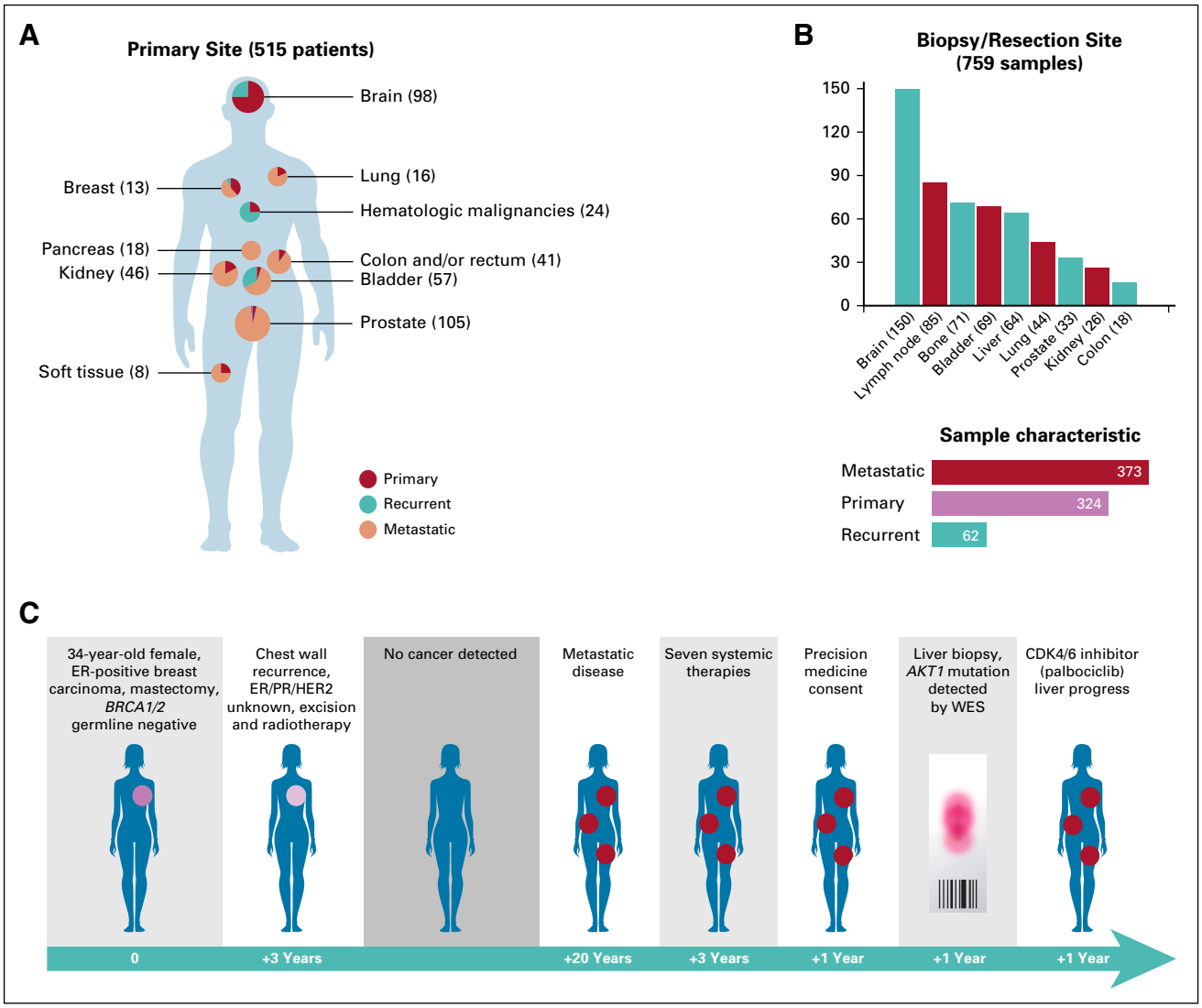

FIG 1. Selected primary tumor sites of 515 Englander Institute for Precision Medicine patients and patient status at time of enrollment. (A) The majority of patients with non-CNS tumors presented with metastatic disease. (B) Most common biopsy or resection sites and information, whether a sample was procured from a primary, metastatic, or recurrent site. (C) Clinical history of a female patient with breast cancer who participated in the precision medicine trial. An *AKT1* mutation was discovered in a liver sample and she was treated with an AKT1 inhibitor, albeit without achieving disease remission. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor; WES, whole-exome sequencing.

WES Results

Tier 1, 2, or 3 mutations were identified in 27.4%, 72.3%, and 95.1% of all samples, respectively. Tier 1, 2, or 3 somatic copy number alterations were found in 52.4%, 88.4%, and 100% of all samples, respectively. Figure 1C shows an example of a patient treated according to WES results. Mutation rate across tumor types ranged from zero to 37 mutations per megabase, with a mean of 1.2 mutations/Mb. The highest mutation rates were observed in tumors of the colon/rectum and bladder. Copy number burden (megabase altered by copy number variation) ranged from zero to 2,098 per sample (mean, 155.59 Mb). Higher somatic copy number alteration (SCNA) values were found in samples from endometrial and colorectal primary tumors. The most common somatic alterations were found in *TP53* (33%), *CDKN2A* (11%), *APC* (10%), *KMT2D* (8%), *PTEN* (8%), and *BRCA2* (7%; Fig 2).

Multiple Samples

Three hundred eighty-two spatially and temporally heterogeneous samples from 146 patients underwent WES. Of these, 185 (48.4%) were metastatic, 153 (40.1%) were primary, and 44 (11.5%) were recurrent tumor samples. Most samples were paired primary and metastatic tumors, and concordance of alterations between primary and metastatic samples in clinically informative genes is shown in Figure 3. The primary tumor was sequenced at a different timepoint in 29 patients—for example, before and after

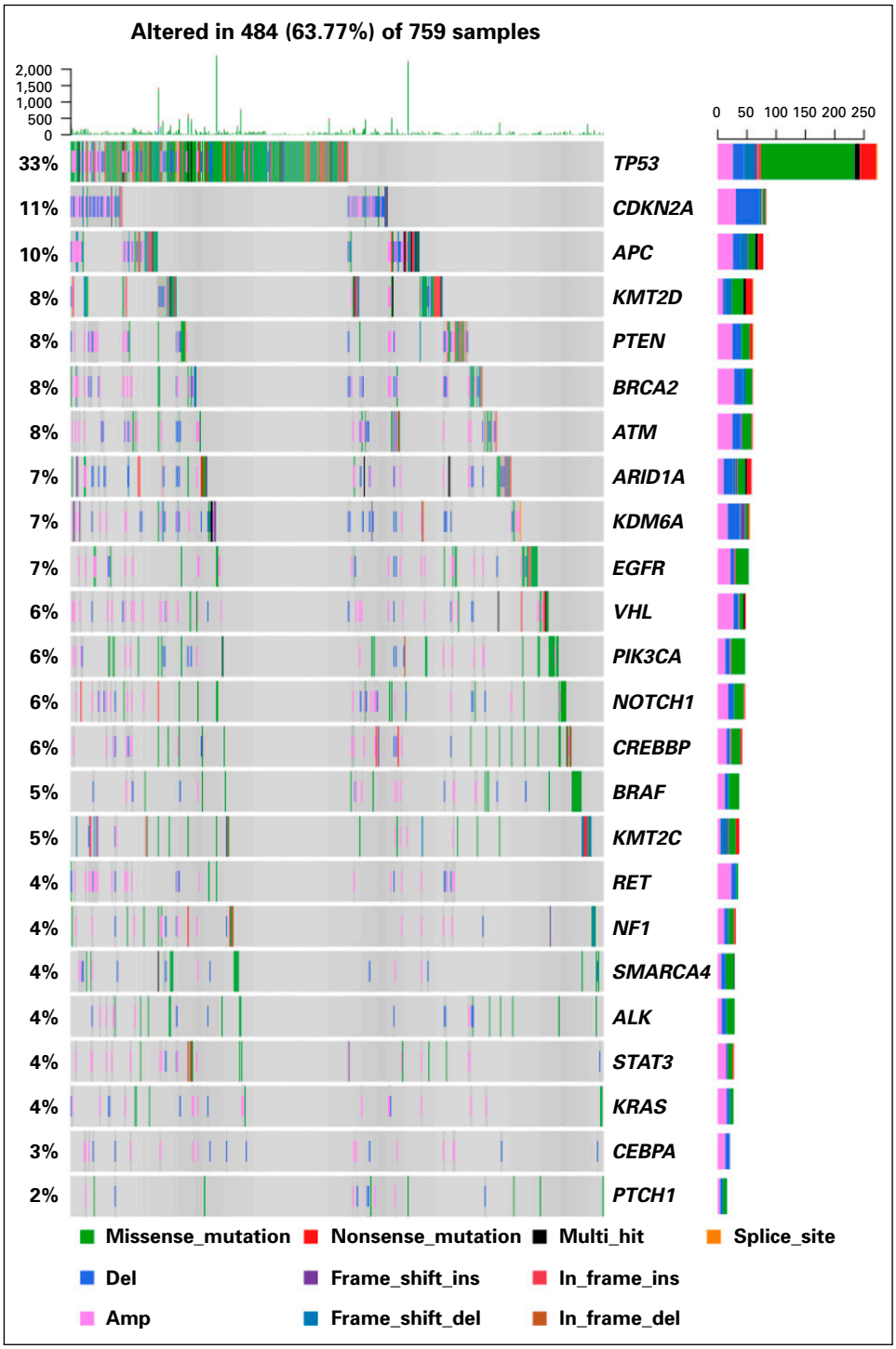

FIG 2. Landscape of genomic alterations in the Englander Institute for Precision Medicine cohort. The most common alterations are nonsynonymous *TP53* mutations (Category 2). This alteration is found often in advanced prostate cancer, bladder cancer, and brain tumors. Amp, amplification; Del, deletion; Ins, insertion.

systemic therapy. Employing a variant allele frequency cutoff of 20% for genomic alterations, multiple samples from 132 patients could be evaluated. Of Category 1 and 2 alterations, 84.62% and 85.75%, respectively, of mutations and SCNA variations were shared.

Germline Findings

WES of germline DNA was performed in all patients to filter single-nucleotide variants and SCNAs that are only present in the tumor (Fig 4). Sequencing germline DNA also serves as an internal quality control to determine whether both samples truly originate from the same patient.[22] The ClinVar and ExAC databases are our main sources for germline variant evaluation.[23,24] Variants are reported in an internal report using a five-tiered system as recommended by the ACMG and the Association for Molecular Pathology.[19] Deleterious germline DRDs involving 12 genes were identified in 55 patients, comprising 10.7% of patients in

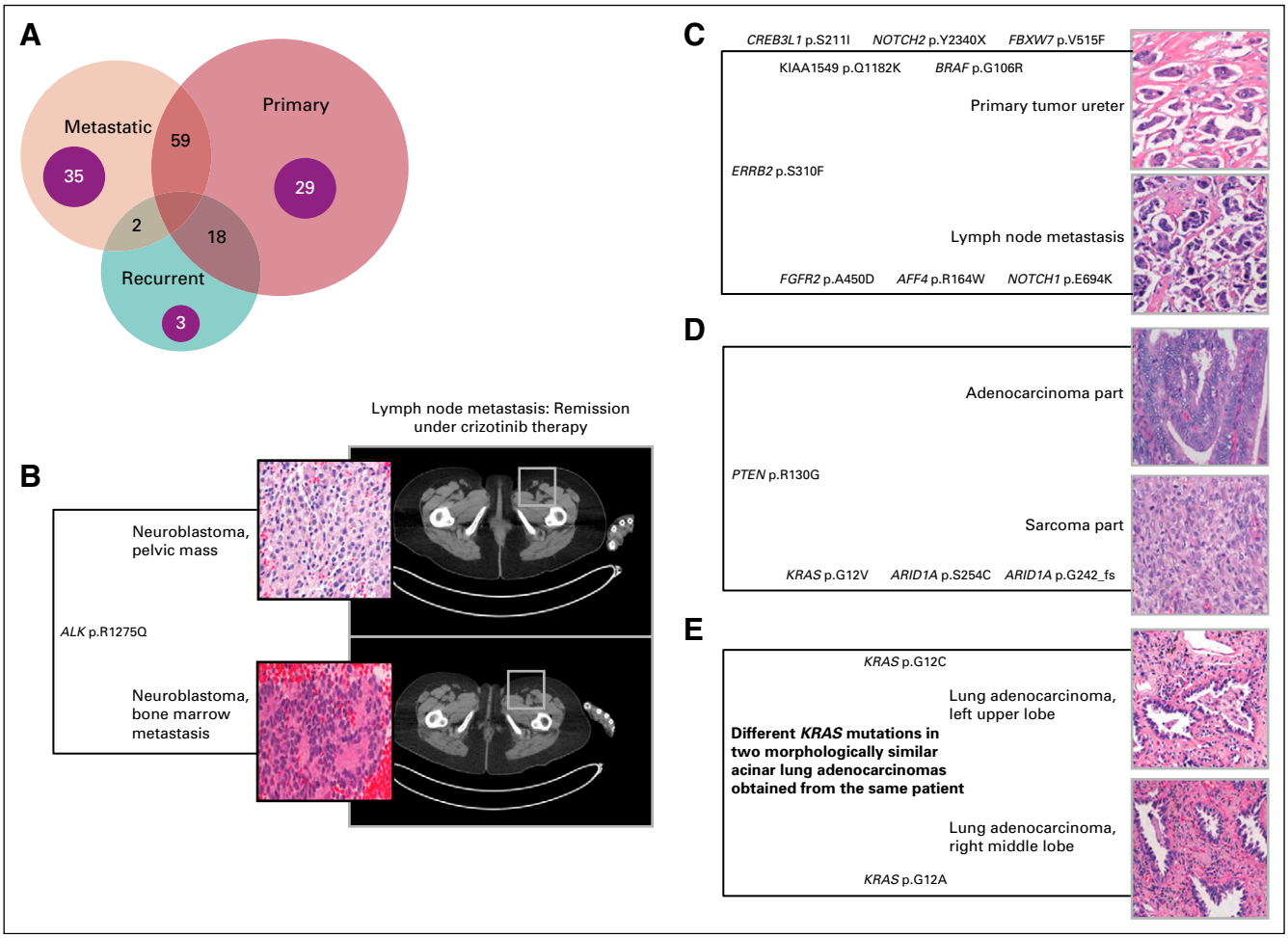

FIG 3. Multiple samples from same patients enrolled in the precision medicine trial at the Englander Institute for Precision Medicine (EIPM) and clinically relevant examples. (A) Venn Diagram showing the relationship of multiple tissue samples from individual patients. Multiple samples from 146 individuals in the EIPM cohort underwent whole-exome sequencing. Paired primary–metastatic tumor samples from 59 patients were the most frequent combination. Spatially and/or temporally heterogeneous metastases and primary tumors from 35 and 29 patients, respectively, also underwent sequencing. Primary tumors were usually sequenced at different timepoints—for example, tumor samples at initial diagnosis and subsequent residual tumor postneoadjuvant treatment. (B) Activating *ALK* mutation in both primary tumor and bone marrow metastasis; partial remission after crizotinib therapy. (C) Shared and potentially actionable mutation in the *ERBB* gene in both primary tumor and lymph node metastasis of an individual with micropapillary urothelial carcinoma. (D) Early divergent evolution in both histologically distinct epithelial and mesenchymal components in an individual with uterine carcinosarcoma. (E) Different *KRAS* mutations in two morphologically similar lung cancer samples from the same patient, thus confirming two separate primary tumors.

this cohort (Data Supplement). The most frequently mutated genes were *CHEK2* (11 patients), *BRCA2* (nine patients), *BRCA1* (nine patients), *MSH6* (nine patients), and *ATM* (four patients; Fig 2). Twelve patients had additional loss of function in the other allele. Positive germline findings were confirmed by targeted sequencing for 53 patients. In addition, 44 additional likely pathogenic variants in 38 patients in *MSH6* (14 cases); *APC* (seven cases); *CHEK2* (five cases); *POLE*, *PMS2*, *MSH2 BRCA1*, and *ATM* (three cases each); and *TP53*, *CDH1*, and *BRIP1* (one case each) were discovered in our cohort.

The prevalence of DRD in our cohort of patients with metastatic prostate cancer was 14.3%, with *BRCA2* mutations being the most frequent variants (Data Supplement). Pathogenic germline DRDs were found in 9.2% of patients with primary brain tumors. The majority of these were in astrocytic neoplasms with WHO classification grades I to IV. We did not identify pathogenic mutations involving mismatch repair genes, which have been described in primary brain tumor patients, in particular as biallelic losses.[25] *CHEK2* was altered in four cases, including one medulloblastoma. The detected c.1100delC mutation has not been described in medulloblastoma patients to date.

DRDs involving homologous recombination genes may result in increased sensitivity to DNA-damaging agents, such as poly(APD-ribose) polymerase (PARP) inhibitors or platinum-based chemotherapy.[26] Of the 55 patients with

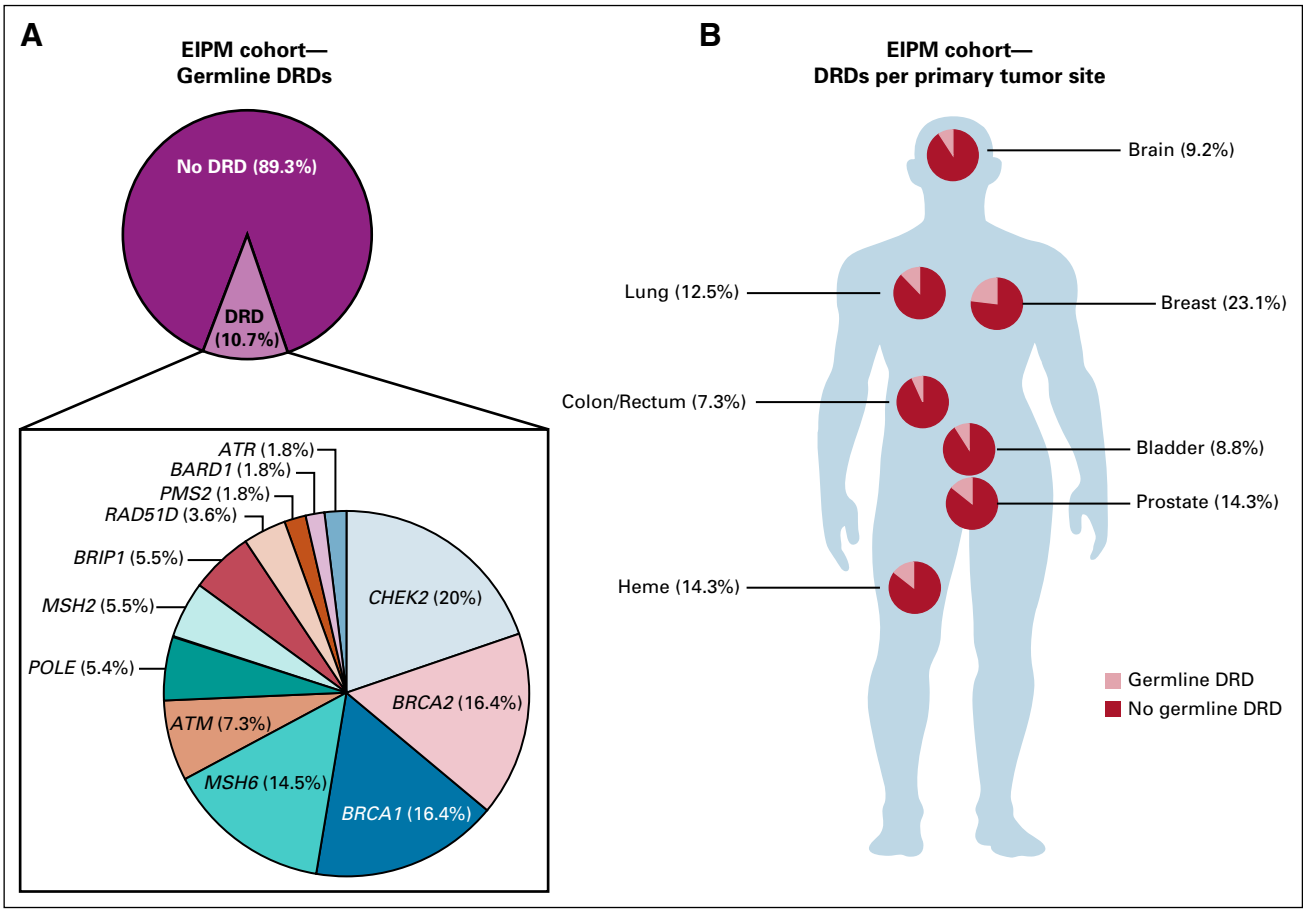

FIG 4. Germline DNA-repair defects (DRDs) in Englander Institute for Precision Medicine (EIPM) cohort. (A) The frequency of germline DRD alterations in our cohort is 10.7%. *CHEK2*, *BRCA1/2*, and *MSH6* are the most frequently mutated genes. (B) High frequency of germline mutations in breast, prostate, and lung tumors is observed.

germline DRD defects, 12 had received platinum-based chemotherapy with follow-up available. Ten patients showed benefit in the form of stable disease or radiographic response.[27] Sixteen patients (28.6%) with pathogenic germline defects succumbed to their disease. Median time from cancer diagnosis to death was 2 years.

RNA Sequencing

RNA sequencing was performed when sufficient fresh frozen tumor tissue was available after WES. RNA sequencing was performed successfully in 235 samples from 219 patients. We identified druggable outliers and potentially targetable gene fusions in 89 patients (17.3%). Of these 89 patients, 50 did not harbor a targetable genomic alteration identified by WES, resulting in an increase in the rate of actionable alteration detection of approximately 15%. We confirmed the drug sensitivity of select outlier genes using cell line experiments compared with randomly selected drugs (Fig 5).

Nine novel fusions in a variety of cancer types were detected. A novel *RBM47-CDK12* gene fusion was found in a prostate cancer bone metastasis, which was confirmed by reverse-transcription polymerase chain reaction and Sanger-Sequencing (Fig 5). An additional targetable *NCOA4-RET* fusion, which has been described in papillary thyroid cancer, non–small-cell lung cancer, and colorectal cancer, was found in a brain metastasis of a patient with unknown primary.[28]

Organoids

Part of the program was the development of patient-derived organoids from patient biopsies for high-throughput drug screening.[4] Altogether 60 organoids were developed from 98 patients with an overall success rate of 61%. High-throughput drug screening was performed in a subset of patient-derived organoids as previously described.[4]

Case Studies

Germline results. A pathogenic *MSH6* mutation was detected in a 26-year-old patient with metastatic breast carcinoma who had undergone previous outside testing for *BRCA1/2* germline mutations with a negative result. Although the association between mismatch repair mutations and breast cancer is not sufficiently established, these patients are at risk for secondary cancers, like colorectal or

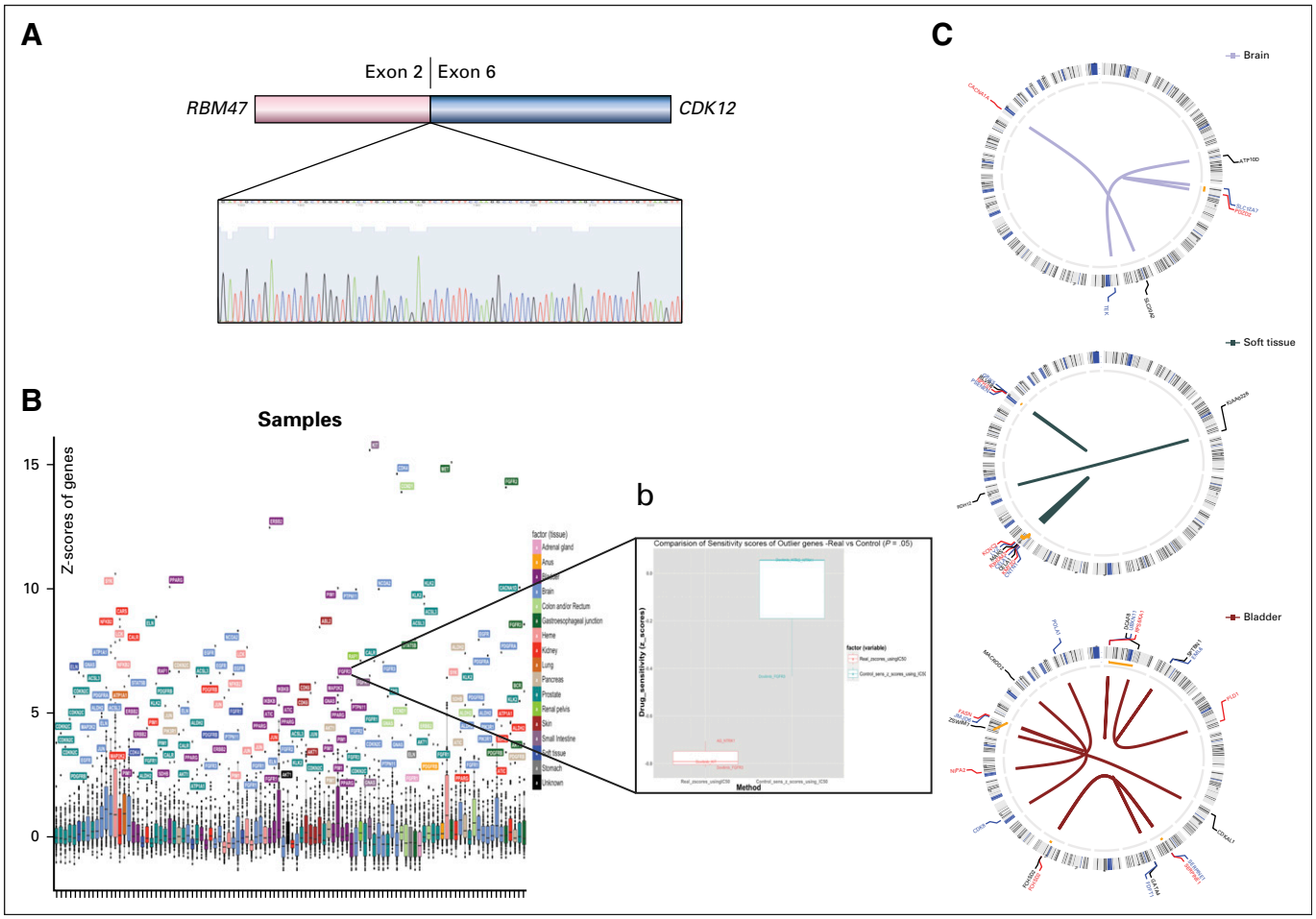

FIG 5. Detection of potential clinically relevant alterations through transcriptome analysis. (A) Novel *RBM47-CDK12* gene fusion in a metastatic prostate cancer sample, detected by RNA sequencing and confirmed by polymerase chain reaction. (B) RNA sequencing outlier analysis and investigation of *FGFR3* outlier in a bladder cancer cell line. (C) Circos plots of gene fusions identified in select cases (brain, soft tissue, bladder).

endometrial carcinoma.[29] As testing was limited to *BRCA1/2* in this case, this important germline finding in *MSH6* was originally undetected. Other potentially significant variants in DRD genes, such as *PALB2*, would have been missed as well.[30] This case underlines the importance of multigene panel or WES testing in patients not only with suspected hereditary cancer but also in those, especially young patients, with metastatic disease to detect underrecognized germline alterations.[31]

Tumor evolution and clonality. Four illustrative cases are highlighted in Figure 3. We sequenced both the sarcoma and adenocarcinoma components from a primary uterine carcinosarcoma and discovered distinct molecular differences. Uterine carcinosarcomas are thought be of monoclonal origin.[32] We detected a shared *PTEN* R130G missense mutation in both histologic subtypes. A deleterious effect has been predicted for this variant.[33] In a previously published analysis of 13 uterine carcinosarcomas analyzed by targeted sequencing, eight cases demonstrated 100% identical mutations in both the carcinoma and sarcoma part.[34] In contrast, we observed an early divergence with only the one *PTEN* mutation of 54 nonsynonymous shared mutations. Of note, a *KRAS* mutation was only observed in the sarcoma component.

In two samples of micropapillary urothelial carcinoma obtained from the primary tumor and a regional lymph node metastasis of one individual, a shared, potentially actionable mutation involving *ERBB2* was detected.[35]

The problem of multifocal lung tumors and uncertainty regarding clinical management is not uncommon and has been addressed before.[36,37] In one patient, two histologically similar lung acinar adenocarcinomas in different lobes were confirmed to be separate, synchronous primary tumors on the basis of two distinct driver mutations in the *KRAS* gene and other alterations observed by WES, thus resulting in two pT1 tumors rather than one pT4 tumor and eliminating the need to administer adjuvant chemotherapy.[38] Whereas this alteration might also have been detected using a targeted gene panel, a background of different mutated genes in both samples that were

detected by WES resulted in a stronger argument for two synchronous primary tumors. This case again demonstrates the importance of using next-generation sequencing to correctly identify synchronous primary tumors.[39]

Another patient with metastatic neuroblastoma was confirmed to have an activating *ALK* mutation (R1275Q) in both primary tumor and bone marrow metastasis. The patient has had stable disease for 13 months on therapy with sequential *ALK* inhibitors[40] (Fig 3).

DISCUSSION

We have established a PM program for patients with advanced cancer using tumor/normal WES and integrative molecular profiling to detect genomic and other actionable alterations, improve clinical decision making, and study tumor evolution in a pan-cancer cohort. The patient population in our study is distinct from several reported studies in that our focus was on the evaluation of advanced tumors.[41,42] The majority of patients (70.8%) presented with metastatic or recurrent disease at the time of enrollment.

Whereas publicly accessible molecular data for several tumor types are available, both for primary and metastatic cancers,[43,44] these specimens are rarely matched—that is, obtained from the same patient.[45] Our cohort of patients with locally advanced and metastatic cancer allowed for the collection of multiple matched, often primary and metastatic samples, resulting in a unique feature of this cohort: Genomic data from spatially and/or temporally heterogeneous, matched tissue samples were available in 146 patients. Our data contribute to the understanding of tumor evolution and heterogeneity. Of clinical importance is the finding that almost 85% of Category 1 alterations were shared between multiple samples from the same patient. These results are in concordance with published data in specific cancer types. In one study, when comparing actionable mutations between presurgery biopsies and resected specimen in patients with non–small-cell lung cancer, the concordance rate was found to be 79%.[46] Similar findings have been reported for primary and recurrent breast cancer, with 86.6% of mutations and 85.5% of SCNAs being concordant.[47] Our data confirm that it may reasonable to select the most accessible location or even archival material from the primary tumor for molecular analysis for certain cancer types.[48,49]

New research indicates that pathogenic germline mutations are more frequently found in patients with advanced cancer,[9,12] and our data confirm this. Germline mutations that involve the DNA repair pathway, found in 10.7% of our patients, are predictive of response to PARP inhibitors and platinum-based therapy and have important familial implications for cancer screening.[50] Family history was indicative of a heritable component in only one half of these patients. This discrepancy should be addressed to select patients and their families for genetic counseling.

WES provides the additional advantage of uncovering likely pathogenic mutations. According to the ACMG, a likely pathogenic variant is backed up by sufficient evidence to aid in clinical decision making.[19] Our cohort is enriched for patients with metastatic prostate cancer, who have been reported to harbor DRDs in 11.8% of cases.[9] This might explain, in addition to the high frequency of Ashkenazi Jewish ancestry, why we observe a higher rate of germline DRD compared with other pan-cancer cohorts (Data Supplement).[10]

Novel gene fusions were also discovered, including a *RBM47-CDK12* gene fusion in a prostate cancer metastasis. This fusion might result in the loss of *CDK12* activity, which has recently been described to delineate a distinct immunogenic subtype of metastatic castration-resistant prostate cancer.[51] In our study, WES was prioritized over RNA sequencing whenever tissue availability was of concern. Biopsies in our study were usually performed for diagnostic purposes in the context of clinical care, hence the lower availability of fresh frozen tissue for additional RNA sequencing.

Targeted sequencing of cancer-related genes offers several advantages over WES, including deeper coverage, quicker turnaround time, lower cost, and fewer requirements for an elaborate computational pipeline. Here, we show that WES and RNA sequencing may provide an additional and complimentary layer of information in certain settings, particularly for patients with advanced cancer who experience progression after standard therapies. The highlighted cases illustrate the potential utility of WES for uncovering clinically informative somatic and germline alterations. WES also considers the rapidly expanding spectrum of actionable alterations, including alterations for which targeted treatment may not be available at the time of analysis, but for which clinical trials might be planned. Emergence of resistance pathways may also be identified.

Limitations of WES are lower coverage, higher cost, and slower turnaround time. In addition, whereas targeted next-generation sequencing testing of somatic DNA are sometimes ordered as part of clinical care, using germline DNA as normal control for WES necessitates securing informed consent.

Implementing -omic data into clinical care requires an interdisciplinary team. Genomic data and its interpretation in the context of tumor type and primary site must be easily accessible to enable clinicians to integrate the data into everyday patient care. Shared data platforms may also help to close the gap between research and clinical care. With the launch of the American Association of Cancer Research Project Genomics Evidence Neoplasia Information Exchange for sharing genomic and clinical data sets, there are now efforts toward developing a unified database to ultimately advance clinical care.[44]

One limitation of our study is the lack of uniformity of the cohort in terms of tumor type and therapy, which prevents us from making generalized statements about treatment response. Its strength lies in its ability to identify outliers and N-of-one cases. This data set also reports on a large number of matched temporally and spatially heterogeneous samples, highlighting the concordance of actionable alterations in different samples.

As other PM trials have shown, uncovering genomic alterations may not be sufficient in identifying actionable alterations. This study demonstrates how integrating genomics with transcriptomic and other potential readouts may ultimately improve actionability and contribute toward our growing understanding of the molecular underpinnings of cancer. Data for this study may be accessed through cBioportal (http://www.cbioportal.org).[52]

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Patents, Royalties, Other Intellectual Property: Methods for diagnosing and treating prostate cancer

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Research Funding: Eli Lilly, Janssen Oncology, Millennium Pharmaceuticals, Sanofi
Patents, Royalties, Other Intellectual Property: US Patent (7,767,393 and 7,229,774), Expression Profile of Prostate Cancer, 2007; US Patent (7,332,290), Detection of AMACR Cancer Markers in Urine, 2008; US Patent (7,718,369), Recurrent Gene Fusions in Prostate Cancer, 2010; US Patent (7,803,552 and 7,666,595), Biomarkers for Predicting Prostate Cancer Progression, 2010; US Patent (7,981,609 B2), Methods for Identifying and Using SNP Panels, 2011; US Patent (8,106,037 B2), Identification and Treatment of Estrogen Responsive PCa, 2012; US Patent (9,090,899 B2), Methods of Diagnosing and Treating Prostate Cancer Characterized by NDRG1-ERG Fusion, 2015; US Patent (9,458,213 B2), Compositions and Methods for Diagnosing Prostate Cancer Based on Detection of SLC45A3-ELK4 Fusion Transcript, 2016; US Patent (9,568,483 B2), Molecular Subtyping, Prognosis and Treatment of Prostate Cancer, 2017; US Patent (9,678,077 B2), ERG/TFF3/HMWCK Triple Immunostain for Detection of Prostate Cancer, 2017; US Patent (61,408,341), Exploration of Novel Gene Fusion in Prostate Cancer by RNA-Seq
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