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CLINICAL MEDICINE

Institutional implementation of clinical tumor profiling on an unselected cancer population

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BACKGROUND. Comprehensive genomic profiling of a patient's cancer can be used to diagnose, monitor, and recommend treatment. Clinical implementation of tumor profiling in an enterprise-wide, unselected cancer patient population has yet to be reported.

METHODS. We deployed a hybrid-capture and massively parallel sequencing assay (OncoPanel) for all adult and pediatric patients at our combined cancer centers. Results were categorized by pathologists based on actionability. We report the results for the first 3,727 patients tested.

RESULTS. Our cohort consists of cancer patients unrestricted by disease site or stage. Across all consented patients, half had sufficient and available (>20% tumor) material for profiling; once specimens were received in the laboratory for pathology review, 73% were scored as adequate for genomic testing. When sufficient DNA was obtained, OncoPanel yielded a result in 96% of cases. 73% of patients harbored an actionable or informative alteration; only 19% of these represented a current standard of care for therapeutic stratification. The findings recapitulate those of previous studies of common cancers but also identify alterations, including in *AXL* and *EGFR*, associated with response to targeted therapies. In rare cancers, potentially actionable alterations suggest the utility of a "cancer-agnostic" approach in genomic profiling. Retrospective analyses uncovered contextual genomic features that may inform therapeutic response and examples where diagnoses revised by genomic profiling markedly changed clinical management.

CONCLUSIONS. Broad sequencing-based testing deployed across an unselected cancer cohort is feasible. Genomic results may alter management in diverse scenarios; however, additional barriers must be overcome to enable precision cancer medicine on a large scale.

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Introduction

In cancer medicine, knowledge of specific tumor genomic alterations has become increasingly important in diagnosis, prognosis, and treatment. The clinical benefits of identification and targeted inhibition of driver genomic alterations have been demonstrated in multiple cancer types (1–7). These successes have led to the notion that systematic assessment of "actionable" cancer genomic changes may enable precision oncology (8, 9).

To identify candidates for both FDA-approved targeted therapies and for enrollment into molecularly driven clinical trials, molecular diagnostics laboratories have historically provided a menu of single-target assays. However, sequential testing of multiple genes can rapidly exhaust precious tumor material, and the testing increases complexity, cost, and turnaround time. Massively parallel sequencing (MPS) offers several advantages over the single-gene approach (10–15); most importantly, it offers a more sensitive and more comprehensive genomic profile that does not apply an a priori knowledge of alterations that may be more common in a specific tumor type. Several academic centers have demonstrated that large MPS panels can be employed in practice (11, 16, 17), with a number of groups reporting a high rate of "actionable" (informative or clinically relevant) gene alterations in clinical cancer cohorts (4, 18, 19).

While the high proportions of actionable alterations in cancer cohorts seems encouraging, the clinical utilization of broad genomic profiling is nascent and the eventual clinical utility has yet to be determined. Several recent reports have shown that only a small proportion of patients (~5%–10%) have their cancer genomic data used as a criterion for selection of a targeted therapy or a clinical trial (16, 19, 20). A variety of logistic, operational, and medical reasons (20) have been cited, such as identification of the appropriate genomic target at the right time in a patient's clinical course, time to result, the value of biopsy at the time of progressive disease (21), choice of conventional treatment protocols, access to clinical trials (16, 20) or off-label use of drug (19), and patient preferences (20). Similarly, evaluation of the confidence of physicians at our institutions in using genomic data to make decisions on patient treatment options (22) indicates that not all "actionable" genomic data will be acted upon.

Assessment of the clinical utility (i.e., measuring outcomes) of applying molecularly targeted therapies has delivered generally encouraging, though mixed, results (4, 18, 19, 21, 23–25), likely based in part on the maturity of targeted therapies in different tumor types and small and heterogenous patient populations, often with metastatic disease and multiple prior therapies. To date, the potential for enterprise-wide clinical tumor profiling to alter diagnosis or guide the clinical management of patients remains incompletely ascertained.

Starting in 2011, we have offered genomic profiling to all cancer patients seen at DFCI, Brigham and Women's Hospital, and Boston Children's Hospital. Through this initiative — termed Profile — we have analyzed over 15,000 individual tumors to date, including 5,000 by genotyping (OncoMap) (26) and >12,000 by MPS (OncoPanel), of which the first approximately 3,700 cases are reported here. The Profile initiative captures the entire population of cancer patients seen at our institutes, thereby defining the plausibly actionable pan-cancer genome in clinical practice. We reasoned that a systematic analysis of the clinical, pathologic, and genomic context of tumor mutations from this study might offer diagnostic clarification and identify new genomic predictors of response to targeted therapy in a manner that enables future large-scale cancer precision medicine initiatives, while, at the same time, identifying challenges and barriers to widespread implementation of precision medicine that would inform subsequent phases of our initiative.

Results

Overall program and technical performance of OncoPanel. Our programmatic approach is summarized in Figure 1A. Our overall consent rate was approximately 70%. Tumor material was available for review in 72% and was adequate for sequencing in 53% of consented patients (Figure 1B). Of patients for whom material was in hand, 23% did not have sufficient material for testing for a variety of reasons, including a lack of an invasive cancer diagnosis (e.g., squamous cell cancer in situ, breast ductal carcinoma in situ), inappropriate fixation (decalcification), protocol-excluded specimens (breast core biopsies), or specimens yielding less than 50 ng DNA. A small proportion of pathologically adequate specimens (4%) failed sequencing. 3,892 samples were tested during the first year; cancer types are listed in Supplemental Table 2 (supplemental material available online with this article; doi:10.1172/jci.insight.87062DS1). Once sufficient DNA was obtained, sequencing was successful in 3,727 cases (96% success rate; Table 1). Sequencing failure rates were significantly higher for consult cases (7%) as compared with in-house cases (3%) ($P < 0.001$) (Figure 1B). The success rate in specimens with adequate tumor cells ranged from a low of 91% in breast tumors to

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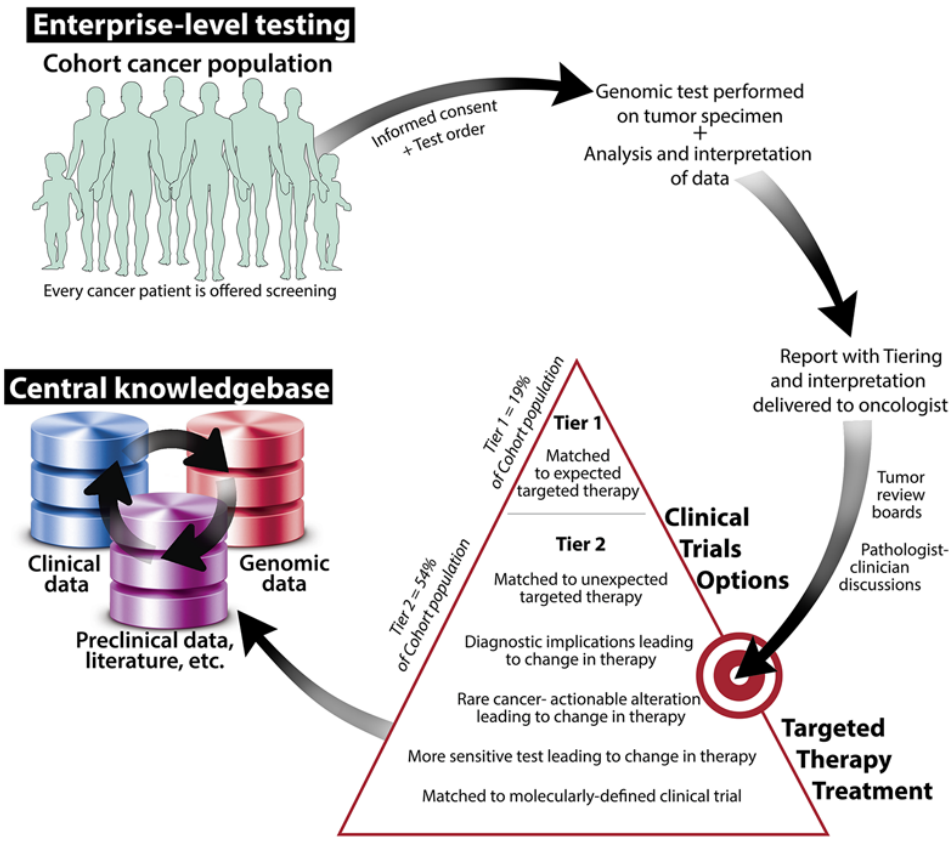

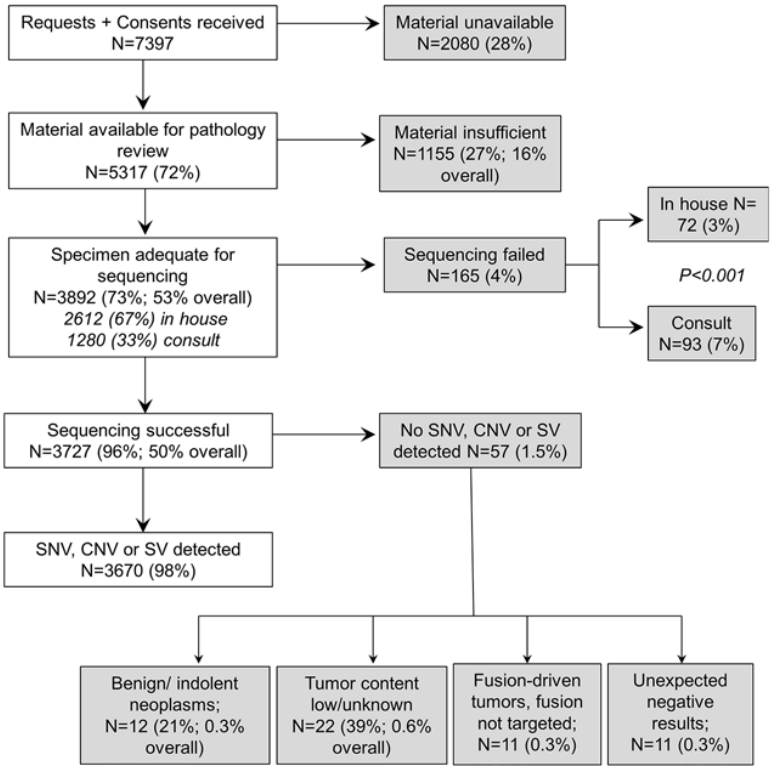

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Figure 1. An overview of the Profile program, including specimen availability, adequacy, and sequencing success. (**A**) For consented patients, a cancer specimen is genomically profiled in a CLIA laboratory. Results are tiered by a team that interprets pathology, incorporating information from each patient's electronic health record and provided to the patient's treating physician(s). Genomic, pathologic, and clinical data are deposited in a central knowledge base that can link to full clinical annotation. The knowledge base can be queried to facilitate development and enrollment of basket trials and inform tumor board discussions. (**B**) Once patient consent and a test requisition are received in the laboratory, pathology records are reviewed for available cancer specimens. Unavailable materials are defined as those not physically located within the participating institutions and not actively requested by the treating physician for OncoPanel testing. Cases are most commonly insufficient because material is too small, tumor content is less than 20%, or generates <50 ng of DNA. Sequencing failure is defined as mean target coverage of less than 50 reads. Tumors lacking single nucleotide variation (SNV), copy number variation (CNV), or structural variants (SV) were rare, but 22 were cases with low tumor content. Reported *P* value calculated by Fisher's exact test.

>99% in hematologic and pancreaticobiliary/liver tumors. The reasons for the substantially higher failure rate in breast tumors are not entirely clear; one potential explanation is that for much of the testing period breast core needle biopsies were excluded from testing in order to preserve tissue for clinical FISH and expression profiling assays. Therefore, most eligible specimens were either mastectomies/lumpectomies or metastatic biopsies. These specimens may be prone to underfixation or harsh fixation, contributing to DNA degradation. The average turnaround time for the test — from receipt of sample in the lab, through plate assembly and chemistries, sequencing, technical review, interpretation, and reporting — was 5.3 weeks.

In addition to evaluating clinical performance in the technical validation (see Methods), we calculated sensitivity and specificity for each category of genomic alteration using hallmark examples that had orthogonal, consecutive clinical testing and known "true positive" specimens. OncoPanel was 100% sensitive (95% CI 0.93–1) and 100% specific (95% CI 0.96–1) for *KRAS* exon 12–13 mutation detection as compared with pyrosequencing. Isolated cases of false-positive, low-level single nucleotide mutation events were detected but were generally attributable to cross contamination rather than sequencing error, an issue that was subsequently addressed by implementation of parallel "fingerprinting," using a genotyping assay on an aliquot of sample DNA (see Methods). Some insertion-deletion events are not readily detected using MPS-based techniques due to informatics challenges relating to sequence alignment (27). We therefore utilized multiple informatics tools (GATK and Breakmer), as well as forced manual review, for selected clinically important variant hot spots, in order to maximize our ability to detect small- to medium-sized insertion-deletion mutations. The forced review process allowed manual recovery of 12.5% of indels in *NPM1* and 100% of indels in *FLT3* (our algorithmic approaches were unable to detect *FLT3* internal tandem duplications). OncoPanel was 100% sensitive (95%CI 0.8–1) and 100% specific (95% CI 0.98–1) for *EGFR* exon 19 deletion mutation detection as compared with PCR-based sizing assays and/or Sanger sequencing with peptide nucleic acid clamps. OncoPanel was similarly 100% sensitive (95% CI 0.85–1) and 100% specific (95% CI 0.92–1) for *EGFR* high-level amplification in glioblastoma. The performance of OncoPanel for detection of single-copy, chromosomal arm-level events was also robust but less specific (97.5% sensitivity [95% CI 0.85–0.99] and 83.3% specificity [95% CI 0.51–0.97]) for detection of the diagnostic 1p/19q deletion event in oligoastrocytomas or oligodendrogliomas, as compared with high resolution aCGH or FISH (28) (Supplemental Table 3 and 4). Low-level copy number calling in particular was adversely affected by sample DNA degradation or low tumor content. Of 190 lung adenocarcinomas tested for ALK fusions by FISH or immunohistochemistry, OncoPanel was 94% sensitive (95% CI 0.69–0.99) and 100% specific (95% CI 0.98–1) (Supplemental Table 5). Moreover, OncoPanel detected an *ALK* fusion in one case with a reported FISH-negative result; the patient was switched to crizotinib therapy and experienced disease stabilization.

Pan-cancer overview of results from the cohort. Overall, the recurrent gene alterations were observed at the expected frequencies (Figure 2). To analyze these genes from the perspective of actionability, we categorized the alterations into tiers (Table 2). 73% of the cohort showed at least one "clinically actionable or informative" alteration (tier 1 or 2; Figure 1A). 19% of alterations would inform standard-of-care therapeutic decision-making based on diagnostic, prognostic, or predictive impact and were grouped into tier 1. 54% of alterations may be used as the basis for recommending enrollment on trials of approved or investigational agents or may be used for informing diagnosis; these were grouped into tier 2 (Figure 1A). Tier 3 alterations were considered to be biologically relevant but were unlikely to inform current treatment decisions. Variants of unknown significance were categorized as tier 4. Known germline polymorphisms that were not filtered by the informatics pipeline were categorized as tier 5.

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Table 1. Assay success and mutation rates across disease sites

Disease site	Number tested	Successful cases (%)	Average mutation count (per Mb[A])	Mutation count range
Breast	291	257 (88)	7.3 (5.4)	0–55
CNS	432	404 (94)	7.7 (5.7)	0–209
Endocrine	122	118 (97)	4.3 (3.2)	0–34
Gastrointestinal	314	304 (97)	10.7 (7.9)	0–155
Gynecology Tract	590	553 (94)	11.1 (8.2)	0–227
Head and neck	169	160 (95)	13 (9.6)	0–410
Heme malignancies	339	336 (99)	5 (3.7)	0–40
Kidney	105	105 (100)	4.3 (3.2)	0–17
Pancreaticobiliary and liver	89	87 (98)	5.5 (4)	0–44
Pediatric	93	93 (100)	7.6 (5.6)	0–174
Prostate	184	170 (92)	3.5 (2.6)	0–41
Sarcoma	181	178 (98)	5.6 (4.1)	0–36
Skin	70	66 (94)	17.6 (13)	0–134
Testis	42	39 (93)	4.5 (3.3)	0–23
Thoracic	732	723 (99)	8.9 (6.6)	0–70
Unknown	12	12 (100)	17.5 (12.9)	2–49
Urinary Tract	127	122 (96)	11.8 (8.7)	1–82
Total	3,892	3,727 (96)	8.6 (6.3)	0–410

[A]This assay sequences 1.358235 megabases (Mb).

The top tier variants for all cases, excluding those cases with only tier 4 variants, are included in Supplemental Table 6. The most common tier 1 alterations were *ERBB2* amplification; *EGFR* L858R, *BRAF* V600E in melanoma and colon adenocarcinoma and *KRAS* codon 12 and 13 mutations in colon and lung adenocarcinomas; *EGFR* exon 19 deletions; and *ALK* and *ABL1* rearrangements. The most frequently observed tier 2 alterations were amplifications of *CDK4*, *CCND1*, and *CCNE1*; homozygous deletion of *CDKN2A*; and single nucleotide variation of *IDH1* R132H and *BRAF* V600E in lung adenocarcinoma and other tumor types and *KRAS* codon 12 outside of the setting of colon and lung cancers. The most common tier 3 alterations were amplification of *MDM2* and single nucleotide variations in *APC*, *ASXL1*, *ATM*, *IDH1*, *TET2*, and *TP53*. In contrast to previous large-scale genomic studies of individual tumor types, most of which are biased toward analysis of primary tumors, this

Table 2. Classification schema according to variant actionability

Tier	Explanation	No. of cases with at least one variant in the tier (%)
1	Published evidence confirming clinical utility in the assigned tumor type. This utility may include the following:	
	(a) Predicting response to an FDA-approved therapy	
	(b) Establishing prognosis in a manner that affects therapeutic decision making	
	(c) Conferring risk of inherited cancer syndrome.	714 (19)
2	(a) Established biomarker for clinical trial eligibility	
	(b) Limited evidence of prognostic association	
	(c) Predictive of response to FDA-approved therapy in another tumor type	
	(d) Similar to another mutation proven to be predictive of response to FDA-approved therapy in this tumor type	2,001 (54)
3	Uncertain clinical utility but some evidence of biologic relevance in the form of the following:	
	(a) Preclinical studies demonstrating association with response to therapy in this tumor type	
	(b) Alteration in a highly conserved region of the protein predicted to alter function	
	(c) Selection of investigational therapy in another tumor type	470 (13)
4	Novel variant of unknown significance in cancer	473 (13)
5	Alteration of no clinical utility (present in ESP at >0.1% frequency)	Not reported
Total		3,658 (98)

ESP, Exome Sequencing Project.

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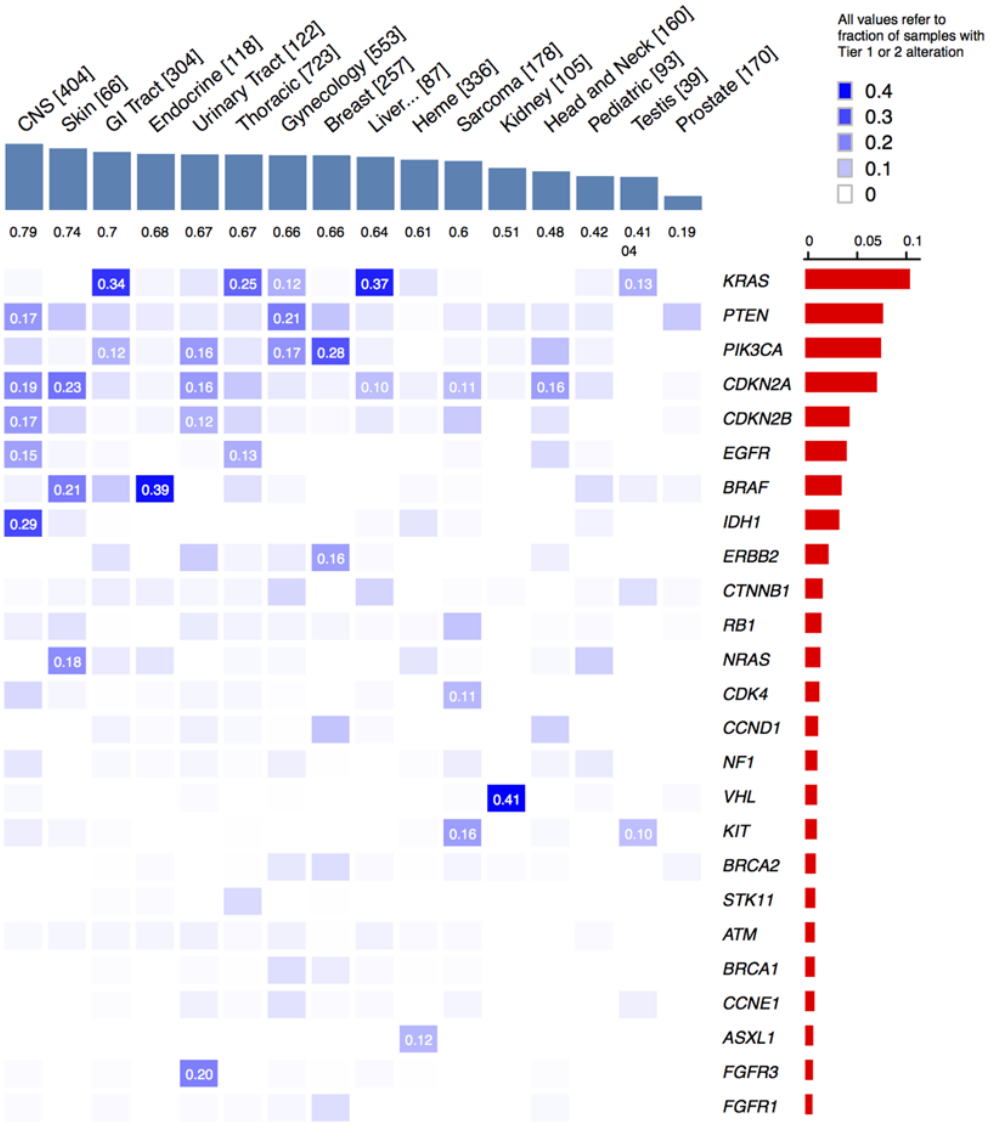

Figure 2. Frequency of alterations across the Profile cohort. The 25 most commonly altered actionable genes across all disease sites (with implications for FDA-approved targeted therapies or clinical trial enrollment) are shown. The frequency of tier 1 and 2 actionable alterations ranged from 79% of primary CNS tumors to 19% of prostate adenocarcinomas.

initiative examined 734 (20%) specimens taken from metastatic sites. As a result, we can begin to discern distinct patterns between primary and metastatic tumors across the cancer genome and within tumor types on the population level. In lung adenocarcinoma, alterations that were significantly more common in metastases as compared with primaries included *TP53* mutations (128 of 281 primaries, 115 of 193 metastases; $P = 0.007$) and *CDKN2A* mutations or homozygous deletions (18 of 281 primaries, 29 of 193 metastases; $P = 0.018$). In colon carcinoma, *TP53* mutations and *MYC* amplification were significantly enriched in metastases (TP53: 99 of 144 primaries, 43 of 48 metastases; $P = 0.045$ and MYC: 6 of 144 primaries, 8 of 48 metastases; $P = 0.036$, respectively). (Supplemental Figure 1). Because variable tumor purity may affect the rates of variant detection, particularly for copy number alterations, we also compared the histologic estimates of tumor content. Although lung adenocarcinoma metastases had a statistically significantly higher average tumor purity as compared with primaries (53% versus 48%, $P = 0.01$), this difference is unlikely to be clinically significant. The average tumor purity was similar between colon metastases and primaries (54% versus 53%, $P = 0.57$).

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Table 3. OncoPanel sequencing leading to a change in diagnosis

Case	Original diagnosis	Patient characteristics	OncoPanel findings	New diagnosis	Clinical implications
1	Peripheral T cell lymphoma, revised to myeloid sarcoma	35 male, refractory to 5 lines of therapy	*FIP1L1-PDGFRA* fusion	*FIP1L1-PDGFRA*-driven AML (confirmed by FISH)	Patient initiated on imatinib therapy with immediate response. Allogeneic SCT with NED at 1 year.
2	Small-cell carcinoma, revised to atypical carcinoid	42-year-old man, light smoker, PD on cisplatin-etoposide	*EWSR1-ERG* fusion	Ewing's sarcoma (confirmed by FISH)	Patient stable on palliative topotecan and cytoxan at 2-year follow-up.
3	Uterine leiomyosarcoma	53-year-old woman with "STUMP" treated by morcellation; recurred as peritoneal dissemination	*IGFBP5-ALK*	Inflammatory myofibroblastic tumor (confirmed by FISH)	ALK inhibitor therapy
4	Undifferentiated sarcoma, high grade	48-year-old woman with rectal bleeding, 6-cm ileal mass	*KIT* exon 11 deletion mutation	Gastrointestinal stromal sarcoma (IHC confirmed KIT and DOG-1 expression)	Patient initiated on imatinib therapy with NED at 6 months
5	GIST	60-year-old woman; high-risk small bowel GIST with peritoneal implants, known KIT exon 11 mutation. New peripancreatic mass on imatinib therapy.	*CTNNB1* Tyr41Ala, no *KIT* mutations	Desmoid fibromatosis (IHC confirmed nuclear β-catenin expression)	Patient maintained on the same dose of imatinib therapy due to improved prognostic implications
6	Undifferentiated sarcoma vs. carcinoma	49-year-old woman with large tumor masses involving bilateral fallopian tubes, ovaries, uterus, and sigmoid colon	MMRd mutation signature	Undifferentiated endometrial adenocarcinoma	Patient DOD; however, follow-up testing advised to determine if sporadic or germline MMR defect for family counseling
7	Lung squamous cell carcinoma	56-year-old man with multiple right middle lobe lung masses	UVA mutation signature	Metastatic cutaneous squamous cell carcinoma	Triggered chart review: patient with remote history of widely invasive cutaneous basosquamous carcinoma

AML, acute myelogenous leukemia; DOD, dead of disease; GIST, gastrointestinal stromal tumor; IHC, immunohistochemistry; MMRd, mismatch repair deficiency; NED, no evidence of disease; PD, progressive disease; SCT, stem cell transplant; STUMP, smooth muscle tumor of unknown malignant potential

Fifty-seven samples (1.5% overall) had no detectable alterations. Of these, 22 were estimated to have 20% tumor or less on additional review. These data correlate with our limit of detection experiments. The remaining cases with no detectable alterations may be genomically silent or driven by epigenetic or other alterations (including fusions) not captured by this panel.

Diagnostic revisions enabled by tumor mutation profiling. Despite having undergone expert pathology review in a high volume tertiary center, where our rate of major diagnostic revisions based on histopathologic and immunohistochemical review is about 4%, sequencing results provided clarification or revision of the diagnosis in a number of cases, often with significant implications for clinical management and prognosis. Exemplary cases are listed in Table 3, highlighting the potential utility of sequencing in tumors with unclear pathologic diagnoses. In case 1, a patient with extensive lymphadenopathy and marrow infiltration received various diagnoses at different institutions, including peripheral T cell lymphoma and myeloid sarcoma. Unexpectedly, OncoPanel data led to a revised diagnosis of *FIP1L1-PDGFRA*-driven AML. Consequently, imatinib therapy was administered, and a dramatic and sustained clinical response ensued (29). The identification of relatively specific driver alterations was particularly informative in the context of sarcoma, as might be expected based on the frequency of characteristic fusion and mutational alterations in subsets of these tumors. In case 2, a small round blue cell tumor in a nonsmoking man was originally classified as small-cell carcinoma and then as atypical carcinoid tumor; this diagnosis was revised to Ewing's

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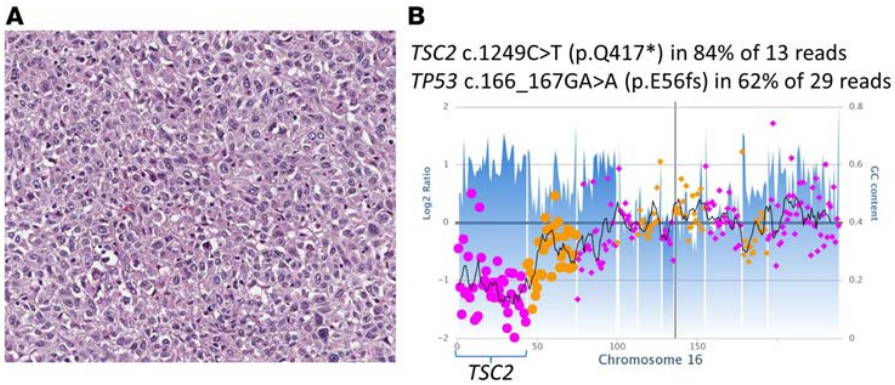

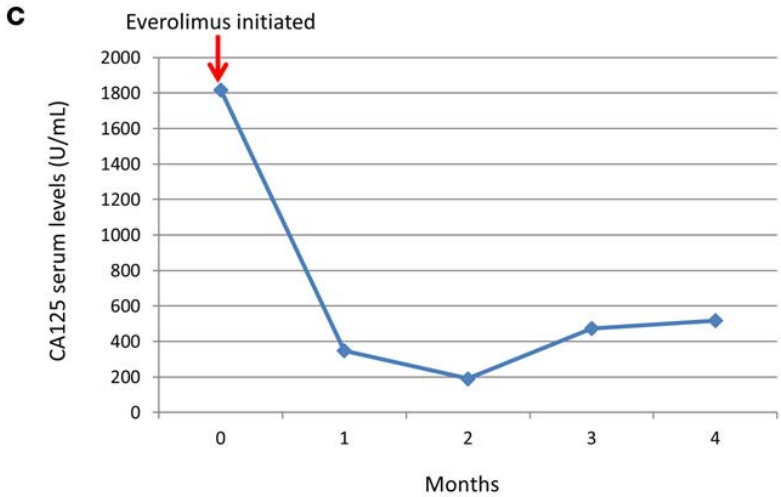

Figure 3. Targeting TSC2 loss of function. (**A**) A woman with high-grade serous ovarian carcinoma (original magnification, ×200) has evidence for (**B**) biallelic TSC2 loss, including a truncating mutation and loss of one copy of the gene, based on OncoPanel sequencing. Magenta and gold dots indicate log2 ratio of sample copy number relative to a pooled normal at the level of individual exons and selected introns for each of the targeted genes. Pale blue tracing shows the percent guanine and cytosine (GC) content in the targeted region. (**C**) Following therapy with everolimus, the woman's CA-125 levels dropped markedly following one cycle of therapy, and CA-125 levels remained low beyond the third cycle of therapy.

sarcoma following identification of an *EWSR1* rearrangement by OncoPanel (30). The identification of an *ALK* rearrangement in a uterine leiomyosarcoma led to a revised diagnosis of inflammatory myofibroblastic tumor at the time of peritoneal dissemination in case 3. The patient has not required further treatment to date; however, if therapy is required in the future she will receive an ALK inhibitor rather than standard chemotherapy for leiomyosarcoma. In case 4, identification of a *KIT* exon 11 in-frame deletion in a small bowel undifferentiated sarcoma triggered additional immunohistochemistry studies and led to a revised diagnosis of gastrointestinal stromal sarcoma. As a result, the patient was initiated on imatinib therapy, with no evidence of recurrent disease at last follow-up. In case 5, a patient with a history of high-risk gastrointestinal stromal tumor (GIST), identification of a *CTNNB1* mutation in a "metastatic peripancreatic GIST," led to reevaluation of the pathology and immunohistochemistry, with revision of the diagnosis to desmoid tumor and maintenance of current therapy.

Most adult carcinomas lack site-specific translocation or mutational events; however, the etiologic associations of hypermutation signatures can provide diagnostic insights. In case 6, identification of a mismatch repair deficiency signature in a high-grade uterine malignancy suggested a diagnosis of undifferentiated endometrial carcinoma and raised the possibility of Lynch syndrome. In case 7, a patient diagnosed with primary lung squamous cell carcinoma, the presence of a UVA signature indicated that the tumor was actually a metastasis from a cutaneous site.

Patient selection for clinical trial enrollment. Several studies have shown that the frequency of actionable cancer gene alterations follows a "long tail" distribution (13), that is, alterations that may specify response to targeted therapy, and with significant clinical implications, may be seen rarely at individual cancer centers. To explore this hypothesis, we sought to mine our genomic and clinical knowledge base to identify exemplary cases that might inform studies of therapeutic utility in particular genetic contexts.

Tumor suppressor genes as therapeutic targets. A 59-year-old woman with advanced ovarian papillary serous carcinoma was followed over 5 years, during which time she underwent radical cytoreductive surgery and multiple courses of chemotherapy, a clinical trial of olaparib and cediranib, surgical debulking, and ongoing catheter drainage due to ascites. Testing of archival tumor showed biallelic *TSC2* inactivation (Figure 3A). The woman was enrolled on a clinical trial of everolimus and had a dramatic response within a few weeks, including a decrease in her ascites from 2 l to 60 ml, CA-125

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Table 4. Tumors with *TSC1* or *TSC2* mutation and copy number loss

Diagnosis	Tumor fraction (%)	Alteration	AF % (reads)	Comment	Clinical follow-up
Ovary, high-grade serous carcinoma	90	*TSC2* Q417*	84 (13)	Biallelic loss	Everolimus therapy, response
Ovary clear cell carcinoma	80	*TSC2* R1743W	71 (63)	Biallelic loss	Lost to follow-up
Invasive papillary urothelial carcinoma of bladder, high grade	50	*TSC1* K834_splice	47 (123)	Biallelic loss	BCG therapy only
Noninvasive papillary urothelial carcinoma of bladder, low grade	50	*TSC1* K875_splice	59 (164)	Biallelic loss	Surgery only
Noninvasive papillary urothelial carcinoma of bladder, low grade	80	*TSC1* Q55*	63 (128)	Biallelic loss	Surgery only
Invasive papillary urothelial carcinoma of bladder, high grade	60	*TSC1* D36_splice	65 (90)	Biallelic loss	Surgery and chemotherapy with NED
Noninvasive papillary urothelial carcinoma of bladder, high grade	70	*TSC1* S836*	72 (87)	Biallelic loss	Surgery only
Invasive urothelial carcinoma of renal pelvis, high grade	90	*TSC2* homozygous del	NA	Biallelic loss	Surgery only
High-grade uterine sarcoma, favor malignant PEComa	90	*TSC2* homozygous del	N/A	Biallelic loss	DOD
Poorly differentiated NSCLC, possible LCNEC	40	*TSC1* A68fs	34 (228)	Biallelic loss	DOD
Poorly differentiated carcinoma, favor lung primary	90	*TSC1* W247fs	62 (191)	Indeterminate copy loss	DOD
		TSC1 S1080L (VUS)	69 (186)	Indeterminate copy loss	DOD
Angiomyolipoma	90	*TSC2* R786_splice	79 (96)	Copy neutral LOH	Surgery only
Merkel cell carcinoma	90	*TSC2* A100fs	9 (43)	Subclone	Chemoradiation with PD

AF, allele fraction; DOD, dead of disease; LCNEC, large-cell neuroendocrine carcinoma; LOH, loss of heterozygosity; NED, no evidence of disease; NSCLC, non-small-cell lung carcinoma; PD, progressive disease; PEComa, perivascular epithelioid cell tumor; VUS, variant of unknown significance.

levels that dropped from 1,816 units/ml to 348 units/ml after 4 weeks of treatment (Figure 3, B and C), and a decrease in index lesions by 10% after 2 months. She had substantial improvement in her quality of life and was able to return to work and her normal activities.

The OncoPanel database was analyzed for other tumors with similar evidence of 2-copy *TSC2* loss. A total of 56 loss-of-function (LOF) variants in *TSC1* or *TSC2* were detected across 43 tumors (1.1%; Supplemental Figure 2). Of these, 22 variants in 10 tumors occurred in hypermutated samples. To reduce the risk of false discovery (31), these were removed from further analysis. Of the 33 remaining, 12 showed evidence for both a LOF coding mutation and loss of one copy of the gene or homozygous gene deletion (Table 4). Using variant allele fraction to exclude predicted subclonal events, 10 (0.2% overall) showed biallelic *TSC1* or *TSC2* loss, including 6 urothelial carcinomas (representing 5% of 122 total).

Rare activating ERBB2 mutations in ovarian cancer. A 48-year-old woman with low-grade ovarian serous carcinoma (originally diagnosed age 18) was managed at progression with chemotherapy, followed by multiple phase I clinical trials, including monoclonal antibody therapy against HER3 and combined MEK1/2 and PI3K inhibitors, all with symptomatic and radiographic progression. Analysis of her recurrent tumor identified an *ERBB2* Tyr772_Ala775dup mutation (Figure 4A), which prompted off-label use of trastuzumab and navelbine with symptomatic improvement and radiographically stable disease for 21 months at last follow-up.

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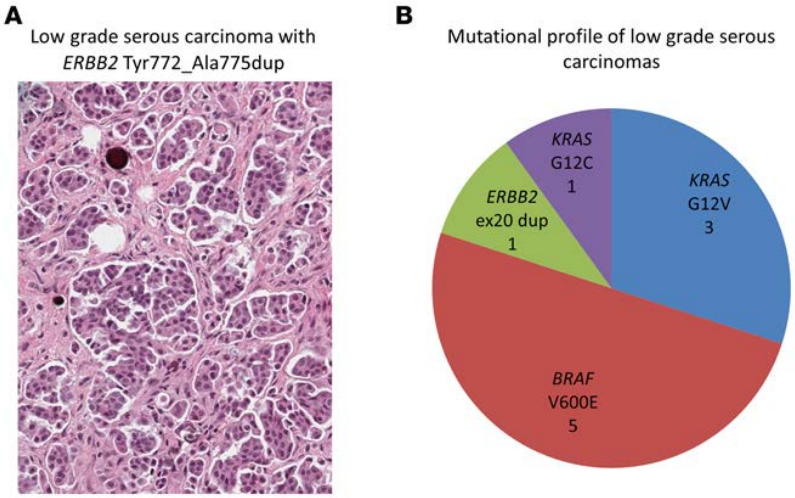

Figure 4. Achieving disease control using ERBB2-targeted therapy in low-grade serous carcinoma. (**A**) Peritoneal biopsies in a 48-year-old patient with a longstanding history of low-grade serous carcinoma show a monomorphic proliferation of epithelial cells with bland cytologic features and frequent psammoma bodies (original magnification, ×400). Tumor genotyping revealed an ERBB2 duplication mutation in exon 20. (**B**) Ten cases of low-grade serous tumors were sequenced: a mean of 3 single nucleotide variants (SNV) (2.4 SNVs per Mb) per case was seen. Two KRAS G12V-mutated cases showed an additional activating PIK3CA mutation; one KRAS G12V-mutated case showed an additional BRAF D594G mutation. Notably, none had concurrent mutations in tumor suppressor genes.

Low-grade ovarian serous carcinomas in this cohort were characterized by mutations in *KRAS*, *BRAF* V600E, or *ERBB2* and a few other genomic alterations (Figure 4B), raising the possibility that the prolonged disease control with ERBB2 inhibitors observed in this patient's tumor may be attributable to single-pathway dependency. Known activating *ERBB2* mutations were detected in 49 cases (1.3% of the OncoPanel cohort) and were enriched in lung, bladder, and low-grade gynecologic tract tumors (Table 5 and 6). All 6 endometrial adenocarcinomas with activating *ERBB2* mutations occurred in the context of hypermutation. In breast, colon, and gastric cancers, *ERBB2* mutations co-occurred with activating mutations in *KRAS*, *BRAF*, and *EGFR*; whether this reflects tumor heterogeneity or simultaneous driver events in the same clone is unknown, though the mutant allele fractions did not suggest subclonal events. *ERBB2* activating mutations were the only clear functional alterations in a subset of tumors, most commonly lung adenocarcinomas, as well as in a case of spinal cord neurofibroma and soft tissue schwannoma. This genomic context may lend some insight into the relative likelihood of success of targeted inhibition of *ERBB2* mutations in different tumor types (32).

An EGFR splice mutation associated with erlotinib response. A 59-year-old male never smoker presented with lung adenocarcinoma metastatic to bone and brain. The patient underwent resection of the brain mass and radiation to the brain and bony metastases. Genotyping was negative for common *EGFR* mutations and *ALK* FISH was negative. He was started on first-line cisplatin/pemetrexed therapy. After 5 months, brain MRI showed asymptomatic slow progression. The brain metastasis was profiled, revealing an unusual *EGFR* intron 19 splice mutation (EGFR c.2283+1G>A [p.D761_splice] and c.2283+12_149del) in addition to high-level amplification of *EGFR* (Figure 5A). Based on its location within the splice site sequence, this variant is predicted to abrogate the canonical splice site, leading to read through to an alternative splice donor site in the intron that would result in a 4–amino acid insertion that translates to A763_Y764insMSSW. Other insertions reported at this site are associated with sensitivity to EGFR inhibitors, unlike most other EGFR exon 20 insertion mutations (33). Although this particular *EGFR* alteration has not been classified as tyrosine kinase inhibitor (TKI) sensitizing, the patient was started on 150 mg erlotinib daily, with reduction in the lung mass on initial restaging (Figure 5A). The brain metastases progressed after 5 months, after which the patient was treated with whole-brain radiation therapy. He experienced durable extracranial response on erlotinib for a total of 12 months before developing systemic progression in bone and lung (34). When erlotinib was stopped in preparation for subsequent therapy, systemic disease rapidly progressed. The patient was refractory to further therapy and died 2 months after stopping erlotinib.

Amplification of AXL associated with response to an Axl kinase inhibitor. A 56-year-old male, never smoker, was diagnosed with metastatic lung adenocarcinoma, with bilateral mediastinal lymphadenopathy and pleural carcinomatosis. Genomic analysis demonstrated focal gain of 19q12-13.2, including *CCNE1*, *AKT2*, and *AXL* as well as *CDKN2A/B* loss. The tumor was wild-type for *EGFR*, *ALK*, *ROS1*, *RAS*, and *MET*. He was treated with carboplatin/pemetrexed and bevacizumab, followed by docetaxel, with a best response of stable disease. Based on *CCNE1* gain, he was enrolled in a clinical trial combining an ATR inhibitor with cisplatin. His course was complicated by pneumonia and continued disease progression. The mean log2 copy ratio for *AXL* was 0.94, and tumor purity based on pathologist's review was estimated at 60%, with approximately 5 normalized copies

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Table 5. Tumors with *ERBB2* activating mutations across the cohort with clinical follow-up

Diagnosis	*n* (%)[A]	Other tier 1–3 alteration	HER2 TX	Standard TX	Other clinical trial	Surgery only	DOD	Lost to f/u
Lung	13 (3)							
ADC stage I		*ERBB2* amp				1		
ADC stage I		None				1		
ADC stage IIIA		None		1				
ADC stage IV		*ERBB2* amp						
ADC stage IV		*NF1*					1	
ADC stage IV		None	1					
ADC stage IV		None	1					
ADC stage IV		None	1					
ADC stage IV		None		1				
ADC stage IV		None					1	
ADC stage IV		None					1	
ADC stage IV		None					1	
ADC stage IV		None						
Bladder	11 (9)							
HG PUC stage I		*RB1*, *TP53*				1		
HG PUC stage I		*ERBB2* as subclone						1
HG PUC stage I		*TP53*, hypermutated						1
HG PUC stage III		*TP53*				1		
HG PUC stage IV		*RB1*, *PIK3CA*					1	
HG PUC stage IV		*RB1*		1				
HG PUC stage IV		*RB1*			1			
HG PUC stage IV		*TP53*					1	
HG PUC stage IV		*PIK3CA*, *PTEN*, hypermutated		1				
HG PUC stage IV		*CDKN2A* and *CDKN2B* del		1				
HG PUC stage IV		NA[B]						1
Gynecologic tract								
Endometrium	6 (4)							
EMC grade 1		MMRd signature				1		
EMC grade 1		MMRd signature				1		
EMC grade 1		MMRd signature				1		
EMC grade 2		MMRd signature				1		
EMC grade 3		POLd signature				1		
EMC grade 3		MMRd signature		1				
Mixed EMC/serous		*TP53*, *KRAS*, *NF1*		1				
Cervix, SCC	1 (3)	*PIK3CA*		1				
Ovary, low-grade serous CA, recurrent	1 (10)	None		1				
Total	32 (1.3)		3	9	1	9	6	3

[A]Percentage of tumors with this diagnosis. [B]Tumor content was at the minimum accepted, therefore other alterations cannot be excluded. ADC, adenocarcinoma; amp, amplification; CA, carcinoma; del, deletion; DOD, dead of disease; EMC, endometrial adenocarcinoma; f/u, follow-up; HG PUC, high-grade papillary urothelial carcinoma; MMRd, mismatch repair deficient; POLd, polymerase defect; TX, treatment.

relative to an assumption of diploidy (see Methods). He was enrolled on a phase I clinical trial of MGCD265, a TKI targeting Met and Axl. Within a few days of starting treatment, he had a dramatic response and no longer required oxygen support; he was able to bike 7 miles a day after 1 month of treatment. Restaging scans after 8 months of treatment demonstrated a 66% decrease in index lesions (Figure 5B).

Based on this response, we investigated further *AXL* amplification events. A similar level of copy gain (approximately 3–6 copies), minimally including *AXL* but often extending to *CCNE1*, was identified in 7 cases (0.2% of our population; Table 7). High copy gain of *AXL* (copy number >6) was detected in one case in the cohort (0.02%) — an endometrial serous carcinoma with approximately 12 copies.

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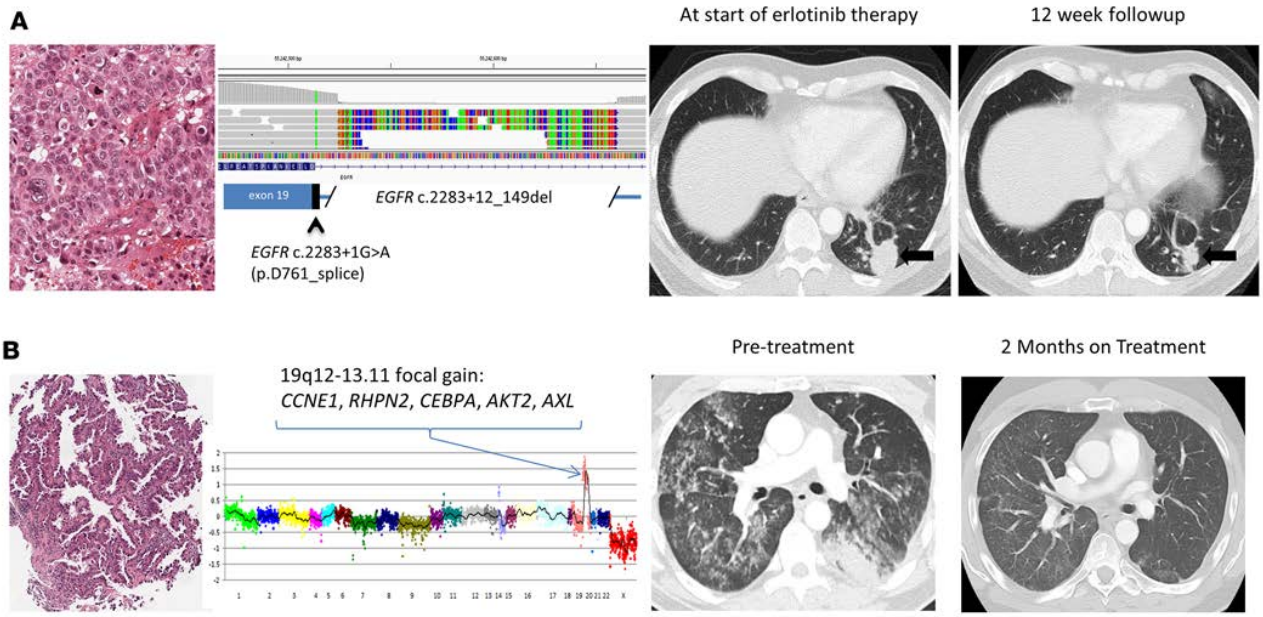

Figure 5. Clinical effect of genomic profiling. (**A**) A nonsmoking man with metastatic lung adenocarcinoma to the brain (original magnification, ×400) had a complex EGFR intron 19 mutation involving the splice junction (EGFR c.2283+1G>A [p.D761_splice] and c.2283+12_149del) in 92% of 780 sequencing reads. The read count is consistent with high-level amplification of the mutated EGFR allele. Baseline chest CT prior to the initiation of erlotinib therapy demonstrated a 3.8-cm mass in the left lower lobe (arrow). On a follow-up chest CT at 12 weeks of erlotinib therapy, the mass has decreased in size, measuring 2.5 cm (arrow), representing response to therapy. (**B**) A nonsmoking man with lung adenocarcinoma (original magnification, ×100) with pleural carcinomatosis had focal gain of chromosome 19q12-13.11, including AXL. Baseline chest CT prior to initiation of MET-AXL inhibitor therapy demonstrated bilateral lung nodules and ground glass infiltrates. Follow-up chest CT at 2 months shows near-complete resolution of the lung infiltrates.

We used publicly available data from The Cancer Genome Atlas (TCGA) (35, 36) to further evaluate the frequency of *AXL* amplification across 11,613 tumors representing 34 distinct diseases. We included any tumor with an *AXL* log2 ratio of ≥0.36 (i.e., corresponding to a single-copy gain in a tumor of at least 60% tumor content), filtering to exclude whole chromosome or arm-level copy number changes. Focal amplifications of *AXL* were identified in 0.65% (n = 76) of tumors (Supplemental Figure 3); high-level amplifications were uncommon (n = 3), with an average size of 1.52 ± 1.23 Mb, and identified exclusively in ovarian serous carcinomas. Low-level amplifications occurred in 0.63% (n = 73), including in ovarian cancer (n = 19), sarcoma (n = 13) lung squamous cell carcinoma (n = 11), breast cancer (n = 8), and lung adenocarcinoma (n = 3). Axl activation has also been reported as a mechanism of resistance following EGFR TKI therapy in lung cancer, with preclinical studies suggesting a therapeutic role for Axl inhibitors in this setting; in our cohort, we identified one such case (Table 7) (37–39).

In the first year of study, 50 patients (n = 50) had OncoPanel testing initiated by clinicians in the Early Drug Development Center, the facility for phase I clinical trial enrollment at DFCI. Because patient selection for many of these phase I trials requires a genomic biomarker, we looked at the frequency of potentially actionable variants in this cohort and clinical trial enrollment. The patients in this cohort had a variety of cancer diagnoses (Supplemental Table 7). 43 OncoPanel reports were generated (7 had insufficient material for testing). 31 of 50 (62%) patients had at least one tier 2 single nucleotide variation or copy number variant detected, similar to the results of our cohort overall. In this setting, 16 (32%) of these patients — just over half (52%) of those with an actionable or informative result and 32% of the full group — were enrolled on a genomic-based clinical trial (Supplemental Table 7).

Discussion

In recent years, a number of academic cancer centers, hospitals, and commercial entities have established genomic profiling programs (11, 12, 16, 17, 20, 24, 26, 40) that differ in terms of technology and gene panel content (varying from tens to hundreds or thousands of genes) as well as target patient populations (specifying cancer type or stage of disease, for example) (16, 21) but are similar in that they apply multigene testing

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Table 6. Tumors with *ERBB2* activating mutations across the cohort with clinical follow-up

Diagnosis	*n* (%)[A]	Other tier 1–3 alteration	HER2 TX	Standard TX	Other clinical trial	Surgery only	DOD	Lost to f/u
Gastrointestinal tract								
Colon	4 (2)							
ADC		*BRAF* (V600E)						1
ADC		*KRAS*, *TP53*					1	
ADC		*TP53*		1				
ADC		*TP53*		1				
Anus SCC	1 (10)	*PIK3CA*			1			
Stomach								
Signet ring ADC	1 (3)	*KRAS*, *EGFR* (L861Q), *CDH1*					1	
Upper GI unknown primary ADC	1 (14)	*CTNNB1*, *PTEN*, hypermutated					1	
Pancreas ADC	1 (4)	*ERBB2* amp					1	
Breast	4 (2)							
PD IDC, ER+, stage IV		*PIK3CA*					1	
PD IDC, ER+, stage IV		*TP53* & *BRCA1*			1			
TNBC, stage IV		*KRAS*, *PIK3CA*,*TP53*				1		
ILC, ER+, stage IV		*CDH1*				1		
Other								
Spinal cord neurofibroma	1 (100)	None		1				
Soft tissue schwannoma	1(100)	None				1		
Skin apocrine CA	1 (3)	*TP53*	1					
Head and neck HPV+ SCC	1 (0.6)	*ERBB2* amp	1					
Total	16 (1.3)		2	3	2	3	5	1

[A]Percentage of tumors with this diagnosis. ADC, adenocarcinoma; amp, amplification; DOD, dead of disease; ER, estrogen receptor; f/u, follow-up; GI, gastrointestinal tract; HG PUC, high-grade papillary urothelial carcinoma; ILC, invasive lobular breast cancer; PD IDC, poorly differentiated invasive ductal carcinoma; SCC, squamous cell carcinoma; TNBC, triple-negative breast cancer; TX, treatment.

to cancer patients, with a goal of more effectively triaging those patients to the appropriate therapeutic regimen — whether on-label, off-label, or experimental/clinical trial. In most cases, a categorization strategy is applied to the genomic profile in order to obtain a shortlist of potentially "actionable" or "informative" alterations that can be used to tailor treatment options in an individualized manner. Depending on the number of genes tested, and the strategy for categorizing such events, the proportion of patients that have a potentially actionable alteration varies but is in the 75%–90% range. While encouraging, the proportion of patients that actually have this information used for clinical management remains much lower, in the 5%–10% range. There are a multitude of reasons for this, ranging from patient preferences (20) and physician comfort with genomic data (22), to biologically relevant information, such as time of testing in the course of a patient's disease, availability of clinical trials, and cost of off-label use of drugs. Despite these barriers, there is evidence to support the clinical utility of molecularly targeted therapy in specific indications (4, 21, 24, 25), though heavily pretreated patients may not benefit to the same degree (25).

We report a large multi-institution clinical sequencing effort using a several-hundred-gene assay that profiles a broad, unselected cancer patient population, spanning adult and pediatric patients, early- and late-stage

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Table 7. Pathology and follow-up for tumors with *AXL* copy number gain

Diagnosis	Tumor fraction (%)	*AXL* CN	Genes in amplicon	Other alterations	CNI	Clinical follow-up
Primary peritoneal high-grade serous carcinoma	80	3.3	*CCNE1*, *RHPN2*, *CEBPA*, *AKT2*, *AXL*	*TP53* P278L, *PTEN* Q171*[A], *PTEN* G209*[A]	Y	Failed PTEN inhibitors, progressive disease on chemotherapy
Follicular thyroid carcinoma, angioinvasive	90	3.4	*CCNE1*, *RHPN2*, *CEBPA*, *AKT2*, *AXL*	*TP53* R213Q	N	Li Fraumeni syndrome, NED at 1 year follow-up
Endometrial serous carcinoma	80	3.8	*CCNE1*, *RHPN2*, *CEBPA*, *AKT2*, *AXL*	*TP53* V272L	Y	Abraxane therapy with PD
Lung adenocarcinoma	80	4.2	*RHPN2*, *CEBPA*, *AKT2*, *AXL*	*EGFR* L858R, *EGFR* T790M, *CCND1* amp	N	Progressed through AZD9291 and pemetrexed therapy
Female genital tract carcinosarcoma	80	5.2	*AKT2*, *AXL*	*TP53* P278S	Y	DOD
Extremity leiomyosarcoma	90	5	*RHPN2*, *CEBPA*, *AKT2*, *AXL*	*RB1* Q846fs	Y	Surgery only
Lung adenocarcinoma	60	5.1	*CCNE1*, *RHPN2*, *CEBPA*, *AKT2*, *AXL*	*CDKN2A*, *CDKN2B* del	N	Increased AXL copy gain on progression biopsy, AXL inhibitor with response
Endometrial serous carcinoma	60	12	*CCNE1*, *AKT2*, *AXL*	*TP53* V274F	Y	Progression on chemotherapy, DOD

[A]*PTEN* variants present at subclonal levels (4%–8% allelic fraction). amp, amplification; CN, copy number; CNI, copy number instability; del, deletion; DOD, dead of disease; NED, no evidence of disease.

disease, solid tumors, and hematologic malignancies. Our cohort is reflective of the full spectrum of patients seen at our institutions but also incorporates biases in terms of the ordering physician, perceived utility of the test in a disease-specific context, and the availability of specimen to be tested. All genomic data generated in our CLIA-certified laboratory is deposited into a research knowledge base that enables linkage to clinical information and will enable future studies as well as enabling the prospective identification of patients for genomically matched clinical trials (Figure 6).

Our data show that, across an unselected cancer cohort, genomic profiling in a CLIA-certified laboratory is technically feasible. This requires rigorous validation of the lab chemistries and the development and implementation of comprehensive analytical algorithms, followed by manual review and signout by a pathologist. Our OncoPanel test performs with high sensitivity, specificity, accuracy, and reproducibility for the detection of mutations, copy number changes, and structural rearrangements.

Because we introduced an updated gene menu and sequencing chemistries after 1 year of sequencing, we have chosen to restrict our genomic and correlative analyses to data collected in that first year; however, many features of the operation have remained steady since 2013–2014. The consent rate for patients across our institutions is approximately 70%. Over the course of sequencing more than 12,000 samples to date, we have observed a 74% rate of available pathology material, a 59% rate of adequacy for sequencing, and a 56% rate overall of generating a sequencing report with a 95% sequencing success rate. These metrics are similar, albeit with a somewhat higher rate of specimen retrieval and histologic adequacy overall as compared with the first year of testing (Figure 1B). Overall, just over half of patients with consent and a test order receive a result. The largest barrier to obtaining adequate tumor tissue for sequencing under this research protocol was lack of access to many patient samples that were located at outside institutions and were not actively retrieved for OncoPanel testing. We anticipate that moving toward clinical testing should significantly improve our ability to obtain and test tumor specimens; once sufficient material was received into the laboratory for pathology review, 73% were scored as sufficient for genomic profiling. Advances in alternative testing approaches, including testing of circulating tumor cell–free DNA, should bring genomic testing to a broader population of patients lacking adequate tissue biopsy samples. The median turnaround time for a result was 5.3 weeks after receipt of sample — this is reasonable for a research test but not a clinical assay. Streamlining processes and making improvements in lab chemistries and analytical pipelines will

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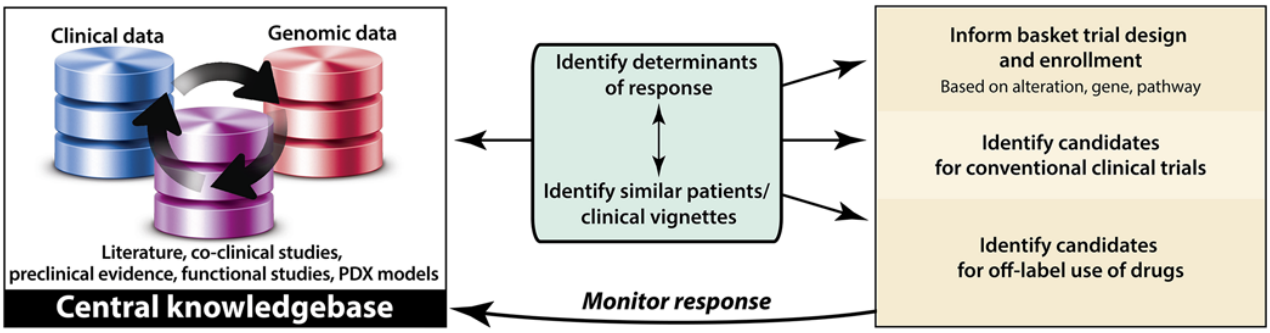

Figure 6. A knowledge base is key to maximizing the effect of genomic testing on precision medicine. Genomic data are tiered according to clinical actionability based on available evidence, incorporating published literature and supporting evidence from preclinical and functional studies, and feed into a central knowledge base that interfaces with clinical data repositories. These data can be queried as part of IRB-approved clinical trials and research studies and used to identify determinants of response as well as patients with similar genomic profiles that may be candidates for targeted therapies. Going forward, this mechanism can also be used to enable more rational design of basket trials.

allow us to achieve a more reasonable clinical timeframe for the next phase of our program.

Biologically, broad genomic profiling across cancers builds upon the results of large, multi-institutional research studies, like TCGA and International Cancer Genome Consortium, and also enables the identification of potentially actionable alterations in rarer cancers. Unique to this cohort is the fact that few restrictions were imposed on the time (in course of disease, grade, or stage) at which genomic testing was ordered, which allows us to broadly compare genomic profiles of primary and metastatic disease. Our data suggest that for certain tumor types, patterns of actionable genomic alterations may vary between primary and metastatic sites and raise the possibility that different information may be gleaned from different sites of disease. Future studies examining serial biopsies from individual patients should further clarify the clinical significance of genetic heterogeneity.

In this study, we demonstrated that enterprise-level genomic profiling was technically feasible and useful for retrospective research purposes. The ultimate goal of implementing such a program, however, is to determine clinical utility — i.e., to improve outcomes for cancer patients. As this was a research test (rather than a clinical trial), we are not systematically able to evaluate the clinical impact of genomic profiling; rather, our analyses are anecdotal. Nonetheless, these "exceptional responder" cases allow us to make new observations about driver alterations in cancer, and retrospective analyses of similar genomic events allow us to make inferences about larger patient cohorts and additional challenges that need to be addressed to maximize utilization of precision cancer medicine.

Although our sequencing program was not originally conceived as a diagnostic service, the unbiased approach to tumor genomic analysis uncovered multiple instances in which sequence-level findings revealed genomic changes that are characteristic of certain tumors not originally considered in the pathologic differential diagnosis and led to clarified or revised diagnoses. The frequency with which sequencing can be expected to lead to a change in diagnosis is difficult to estimate from this study, as we did not systematically capture these types of events. However, it is clear that this type of assay can bring added diagnostic value, even in a setting in which expert pathology review is standard. Furthermore, the results of implementing profiling across all cancer types provide evidence that the systematic use of broad-scale genomic sequencing enables precision therapeutics and is likely to be highly valuable at institutions that offer a broad portfolio of clinical trials. An exemplary finding in our cohort was the presence of biallelic *TSC1*/*TSC2* inactivation in a small but appreciable percentage of bladder carcinomas, consistent with previous reports of response to everolimus (41, 42), indicating that these should be taken into account in the design of mTOR inhibitor trials, including ongoing basket trials (as is the case in our institution).

More strikingly, our comprehensive testing strategy led to the detection of uncommon and unanticipated driver events. For example, we found an *EGFR* intron 19 splice mutation in lung adenocarcinoma associated with a modest but prolonged response to erlotinib therapy; further investigation is needed to determine the degree to which *EGFR* splice variants contribute to EGFR activation and TKI sensitivity in patients with lung adenocarcinomas. In another example, we identified a case of

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amplification of the *AXL* tyrosine kinase gene in the absence of other known driver alterations in lung adenocarcinoma associated with clinical response to an Axl inhibitor. The rarity of this alteration in both our cohort and TCGA raises the issue of identifying sufficient numbers of a genotype to enable clinical trials. One possible solution is enabling multi-institutional basket trials and sharing of genomic data to identify such patients — an endeavor that has been endorsed by the American Association for Cancer Research in Project GENIE, to which we are a contributing institute. Incorporation of complementary technologies, such as RNA sequencing, may be particularly fruitful when seeking targets such as Axl that are subject to epigenetic or RNA-based regulation (39).Finally, our analysis provides some understanding of the feasibility and actionability of systematic cancer profiling of a large, unselected patient population. Analysis of the frequency of gene alterations across all tumor types suggests that over two-thirds of tested tumors have some potentially targetable alteration. This agrees with previously published studies that found a similarly high proportion of patients with potentially actionable alterations (64%–90%, refs. 4, 18, 19), though it has also been reported that a much smaller proportion of patients (5%–11%) are actually treated based on the mutational profile or enrolled on a clinical trial (16, 19, 20). Our focused examination of patients with targetable *TSC1/2* or *ERBB2* or *AXL* alterations suggests that there are patients with actionable alterations in these select targets across disease types, but only approximately 10% had their genomic profile used for clinical management (1 of 13 *TSC1/2* alterations [Table 4]; 5 of 49 *ERBB2* activating mutations [Tables 5 and 6]; 1 of 8 *AXL* copy gain [Table 7]; and total 7 of 70 cases in which genomic profile was used clinically). We identified several reasons for the low rates of trial enrollment, including standard of care therapy providing effective disease control, death due to disease before genomically driven therapy could be considered, or enrollment in a clinical trial not related to the genomic results. Specific to the research nature of this program, our cohort contains many early-stage patients that require no further therapy beyond surgical resection (Tables 4 and 5). In addition, certain limitations on return of results imposed by the research protocol meant that some potentially actionable alterations in patients' cancers could not have clinical impact, suggesting the importance of migrating our research genomic test to a routine clinical test. In a chart review of 50 advanced cancer patients who had an OncoPanel test ordered in the phase I clinical trials group, 16 (32%) were found to have changes in recommendation for trial options, though additional factors (such as progressive disease) must be considered before a patient is enrolled on a trial. Although the number of patients analyzed was small and the data are context specific, this demonstrates that the rate of genomically driven enrollment in this setting is relatively high, reflecting the specific expertise of this group of oncologists and the access to a portfolio of genomic-based trials, and indicates that the combination of comprehensive molecular testing and ready access to genomically driven trials can improve rates of clinical trial enrollment.

There are many open questions regarding the appropriate scope and scale of cancer genome profiling in the clinical setting, particularly in relation to the decreasing cost of DNA sequencing. One consideration is the comparison of whole-exome sequencing or whole-genome sequencing to more targeted gene panels. A major advantage of gene panels — as opposed to whole-exome sequencing or whole-genome sequencing — is the option to sequence without a matched normal. While tumor-only sequencing is more feasible for logistical (i.e., ability to obtain a matched normal specimen for each patient) and financial (cost of performing more sequencing) reasons, we recognize that there are limitations in the detection of relevant cancer-susceptibility germline events, among others (43). To address this issue, we developed an informatics pipeline to filter common single nucleotide polymorphisms present in public and internal noncancer populations; to incorporate data on the allelic fraction (with contextual purity and ploidy information) of a mutation; and to apply a tiering strategy to the interpretation of alterations. In a study of a subset of 91 cases comparing our filtering approach, including pathologist review, with matched tumor-normal sequencing (44), 54 germline variants were reported — i.e., not filtered out by our pipeline. 93% of these (50 of 54) were reported as tier 4 "variants of unknown significance," 3 were reported as tier 3, and only 1 was reported as a tier 2 result. This resulted in "false somatic" calls reported as tier 3 or higher in 4 of 91 (4.4%) of patient samples. This finding is in keeping with the rate of significant occult germline variants found in cancer patients undergoing paired tumor-normal sequencing at other institutions (45).

In practice, we report potential germline variants conservatively, according to clinical relevance. Where there was a suspicion of germline status of a pathogenic allele, the data were communicated to

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the ordering physician with a recommendation to correlate with additional medical history and confirmatory testing, if deemed appropriate. We acknowledge that incorporating matched normal sequencing would allow the definitive ascertainment of somatic versus germline status.

Although our initiative was not designed to measure clinical outcomes explicitly, it nonetheless lays the groundwork for more systematic study of the effect of genomics on clinical practice and patient outcomes. Indeed, this initiative has enabled a large number of other more focused studies of common and uncommon tumor types and, in particular, has permitted prospective identification of relatively uncommon genomic variants to facilitate clinical trial enrollment and biomarker studies (46–54). Migrating to a clinical test, identifying when in the course of a patient's disease genomic profiling should be used, and triaging patients in real-time for clinical trial enrollment should contribute to determining clinical utility on a broader scale. Profile enlivens a framework (see Figure 6 for schematic) whereby individual patient experiences can be extrapolated to systematic investigations on genomic correlation with cancer outcomes that lead to new avenues of scientific inquiry, challenge assumptions about cancer diagnosis, and enable extension of novel and more effective treatment approaches to new sets of patients.

Methods

See the Supplemental Methods, and Supplemental Tables 1, 8 and 9, for details on study design, patient recruitment, assay design and technical validation, data analysis and statistics, and clinical use.

Statistics. Categorical comparisons were performed using Fisher's exact or Chi square tests with Bonferroni correction for multiple comparisons. Sample means were compared using a 2-tailed Student's *t* test assuming equal variance. *P* values of <0.05 were considered significant. Sensitivities and specificities with 95% confidence intervals were calculated using a publicly available clinical calculator (vassarstats.net). Confidence intervals were calculated using the Hmisc library in R using the binconf() function set to the "Wilson" calculation method.

Study approval. This study was performed with approval from the DFCI IRB (DFCI IRB protocol 11-104). Written informed consent was obtained from participants prior to inclusion in this study

Author contributions

LMS, MM, LAG, NIL, and LEM wrote the manuscript. WCH, PWK, BEJ, MM, LAG, BJR, NIL, and LEM designed the study. LMS, PS, EC, AMD, FCK, EPG, YJ, LLR, VRR, PD, AHB, AHL, NIL, JDC, and LEM analyzed the data. RPA, TJP, PVH, FCK, ART, and MD created the informatics pipeline and internally developed software tools. KD, KAJ, AC, MH, SR, JMC, UM, GRO, DJK, MN, and GIS contributed clinical data. RC, VT, and JC supported the MGCD265 clinical trial.

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SUPPLEMENTAL METHODS

Patient recruitment

All adult and pediatric cancer patients presenting to our institutions are invited to participate in the Cancer Research Study. Upon receipt of a test requisition and signed informed consent from the patient, samples are selected and analyzed in a CLIA-certified laboratory- the Center for Advanced Molecular Diagnostics (CAMD) at BWH. There were no restrictions imposed on the cancer type (both solid tumors and hematologic malignancies) to be tested, the stage or grade of disease (however in situ lesions were excluded), course of disease, or whether a primary or a metastatic sample was profiled.

OncoPanel

Eligible samples included formalin-fixed paraffin-embedded (FFPE) tissue sections or cytologic cell blocks, freshly frozen tissue or cell pellets, fresh peripheral blood or bone marrow, and slides containing smear preparations. Tumor content was estimated by an anatomic pathologist from corresponding stained slides, when available, or by cell count/flow cytometry for fresh blood or bone marrow samples. Samples with at least 20% malignant cells were analyzed. For tissue sections, including from surgical resections, biopsies, and cytology cell blocks, regions of adequate cancer measuring at least 3 mm in greatest linear dimension were either manually dissected off corresponding unstained sections or cored directly from the paraffin block. Paired germline samples were not analyzed. DNA was isolated with a commercial kit (Qiagen, Valencia, CA), following the manufacturer's instructions. DNA was quantified (PicoGreen, ThermoFisher Scientific, Waltham, MA), and 200 ng of DNA was used for library preparation (with a low input threshold of 50ng).

Hybrid-capture libraries were prepared as described previously (14, 55). DNA libraries were hybridized to a set of custom-designed capture probes targeting the full coding regions of 275 genes plus selected intronic regions of 30 genes, for a total of 282 unique genes/regions (**Supplemental Table 1**). This

report is focused on testing from August 2013 to July 2014; a new version of the test was implemented at the end of this period. Sequencing was performed using an Illumina HiSeq 2500 with 2x100 paired-end reads to a mean target coverage of 187X unique, high quality, mapped reads per sample (range 50 to 844X; 50X minimum required to pass).

In order to confirm patient identity and eliminate sample mix-up and cross-contamination, specimens were genotyped across 48 single nucleotide polymorphisms (SNPs) selected for 45 - 55% heterozygosity. Briefly, stock DNA was PCR amplified across each of the 48 SNPs follow by removal of primers and unincorporated nucleotides by SAP treatment. SNP identity at each of the targeted sites was resolved by single base extension and analysis by MALDI-TOF mass spectrometry using the MassArray System (Agena Bioscience). Genotyping results were correlated with results obtained during OncoPanel sequencing. Passing criteria required >80% concordance across genotyped loci between the two technologies. For specimens failing to meet 80% concordance with the fingerprinting assay, additional investigation is performed to identify similarly contaminated specimens and/or similar genomic profiles in a batch. Barring evidence of laboratory contamination, which will typically trigger specimen failure, additional investigation into the patient's history may be performed to determine if there is a clinical basis for chimerism (i.e. prior hematopoietic stem cell transplantation).

Data analysis

Data was analyzed by an internally-developed bioinformatics pipeline composed of reconfigured publically-available tools and internally-developed algorithms (VisCap Cancer, Phaser, BreaKmer(55)). Pooled sample reads were demultiplexed using Picard (http://broadinstitute.github.io/picard/command-line-overview.html), aligned to Human Genome Reference Consortium reference sequence GRCh37p13 (BWA(56)) and duplicate reads removed. GATK(57) was used to refine alignments around insertion/deletion (indel) sites and to perform quality

score recalibration. Single nucleotide variants (SNVs) were called using MuTect(58) and indels using Indelocator (http://www.broadinstitute.org/cancer/cga/indelocator). Annotation was performed using Oncotator(59). Multiple SNVs identified in the same codon were further analyzed using the GATK ReadBackedPhasing tool to determine if they represent a di- or tri-nucleotide change.

Because tumor tissues were tested without a paired normal from individual patients, additional informatics steps were taken to identify common single nucleotide polymorphisms (SNPS): any SNP present at >0.1% in Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/ accessed May 30, 2013) or present in dbSNP was filtered, however variants also present in at least twice in COSMIC were rescued for manual review. Variants that appeared two or more times in a panel of 150 normal samples sequenced in-house and are not present in COSMIC are also filtered. Following computational filtering, manual interrogation by a molecular pathologist was applied to consider tumor purity, ploidy and estimated allele fraction at each site, along with contextual information such as tumor type.

VisCap Cancer calls copy number changes based on log2 ratios that are calculated using a normalized depth of coverage against a median from a panel of normal (non-cancer) samples. Circular binary segmentation (CBS) is used to segment the data; segments are called via strict thresholding; the algorithm is configurable for different tumor fractions. Estimated (approximate) copy number was calculated using the ABSOLUTE method(60) and the formula $CR = (TC/2)*P + (2/2)(1-P)$, where CR = median copy ratio for all intervals in a gene; TC = number of copies in the tumor sample; P = Purity. The OncoPanel assay does not cover a sufficient number of polymorphic sites in the genome to employ a computational algorithm (such as ABSOLUTE) to estimate tumor purity, therefore purity was calculated based on pathologist review. Pathology-based determination of tumor purity has been shown to have

limited precision between laboratories but reasonable accuracy; this approach tends to overestimate the tumor content(61), suggesting that our approximate ploidy calculation may underestimate the true copy numbers.

Unique, aligned (hg19) sequence reads with PHRED>30 were reviewed, annotated, and interpreted using Integrated Genome Viewer(62) and a suite of internally-developed Web-based tools. (58) A lower threshold of 50X mean target coverage was established for mutation calling- as previously reported, at loci with at least 50 high quality (Q20) reads, the power to detect a 15% allele fraction event is 99%(58).Samples with a mean target coverage of <50X were failed and excluded from further analysis. Individual variants present at <10% allele fraction (AF) or in regions with <50X coverage were flagged for manual review and interpreted by the reviewing laboratory scientists and molecular pathologists based on overall tumor percentage, read depth, complexity of alteration, and evidence for associated copy number alterations. These thresholds were established during assay validation to differentiate between absolute and partial precision in replicate experiments (data not shown). Hypermutated tumors were defined as those that, following filtration for germline variants, fell into the 95th percentile for median number of SNVs and/or indels per sample (>18).

Comparison to existing diagnostic techniques

For many samples, clinical analysis of selected genes was performed during the course of the patient's management. Single gene assays are performed by CLIA-validated methods using orthogonal technologies such as Sanger sequencing (*KIT, PDGFRA, ABL1*), pyrosequencing (*KRAS, BRAF, NRAS, NOTCH1*), allele-specific PCR or RT-PCR (*EGFR* codon 858, *KIT* codon 816, *JAK2* codon 617, *MYD88* codon 265, BCR-ABL1 b2a2 and b3a2), electrophoresis (*EGFR* exon 19, BCR-ABL1, PML-RARA, *IGH-BCL2*), as well

as FISH, array comparative genomic hybridization (OncoCopy(28)) and karyotype analysis for a broad array of structural and copy number variants. Individual methods are available upon request. Where available, these traditional analyses were compared with OncoPanel results.

Technical validation

Technical validation experiments were performed to determine precision, accuracy, sensitivity, specificity and limit of detection, using blood, fresh frozen, and FFPE samples that had existing genomic characterization using an orthogonal clinical test (e.g., OncoMap, pyrosequencing, Sanger sequencing, PCR/electrophoresis, real-time PCR, aCGH array, karyotype or FISH). 120 samples with known somatic mutations, indels, copy number alterations and/or rearrangements were selected. Normal (non-cancerous) FFPE liver and fresh blood was included to verify detection of wild type loci and to identify batch related sequencing artifacts. In order to obtain sufficient quantities of DNA, several isolations were performed from each sample, pooled to ensure sample homogeneity, and then aliquoted for use in validation.

Lower limit of detection was based on analysis of precision for 12 samples that were diluted with non-neoplastic DNA at 100% (no addition of diluent), 50%, and 20% sample. Each sample was run in triplicate at each dilution. For variants with coverage $\geq$50X and allele fraction $\geq$10%, alterations were detected across all three triplicate runs for 1309 of 1330 variants (98.4% concordance). With $\geq$50X coverage and $\geq$10% allele frequency, we saw 100% reproducibility (1014/1015 variants; **Supplemental Table 8**) across 160 genes identified in 10 samples. Clinical sensitivity and specificity across the 120 sample cohort was determined. For SNVs, OncoPanel was 97.8% sensitive (95% CI: 86.5 - 99.9%) and 100% specific (95% CI: 99.9 - 100%; **Supplemental Table 9**); for indels 97.7% sensitive (95% CI: 86.5 - 99.9%) and 100% specific (95% CI: 99.9 - 100%). For copy number changes (combining single copy gains, amplifications and deletions), the assay was 93% sensitive (95% CI: 87.1 - 96.4%) and 97.6% specific

(95% CI: 96.1 - 98.5%). For rearrangement detection, OncoPanel was 74% sensitive (95% CI: 53.4% - 88.1%) and 100% specific (95% CI: 69.9% - 100%). Six of 7 non-concordant rearrangement results mapped to IGH rearrangements. This is not unexpected as our assay covers only a fraction of the immunoglobulin loci that have been implicated in rearrangements.

Statistics

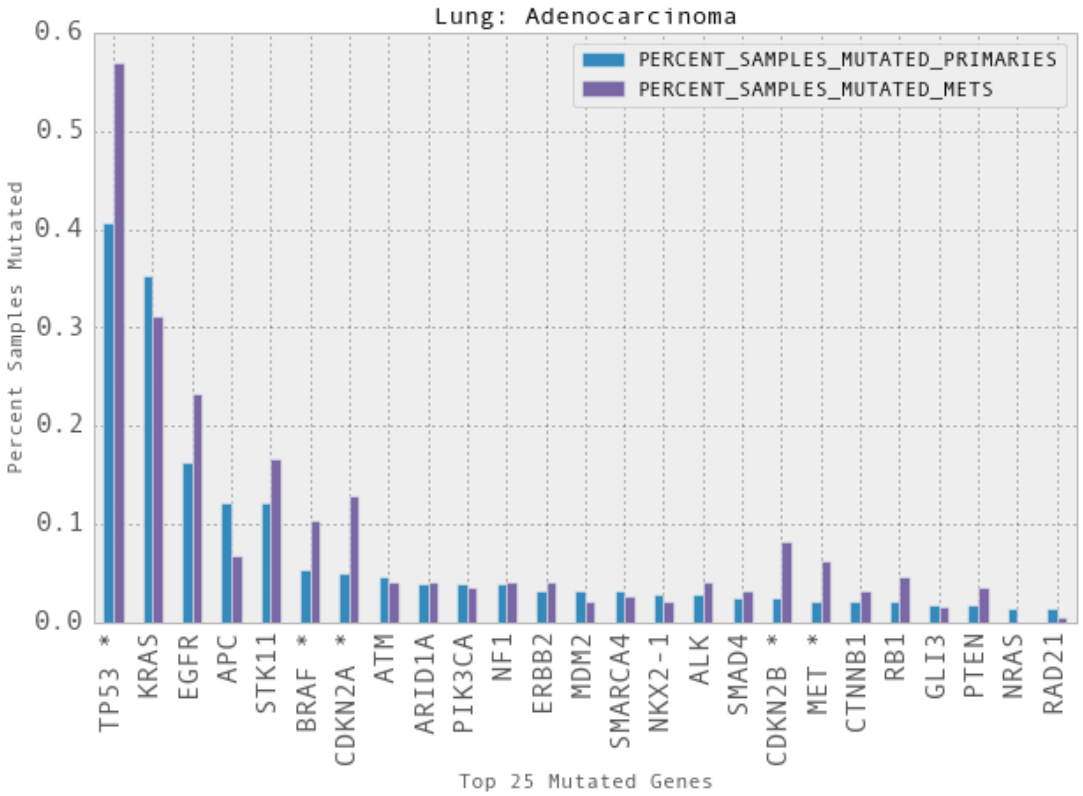
Categorical comparisons were performed using Fisher's exact or Chi square tests with Bonferroni correction for multiple comparisons. Sample means were compared using Student's T test assuming equal variance. P values of <0.05 were considered significant. Sensitivities and specificities with 95% confidence intervals were calculated using a publicly available clinical calculator (vassarstats.net). Confidence intervals were calculated using the Hmisc library in R using the binconf() function set to the "Wilson" calculation method.

Clinical use

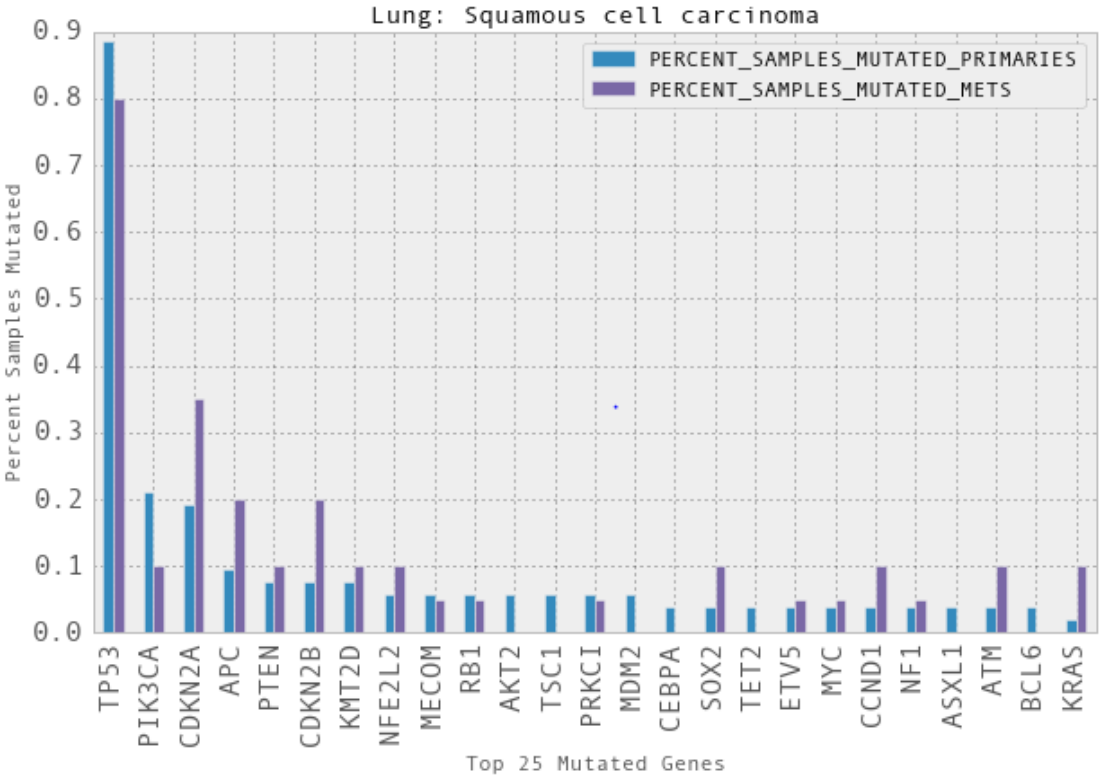
The resulting sequence data are analyzed and classified into 5 tiers of clinical relevance by a team of pathologists with molecular diagnostics expertise, incorporating information from each patient's electronic health record, and then provided to the patient's treating physician(s) as part of this study. Genomic, pathologic, and clinical data is deposited in a central knowledgebase which is linked to a database containing full clinical annotation. The knowledgebase can be queried by oncologists and laboratory scientists to facilitate development and enrollment of basket trials, inform tumor board discussions, and hone variant interpretations.

Supplemental Figure 1. Frequency of alterations in primary versus metastatic tumors among the top 25 most commonly altered tier 1-3 genes in selected diseases. Starred comparisons are significant by uncorrected Chi square test. Comparisons that remain significant after correction for multiple comparisons are noted in the results text.

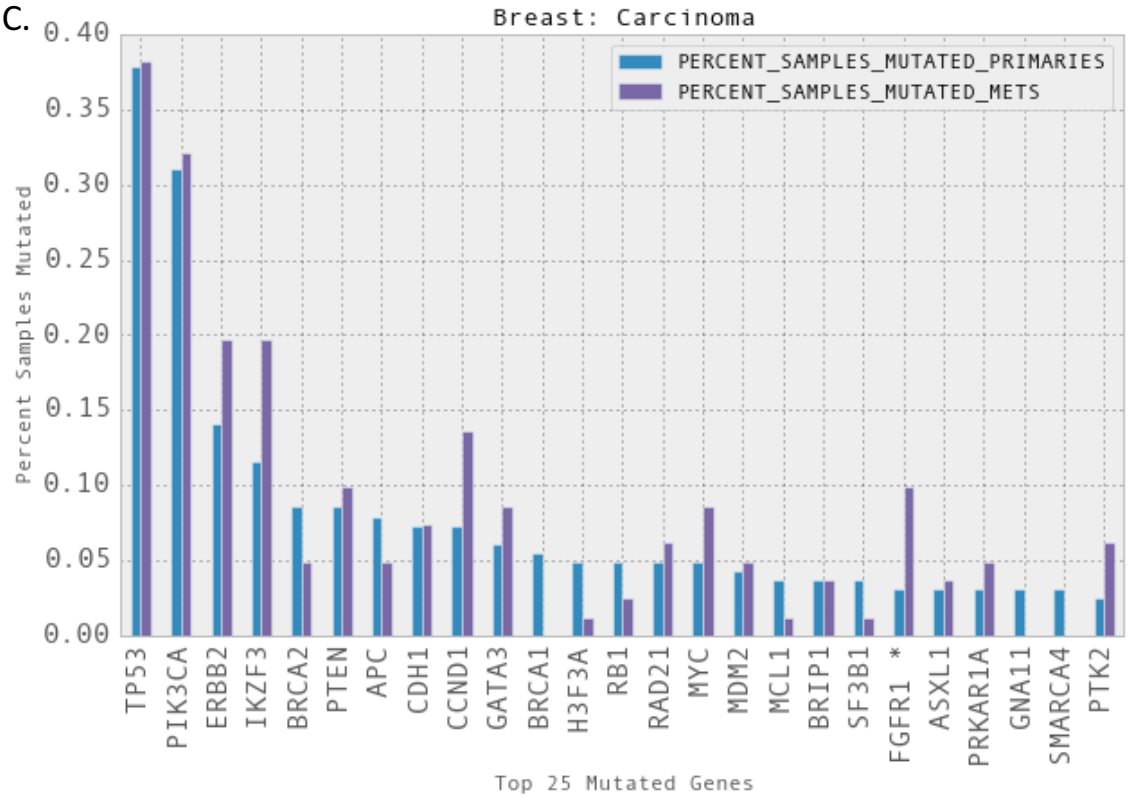
A.



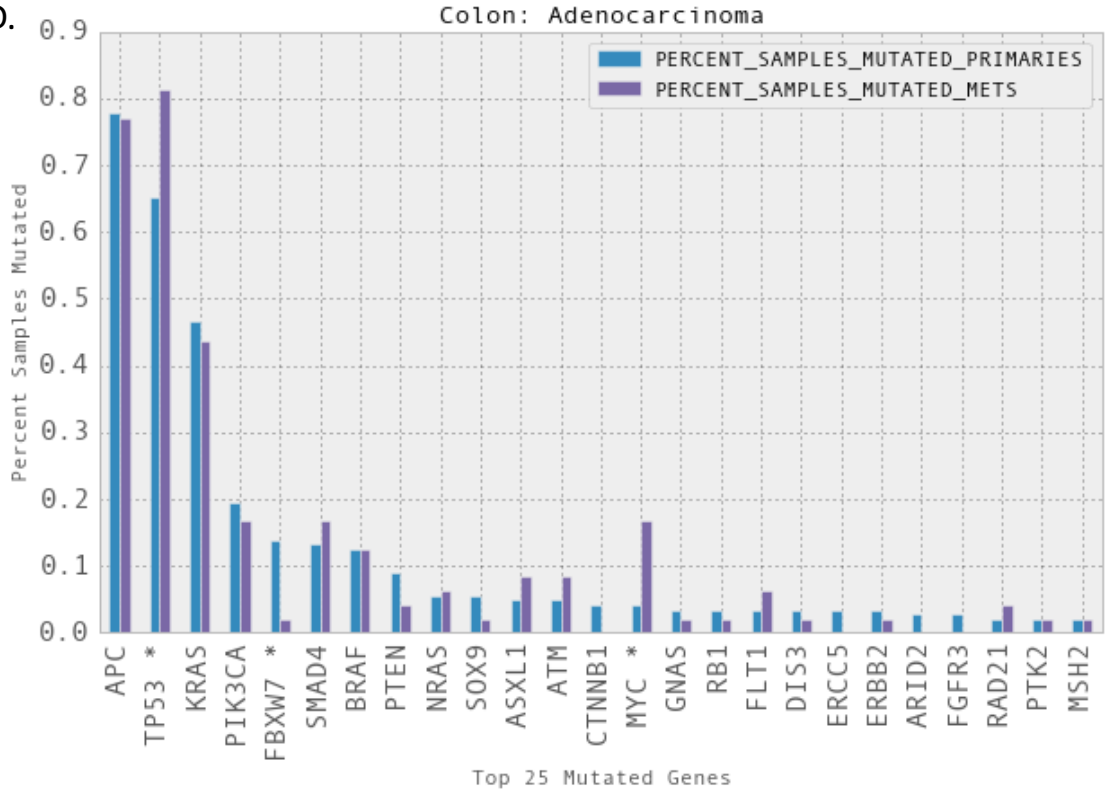
B.



C.


Breast: Carcinoma
PERCENT_SAMPLES_MUTATED_PRIMARIES
PERCENT_SAMPLES_MUTATED_METS
Percent Samples Mutated
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
TP53
PIK3CA
ERBB2
IKZF3
BRCA2
PTEN
APC
CDH1
CCND1
GATA3
BRCA1
H3F3A
RB1
RAD21
MYC
MDM2
MCL1
BRIP1
SF3B1
FGFR1 *
ASXL1
PRKAR1A
GNA11
SMARCA4
PTK2
Top 25 Mutated Genes

D.


Colon: Adenocarcinoma
PERCENT_SAMPLES_MUTATED_PRIMARIES
PERCENT_SAMPLES_MUTATED_METS
Percent Samples Mutated
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
APC
TP53 *
KRAS
PIK3CA
FBXW7 *
SMAD4
BRAF
PTEN
NRAS
SOX9
ASXL1
ATM
CTNNB1
MYC *
GNAS
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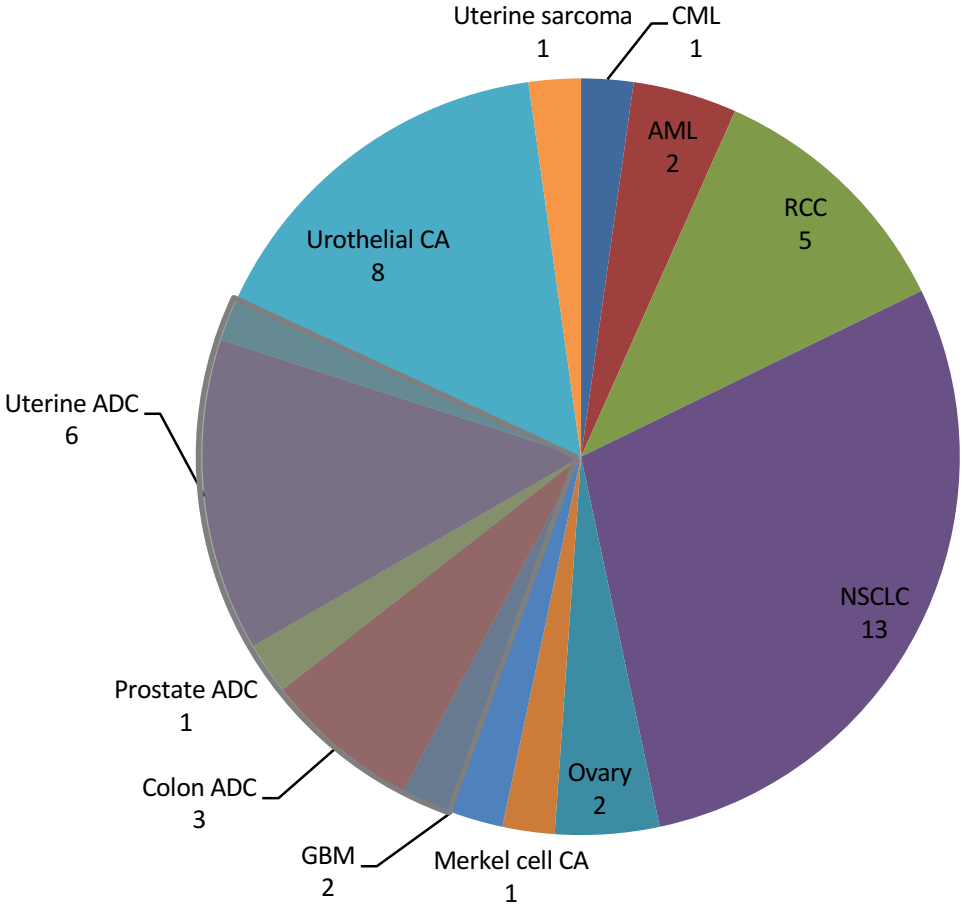
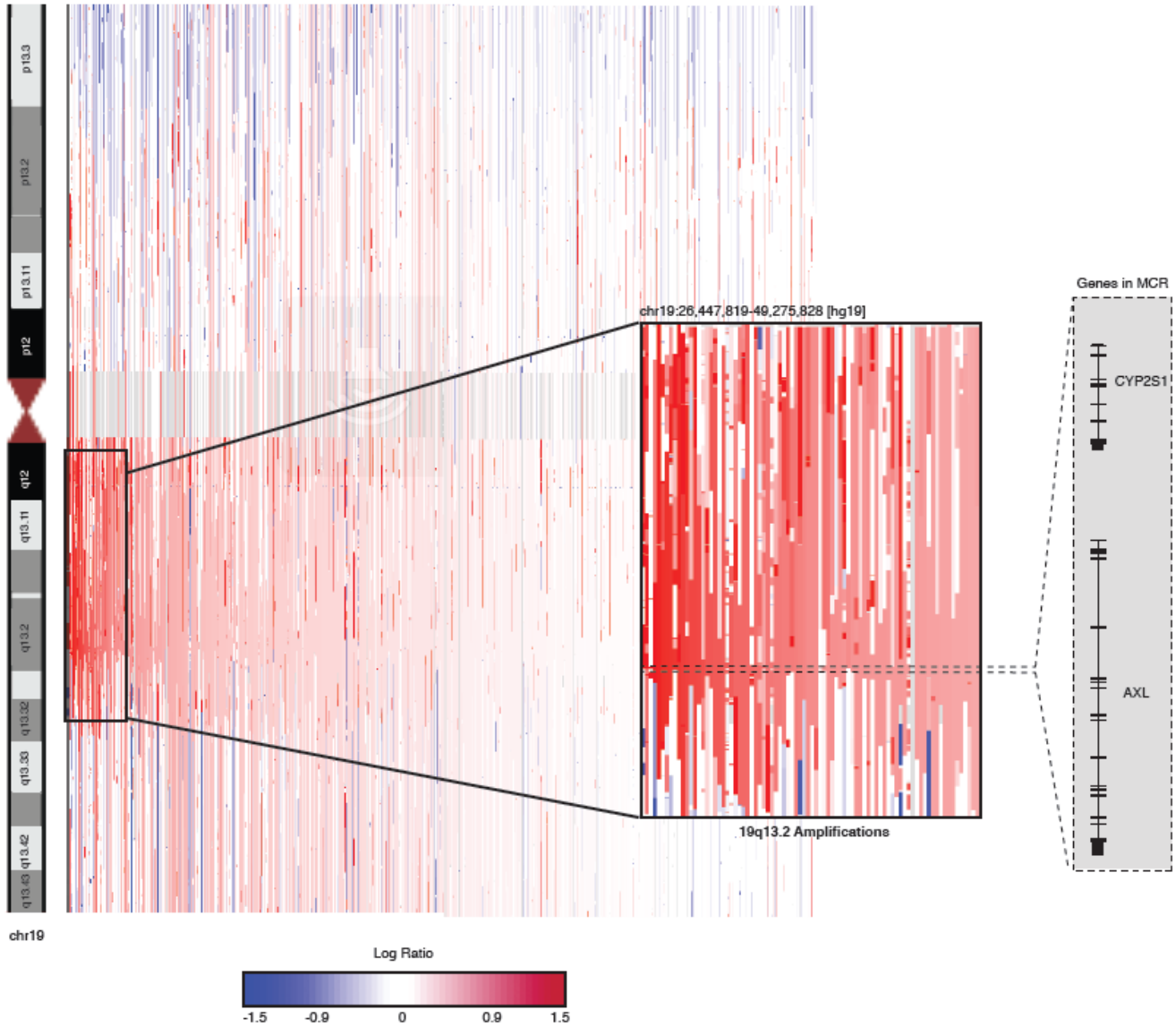
FLT1
DIS3
ERCC5
ERBB2
ARID2
FGFR3
RAD21
PTK2
MSH2
Top 25 Mutated Genes



Tumor types	N (% of Oncopanel cohort)
All	43 (1.1)
Non-hypermutated	33 (0.9)

Supplemental Figure 2. Tumors with loss of function TSC1 or 2 variants. The case types containing at least one loss of function TSC1 or TSC2 variant are shown in the pie chart. The grey wedge overlay demarcates the tumors with evidence for hypermutation.



Supplemental Figure 3. The patterns of chromosome 19 copy number alterations across all tumor types tested by The Cancer Genome Atlas are shown. Individual tumors are arranged in columns, and sorted according to degree of gain at the *AXL* locus. In most cases containing *AXL* gain, the amplicon extends to include other genes located 19q. The minimally amplified region is shown in the inset.