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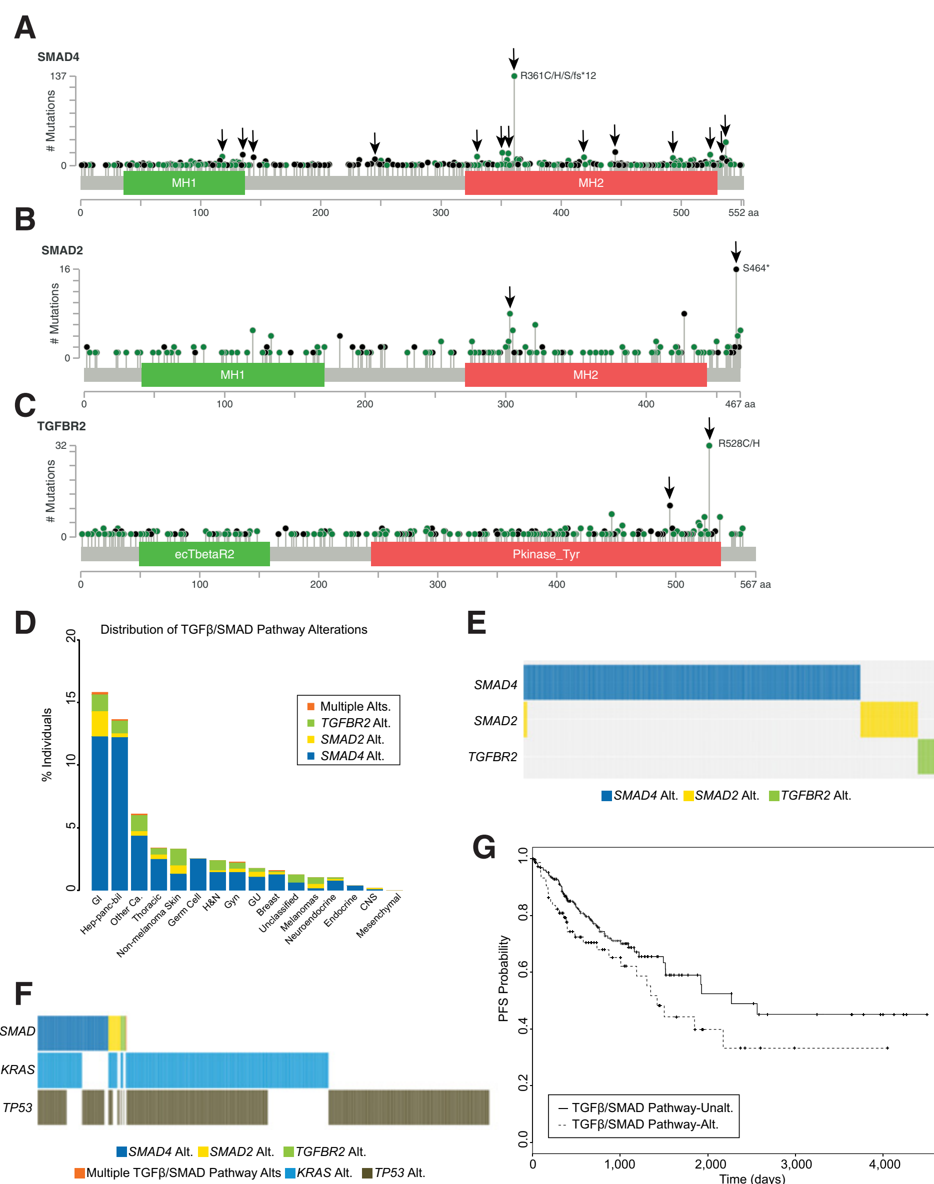

Figure 3.
Novel variants in *SMAD4* and SMAD signaling pathway components. **A,** Hotspot alterations within *SMAD4* identified by a hotspot analysis. These include both known hotspots (D351A/G/V/Y, R361C/H/S, P356H/L/R/S, D537A/E/G/H/V/Y), and novel hotspots (A118V, E330K/*, G419M/R/W, and D493H/N, W524C/G/R). **B** and **C,** Alterations within SMAD pathway genes *SMAD2* and *TGFRB2* were also analyzed. **D–F,** Analysis of all hotspot and loss of function (truncation and homozygous deletions) mutations in these three genes (**D**) for incidence across disease groupings and (**E**) cooccurrence in colorectal adenocarcinoma. **F,** Cooccurrence of TGFβ/SMAD pathway alterations in colorectal adenocarcinoma with *KRAS* alterations and significant mutual exclusivity with *TP53* alterations is shown. **G,** TCGA colon adenocarcinomas were separated into TGFβ/SMAD pathway altered ($n = 75$ hotspot mutations, truncating mutations, and homozygous deletions) versus TGFβ/SMAD pathway unaltered ($n = 254$) groups. The TGFβ/SMAD mutated group exhibited reduced PFS that trended toward significance ($P = 0.06$; log-rank test).

To capture rare alterations, we also analyzed the functional impact of VUS point mutations occurring in at least 5 samples using MutationAssessor and PolyPhen-2 tools (12, 13). A merge of these outputs identified 23 unique point mutations in 11 genes with predicted functional impact across both algorithms (Supplementary Table S5). All mutations also reached significance using the hotspot caller described above. In addition to the mutations described above, this VUS analysis identified multiple alterations in *ERBB3* (T355I and T389I), *BRIP1* (R762C and R251C), *KEAP1* (G523V, R413C, and G419W), and *SMARCA4* (E882K, P913L, R973W, R1135Q, and R1192H) and intriguing variants in *PTEN* (D24H), *FLT1* (E432K), *STK11* (P179R), *LRP1B* (G401E), *ESR1* (A361V), and *CDKN2A* (G101V) predicted to have functional impact. Interestingly, these variants tended to occur in tumor types associated with alterations in that gene. For example, 3 of 6 *FLT1* E432K alterations were in melanomas, 21/32 of the various *SMAD4* alterations were found GI and hepatobilliary cancers, and 9 of 15 of the *KEAP1* alterations were found in thoracic cancers. The nonrandom distribution of these VUSes strongly implicates them as functional alterations. Of note, as matched germline DNA was not tested, we cannot completely discount the possibility that some of these alterations may be rare germline alterations. However, somatic/germline status was predicted with a novel, internally developed algorithm that assesses germline status based on allele frequency and tumor purity/ploidy (Sun and colleagues, in review 2016). For example, in a sample with moderate cellularity (~50%), in copy neutral, diploid regions, somatic alteration allele frequencies will be impacted by cellularity and have allele frequencies approximately 25%. In contrast, heterozygous germline variants will not be impacted by cellularity and will have allele frequencies approximately 50%. These estimates suggest that >90% of the VUSes predicted to have a functional impact are somatic events (data not shown).

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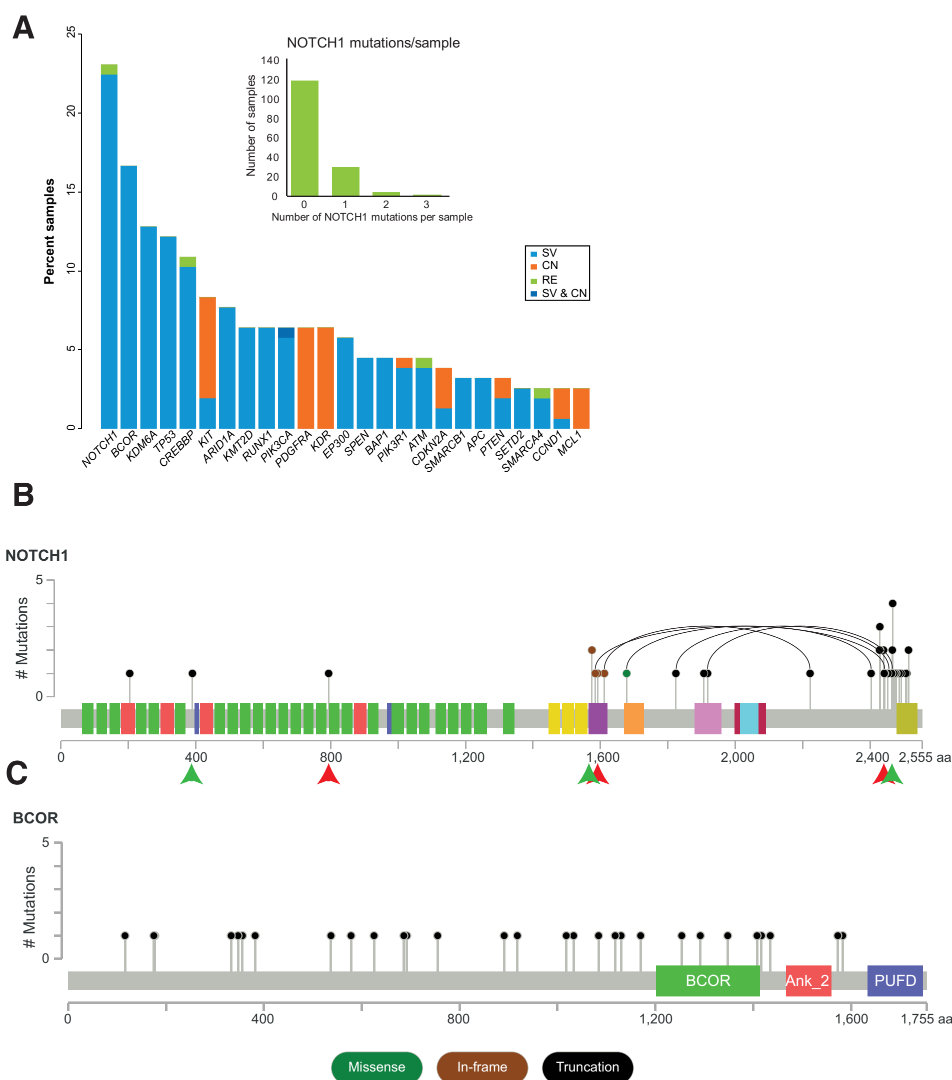

Figure 4.
NOTCH1 and *BCOR* variants in adenoid cystic carcinomas. **A,** A long tail plot of alterations within ACCs shows a high prevalence of alterations in *NOTCH1* and *BCOR*. **B,** The distribution of alterations within *NOTCH1* reveals a clustering of inactivating mutations within the C-terminal PEST domain. Arcs represent mutation pairs cooccurring in the same tumor. Mutations in tumors with 3 *NOTCH1* mutations ($n = 2$) are highlighted with arrowheads color coded by sample. Protein domains are: EGF-like (green), calcium-binding EGF (red), hEGF-like (blue), LNR (yellow), NOD (purple), NODP (orange), Ank (fuchsia, dark red, cyan), and DUF (mustard). NOD and NODP together represent the homopolymerization domain and DUF is contained within the PEST domain. **C,** The distribution of alterations in *BCOR* reveals inactivating events across the gene. Diagrams were generated using MutationMapper (see Materials and Methods).

Genomic analysis of rare diseases uncovers higher frequencies of *NOTCH1* and *BCOR* alterations in adenoid cystic carcinomas

We investigated the genomics of rare tumors given that many of the subtypes within the FM dataset were excluded from large-scale analyses (i.e., TCGA) or profiled only as part of small cohorts. Interesting results were observed in adenoid cystic carcinomas (ACC) of the head and neck region ($n = 156$ total), including head and neck ACCs ($n = 78$), salivary gland ACCs ($n = 57$), tracheal ACCs ($n = 7$), and unknown primary ACCs ($n = 14$). In agreement with recent findings of 36 recurrent and metastatic ACCs (17), the most frequent alterations occurred in *NOTCH1* (23%; Fig. 4A). *NOTCH1* alterations were clustered in the C-terminal PEST domain of the protein (Fig. 4B), and were significantly enriched within this disease (Supplementary Table S6). *NOTCH1* PEST domain alterations are weakly activating by themselves (18). However, they are synergistic with HD domain mutations in *cis* and can significantly increase the activity of this protein when this combination of alterations is present. Interestingly, 7 samples harbored mutations in both the PEST and HD domains, suggesting a potential mechanism through which activity of this gene could be altered (Fig. 4B). Unfortunately, phasing of the mutations was impossible to determine definitively due to the distance between mutations and the length of the sequencing reads. However, two tumors contained a third alteration (E794* and N390fs*243) toward the 5′ end of *NOTCH1*, likely disrupting one allele and suggestive that the other two alterations are in *cis*. Collectively, other genomic studies have investigated 111 total ACC tumors, including 28 samples also represented within this dataset, and reported *NOTCH1* missense and nonsense alterations in 5–10% of samples (19–21). This analysis confirms findings from smaller studies that *NOTCH1* is the most commonly altered gene in ACCs at approximately 24% and extends it by providing multiple examples of cooccurring PEST and HD domain alterations. Further work to evaluate the effect of these alterations is warranted as multiple inhibitors of this protein are currently in clinical trials (22).

The second most common alterations in ACCs occurred in the TSG *BCOR* (17%; Fig. 4A). All variants are predicted to inactivate this protein (Fig. 4C). These results agree with previous findings where 4/36 ACCs were found to have truncating mutations in *BCOR*. Together, these results establish *BCOR* inactivation as a signature event in ACC. *BCOR* alterations have been described in

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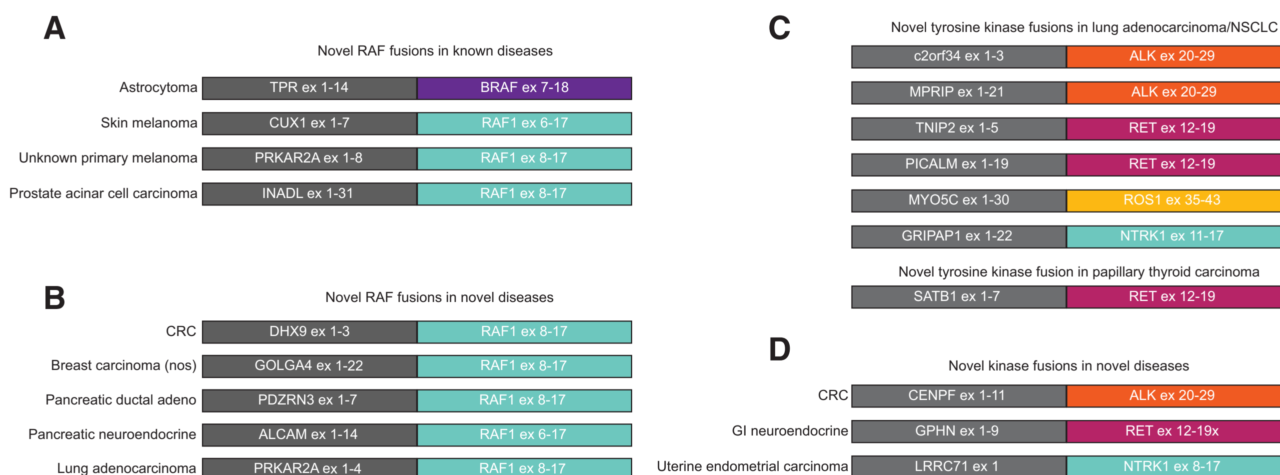

Figure 5.

Novel fusions in known and unknown diseases. **A,** Five novel *BRAF* and *RAF1* (*CRAF*) fusions were identified in diseases where rearrangements involving these genes are known to be oncogenic. **B,** In addition, we also observed five novel *RAF* fusions in other diseases. **C,** Novel fusions involving *ALK*, *RET*, *ROS1*, and *NTRK1* were identified in NSCLCs and a papillary thyroid carcinoma. Kinase fusions within these diseases are known to play a tumorigenic role. **D,** Three novel tyrosine kinase fusions were also identified in other diseases.

myelodysplastic syndromes where they are associated with poor prognosis and shorter overall survival (23). They have also been described in multiple pediatric tumors where they are thought to play a role in chromatin modification (24). Both *BCOR* and *NOTCH1* alterations cooccurred with other events (Supplementary Fig. S16).

Identification of novel fusion events

We investigated the spectrum of fusions involving 8 clinically relevant kinases (*ALK*, *BRAF*, *EGFR*, *NTRK1*, *PDGFRA*, *RAF1*, *RET*, and *ROS1*) with established druggability. In total, we identified 19 novel fusions with structures similar to known oncogenic fusion proteins and multiple known fusions in diseases different from those in which they were reported originally.

Nine novel fusions involved the serine threonine kinases *BRAF* or *RAF1* (Fig. 5). These included fusions with four novel fusion partners in diseases known to be driven by these events, such as astrocytoma, melanoma, and prostatic acinar cell carcinoma (Fig. 5A). *RAF1* fusions were also observed in 5 other disease types (Fig. 5B). Interestingly, the *PRKAR2A-RAF1* fusion was recurrent and observed in both a lung adenocarcinoma and an unknown primary melanoma. In addition, we also observed 7 novel tyrosine kinase fusions involving *ALK*, *RET*, *ROS1*, and *NTRK1* (Fig. 5C) in non–small cell lung cancers and thyroid cancer. Novel fusions involving *ALK*, *RET*, *ROS1*, and *NTRK1* were also observed in a colon adenocarcinoma, a GI neuroendocrine tumor, and a uterine endometrial carcinoma (Fig. 5D).

Although kinase fusions can be hallmarks of certain cancers, we observed known fusions outside of the diseases in which they were identified originally. *CLTC-ALK* and *STRN-ALK*, have been observed in aggressive thyroid cancers and soft tissue malignancies (25, 26). Within the FM cohort, these fusions were observed in a lung adenocarcinoma and an epithelioid peritoneal mesothelioma, respectively (Supplementary Fig. S17). Diagnosis of the epithelioid peritoneal mesothelioma is supported by IHC staining (positive for cytokeratin 7, calretinin and vimentin; negative for CEA, B72.3, and TTF1). While a subset of lung adenocarcinomas is known to be driven by *ALK* fusions, *CLTC-ALK* has yet to be described in this disease. In contrast, kinase fusions have not been noted in mesothelioma; *STRN-ALK* represents a novel, yet rare (1/184, 0.5%), driver event in this disease. *RET* fusions in thyroid and lung cancers are well characterized (27, 28). Here, two breast cancers were found to harbor the oncogenic *CCDC6-RET* fusion (Supplementary Fig. S17). Two similar onocgenic *RET* fusions (*NCOA4-RET* and *KIF5B-RET*) were also identified in a rare liver cholangiocarcinoma and an ovarian epithelial carcinoma, respectively (Supplementary Fig. S17). *BRAF* fusions have been described in melanoma, thyroid cancers, and pediatric brain cancers (29). A single thyroid papillary carcinoma was found with a *MAD1L1-BRAF* fusion identified previously in melanoma (30). We also observed the known *TMEM106B-ROS1* fusion (31) in a liver cholangiocarcinoma. Interestingly, *GOPC(FIG)-ROS1* has been reported in a glioblastoma cell line (32), in a lung adenocarcinoma (33), and in rare biliary tract carcinomas (34). Here, we observed this fusion in a small intestine adenocarcinoma and confirm its presence in a human glioblastoma sample (Supplementary Fig. S17). Finally, we observed an imatinib sensitive hypereosinophilic syndrome fusion, *FIP1L1-PDGFRA* (35), in a glioblastoma (Supplementary Fig. S17).

Spectrum of known clinically relevant alterations across diseases

Recent publications have suggested a broad spectrum of genomic changes in clinically relevant targets (29, 36–38), and expanded analyses are warranted to identify more patients predicted to be sensitive or resistant to targeted therapies. Furthermore, the identification of drug sensitivity and resistance biomarkers across multiple indications suggests that targeted agents may have broader utility beyond that for which they were approved originally. We surveyed the spectrum of known clinically relevant genomic changes to identify (i) the spectrum of these changes in indications for which testing is currently recommended and (ii) potential opportunities for broader utility of approved targeted agents.

In colorectal cancers (CRC), activating mutations in *KRAS* are predictive of poor response to cetuximab. Alterations in hotspot

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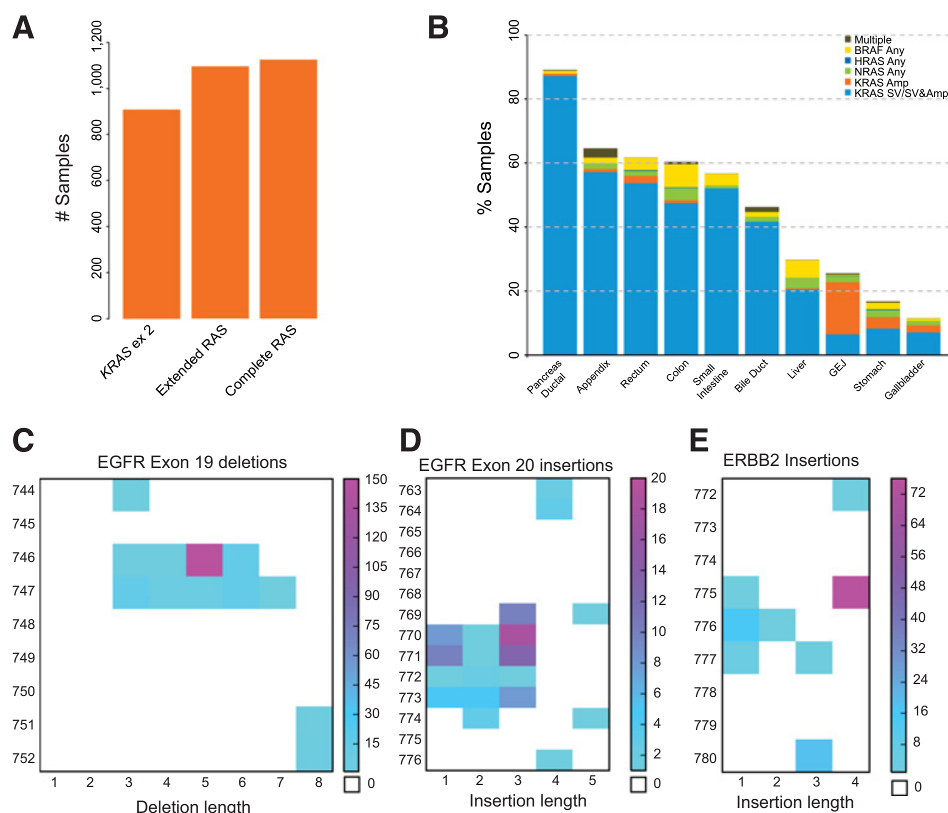

Figure 6.
Diversity of clinically relevant alterations across the dataset. **A,** Distribution of clinically relevant *KRAS* alterations in colorectal adenocarcinomas. **B,** Distribution pattern of all *RAS* alterations in GI cancers. **C–E,** Indel alterations in *EGFR* (**C** and **D**) and *ERBB2/HER2* (**E**) can vary in length. Numbers on the left side of the graphs correspond to the codon positions; heatmaps display the number of samples.

exons 12 and 13 were observed in 908 of 1,986 (45.7%) CRCs. Recently, expanded guidelines adopted by American Society of Clinical Oncology (ASCO) and others recommend testing for alterations in exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146) in both *KRAS* and *NRAS* (39). These extended RAS testing guidelines captured an additional 188 CRC samples (9.5%) within our dataset. Beyond these extended guidelines, we also observed activating *KRAS* and *NRAS* amplifications and mutations at codons 14 and 22 in an additional 29 CRC samples (1.5%; Fig. 6A; refs. 40, 41). These data provide robust estimates of RAS alterations in CRCs, utilizing both ASCO guidelines and the current state of knowledge, which is critical when considering *EGFR* targeted therapies.

As mutations in the MAPK pathway are known drivers of multiple GI cancers, we performed a survey of *KRAS*, *NRAS*, *HRAS*, and *BRAF* alterations across cancers of the GI and hepato-biliary tracts (Fig. 6B). Gastroesophageal junction adenocarcinoma was unique in that it had a high proportion of *KRAS* amplifications without a *KRAS* mutation (16.3%). Appendix adenocarcinomas had a similar rate of MAPK alterations (64.5%) as neighboring small intestine (56.6%) and CRCs (60.3%). MAPK alterations in cancers of the biliary tree (see Fig. 6B) included a high frequency in bile duct adenocarcinomas (46.3%) and pancreatic cancers (89%) and a low frequency in gallbladder adenocarcinomas (11.5%) and liver cholangiocarcinomas (29.8%).

We next investigated the spectrum of clinically relevant insertions and deletions (indels) in *EGFR* (*EGFRvIII* rearrangements were also identified and are discussed in a subsequent section). The most prevalent *EGFR* exon 19 indel was the canonical E746-A750 deletion, although the length of deletions at amino acids 746 and 747 ranged from 3–7 amino acids (Fig. 6C). We also observed rare deletions of 8 amino acids at positions 751 and 752. In contrast, drug-resistant *EGFR* exon 20 insertions lacked a single dominant location and length, but the vast majority occurred between amino acids 769–774 and inserted 1–3 residues within this region (Fig. 6D). Analogous *ERBB2* insertions primarily consisted of a 4 amino acid duplication between residues 772–775, however, these insertions ranged from 1–4 amino acids between residues 772 and 780 (Fig. 6E). The diversity of these mutations has implications for robust diagnostic detection and developing drugs to target this heterogeneous set of insertions (37).

Current data suggest that *ERBB2* amplifications, oncogenic point mutations, and activating insertions can confer clinical sensitivity to *ERBB2* targeted agents (42). We observed recurrent activating *ERBB2* alterations across 15 tumor types (Fig. 7A), including new trends such as *ERBB2* amplifications in cervical cancer and skin squamous cell carcinomas. *ERBB2* point mutations have been described in cervical cancers (43), but amplifications suggest an alternate mechanism through which the gene can be activated in this disease. Consistent with previous studies, *ERBB2* activating mutations in cervical cancers did not cooccur with copy number alterations (Supplementary Fig. S18). To our knowledge, *ERBB2* amplifications in skin squamous cell carcinoma represent a novel therapeutic target in this disease.

Amplification of the *CDK4/6* locus has been associated with response to the CDK4 inhibitor palbociclib in breast cancers and liposarcomas (44, 45). Within this cohort, we observed *CDK4/6* amplification across 43 tumor types (Fig. 7B). Novel findings

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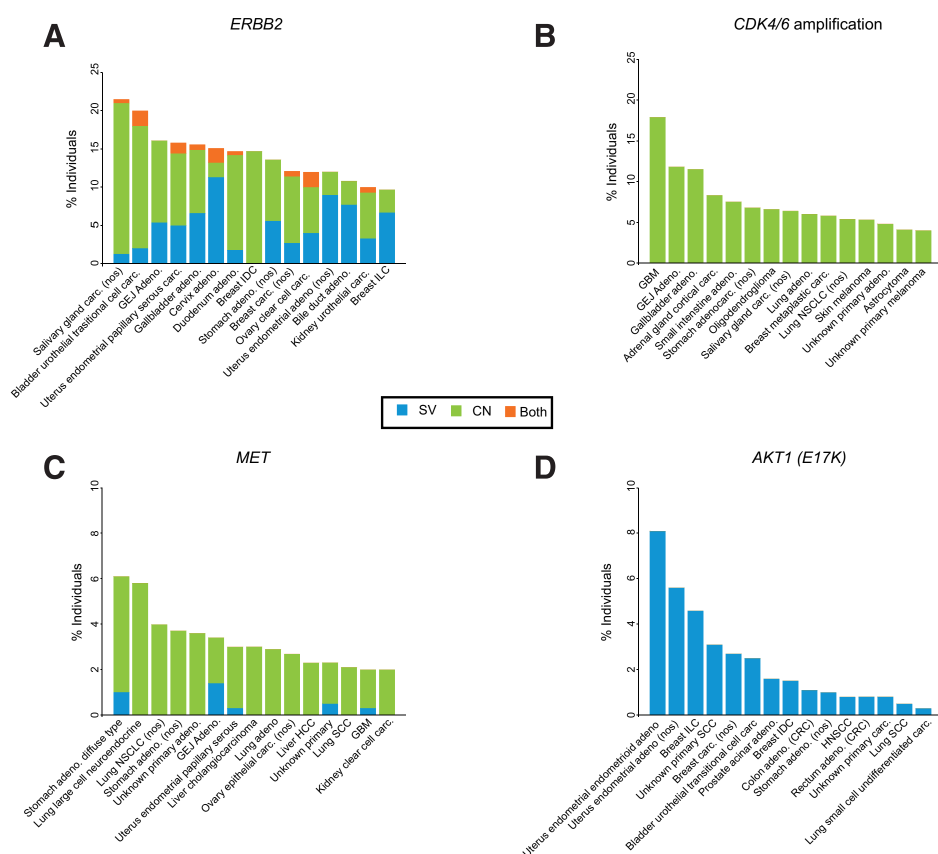

Figure 7.

Spectrum of druggable alterations across cancers. Distribution of alterations across the top 15 disease types for *ERBB2/HER2* (**A**), amplifications in *CDK4/6* (**B**), amplifications in *MET* (**C**), and activating mutations in *AKT1* (**D**).

included amplification of these genes in gallbladder carcinomas (11.5%) and oligodendrogliomas (6.6%). Preclinical models have suggested that this event may contribute to oligodendroglioma formation (46).

MET amplification was observed in glioblastoma samples at an appreciable frequency (2.1%; Fig. 7C). We explored whether this finding correlated with overexpression of *MET* mRNA by incorporating expression data from TCGA glioblastoma samples. Although few TCGA GBM cases exist with *MET* amplification, amplification was associated with increased expression (Supplementary Fig. S19). Interestingly, at least one case report has demonstrated clinical sensitivity to the MET inhibitor, crizotinib, in this disease (47). A similar observation was confirmed in *AKT1* E17K-mutant colorectal adenocarcinomas (Fig. 7D). While this mutation has been observed previously in this disease, frequencies varied from 0%–8.2% (14, 48, 49). Our data confirm the rare occurrence of this alteration in approximately 1.0% of routine CRC samples. Interestingly, in contrast to a previous study (50), we observe *AKT1* E17K mutant colorectal samples to be enriched for *KRAS* alterations ($P = 0.02$) but not *BRAF* alterations (Supplementary Fig. S20).

In addition to potentially targetable alterations that occur across diseases, we also observed striking patterns of disease specificity for certain alterations. *EGFRvIII* rearrangements and extracellular activating mutations were found almost exclusively in glioblastomas while activating indels in *EGFR* occurred almost exclusively in NSCLCs. Although we also saw an appreciable rate of *EGFR* indels in unknown primary adenocarcinomas, it is likely that these represent NSCLC samples for which incomplete pathology information was available (J. Ross, personal communication). A similar trend was also observed for *ROS1* rearrangements. These events were observed primarily in NSCLC and a small proportion of glioblastomas. Both of these rates are consistent with published reports, and suggest that *ROS1* fusions show tissue specificity.

While many oncogenic alterations cluster within diseases and are targetable directly, some inhibitors rely on the status of TSGs as biomarkers of response. For example, deleterious alterations in *BRCA1/2* are associated with sensitivity to PARP inhibitors (51), and multiple trials require intact p53 (*TP53*) as enrollment criteria (NCT01760525, NCT02143635, NCT02264613). Therefore, we investigated patterns of TSG alterations. Unsupervised clustering of alterations within a curated list of TSGs (Supplementary Table S7) identified unique patterns of inactivation across solid tumors (Supplementary Fig. S21). Genes including *TP53* and *CDKN2A/B* were altered uniformly across multiple solid tumors. In contrast, other TSGs displayed disease-specific clustering, such as *APC* alterations within GI cancers. Multiple novel disease-gene associations were also present. For example, alterations in *BCOR*, *NOTCH1*, *KDM6A*, *CREBBP*, and *KMT2D* clustered primarily in ACCs. Collectively, these data suggest patterns of TSG inactivation that may be disease specific, similar to patterns for some oncogenes. Further research to understand this tissue selectivity is warranted.

Discussion

We describe herein a dataset of 18,004 unique adult solid tumors that underwent genomic profiling as part of routine clinical care. This collection represents "real world" specimens that were not selected for any features prior to sequencing. The dataset was composed of 162 disease subtypes, including many rare and unusual tumors not included previously as part of larger

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sequencing efforts. In addition, common tumors (e.g., breast, lung, and colon) are represented by thousands of samples, enabling robust statistical analyses as well as validation of rare variants. Comparison of alteration frequencies to TCGA, where possible, identified some significant differences, mostly in CN frequencies. Detailed examination suggests that both technical (i.e., platform, annotation) and sample differences underlie between the discrepancies between the two datasets.

We also observed an enrichment of treatment refractory samples in FM breast and lung cancer cohorts based on an increased frequency of alterations associated with acquired resistance to targeted therapies in these diseases. To exemplify novelty within the FM dataset, we surveyed the genomic landscape of rare diseases and identified *NOTCH1* alterations in ACCs at a higher frequency compared with previous studies. We also identified multiple potentially druggable novel kinase fusions as well as known fusions in diseases beyond those in which they are currently recognized. Analysis of VUSes identified a clinically significant enrichment of *SMAD4* alterations in colon cancer, as well as multiple other rare alterations predicted to have functional impact. A survey of clinically relevant alterations highlighted the spectrum of molecular changes for which testing is recommended as well as opportunities for expansion of approved targeted therapies. Clustering of alterations in TSGs revealed patterns of disease specificity for certain genes, but broad inactivation of others. This dataset is rich with discovery potential and presents a new resource in which to investigate rare alterations and diseases, validate clinical relevance, and identify novel therapeutic targets.

To our knowledge, this dataset represents the largest collection of tumors to date profiled on a single uniform platform. The high unique sequencing coverage (>600×) across all targets enables accurate detection of all classes of genomic variants, even in impure clinical specimens. Previous validation has optimized this assay for sensitive and specific detection for all classes of variants down to low mutant allele frequencies (11). The samples within this dataset lack sequencing of patient-matched normal tissue, but multiple steps have been taken to enrich for significant cancer-associated variants (see Supplementary Methods). These include inclusion of (i) all truncation events in TSGs, (ii) known pathogenic germline events, and (iii) uncharacterized alterations reported previously in cancer (Supplementary Fig. S2). To minimize the number of benign germline variants, those variants not meeting the criteria above were filtered through online databases (ExAc and 1000 Genomes) to remove events recognized currently as benign polymorphisms. Collectively, the uniformity of the data and the stringent filtering to enrich for cancer-associated alterations facilitate comparisons and enhance the discovery potential for variants contributing to tumorigenesis.

The dataset can be used by basic researchers to identify novel findings for validation and to validate previous observations, especially those involving rare diseases and rare variants. Our preliminary analyses exemplify how hypothesis-generating discoveries within this cohort can be integrated with existing data. For example, the identification of novel *SMAD4* genomic alterations in colorectal adenocarcinomas was expanded using the TCGA cohort to investigate survival differences among patients. In addition, *MET* amplifications in glioblastoma were shown to correlate with mRNA overexpression of this target in TCGA, a finding that may have been unappreciated in the past due to small datasets and the rarity of the event. A data collection of this size also allows for pan-cancer analyses to better understand tissue-specific patterns of alterations, such as TSG inactivation. These findings can be used to plan thoughtful functional follow-up experiments. This resource also has applicability to clinical oncology and drug development. We highlight the spectrum of clinically relevant molecular markers and show that a wide variety of alterations exist in targets for which established routine clinical tests exist. This resource can also be used to explore opportunities for drug expansion with new or approved targeted agents for whom biomarkers of therapeutic sensitivity or resistance are known. For example, we highlight multiple alterations, including *MET* amplification, *ERBB2* amplification, and activating point mutations, and amplification of *CDK4/6*, that occur across multiple diseases. In contrast, we observed that *ALK* rearrangements and *EGFR* activating alterations are confined to specific diseases and very rarely observed outside of those tissues.

The lack of clinical data is a limitation of this dataset. Information about exposure to previous treatments, survival, and response rates was unavailable. As these samples were profiled on a clinical platform, and not as part of a research study, genomic information is only available for those 295 genomic targets deemed to have clinical relevance today. While the role of genomic changes in cancer development and treatment response is well studied, it is likely that other changes in methylation, expression, and noncoding DNA regions may have implications and would not be captured within this dataset.

The National Cancer Moonshot Initiative has emphasized that data sharing is essential to accelerate progress in oncology. Academic, private, and public sectors have an obligation to patients, researchers, and clinicians to share data, knowledge, and insight across the field. Large-scale sequencing projects have profiled many common tumors but often lack robust sample numbers for rare diseases and variants. The public availability of large genomic datasets, such as the one described herein, enables the broad use of this data across multiple disciplines, and is designed to remove barriers to progress. The insights gleaned from this data release will be instrumental in accelerating research and development efforts for targeted agents and immunotherapies.

Disclosure of Potential Conflicts of Interest

R.J. Hartmaier has received speakers' bureau honoraria from Bio-Rad Laboratories and has ownership interest in Foundation Medicine. L. Albacker has ownership interest in Foundation Medicine. J. Chmielecki is a current employee at Foundation Medicine. M. Bailey has ownership interest in Foundation Medicine, Inc. J. He is a senior manager at Foundation Medicine. M. Goldberg is a clinical data analyst and has ownership interest in Foundation Medicine, Inc. J.A. Elvin has ownership interest in Foundation Medicine. G.M. Frampton reports receiving a commercial research grant and has ownership interest in Foundation Medicine. J.S. Ross is a medical director at Foundation Medicine and has ownership interest in Foundation Medicine. V. Miller is a chief medical officer at Foundation Medicine, Inc. P.J. Stephens is a chief scientific officer at and has ownership interest in Foundation Medicine. D. Lipson is a vice president and has ownership interest in Foundation Medicine. No potential conflicts of interest were disclosed by the other authors.

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Study supervision: J. Chmielecki, D. Lipson

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References

1. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61–70.
2. The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014;513:202–9.
3. The Cancer Genome Atlas Research Network. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med 2015; 372:2481–98.
4. Pereira S, Gibbs RA, McGuire AL. Open access data sharing in genomic research. Genes 2014;5:739–47.
5. Brennan CW, Verhaak RGW, McKenna A, Campos B, Noushmehr H, Salama SR, et al. The somatic genomic landscape of glioblastoma. Cell 2013;155:462–77.
6. Verhaak RGW, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 2010;17:98–110.
7. Ettinger DS, Wood DE, Chair Fred V, Aisner DL, Bauman J, Chirieac LR, et al. NCCN Guidelines Version 3.2017 Panel Members Non-Small Cell Lung Cancer-Farber/Brigham and Women's Cancer Center. Fort Washington, PA: National Comprehensive Cancer Network; 2016[cited 2016 Nov 21]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf.
8. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015;348:124–8.
9. Catenacci DVT. Next-generation clinical trials: Novel strategies to address the challenge of tumor molecular heterogeneity. Mol Oncol 2015;9: 967–96.
10. Kris MG, Johnson BE, Berry LD, Kwiatkowski DJ, Iafrate AJ, Wistuba II, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. JAMA 2014;311:1998–2006.
11. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 2013;31: 1023–31.
12. Reva B, Antipin Y, Sander C. Predicting the functional impact of protein mutations: application to cancer genomics. Nucleic Acids Res 2011;39: e118.
13. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7:248–9.
14. The Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012;487: 330–7.
15. Chang MT, Asthana S, Gao SP, Lee BH, Chapman JS, Kandoth C, et al. Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. Nat Biotechnol 2016;34:155–63.
16. Fleming NI, Jorissen RN, Mouradov D, Christie M, Sakthianandeswaren A, Palmieri M, et al. SMAD2, SMAD3 and SMAD4 mutations in colorectal cancer. Cancer Res 2013;73:725–35.
17. Morris LGT, Chandramohan R, West L, Zehir A, Chakravarty D, Pfister DG, et al. The molecular landscape of recurrent and metastatic head and neck cancers. JAMA Oncol. 2016 Jul 21. [Epub ahead of print].
18. Weng AP, Ferrando AA, Lee W, Morris JP, Silverman LB, Sanchez-Irizarry C, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 2004;306:269–71.
19. Ho AS, Kannan K, Roy DM, Morris LGT, Ganly I, Katabi N, et al. The mutational landscape of adenoid cystic carcinoma. Nat Genet 2013;45:791–8.
20. Stephens PJ, Davies HR, Mitani Y, Van Loo P, Shlien A, Tarpey PS, et al. Whole exome sequencing of adenoid cystic carcinoma. J Clin Invest 2013;123:2965–8.
21. Ross JS, Wang K, Rand JV, Sheehan CE, Jennings TA, Al-Rohil RN, et al. Comprehensive genomic profiling of relapsed and metastatic adenoid cystic carcinomas by next-generation sequencing reveals potential new routes to targeted therapies. Am J Surg Pathol 2014;38:235–8.
22. Olsauskas-Kuprys R, Zlobin A, Osipo C. Gamma secretase inhibitors of Notch signaling. Onco Targets Ther 2013;6:943–55.
23. Damm F, Chesnais V, Nagata Y, Yoshida K, Scourzic L, Okuno Y, et al. BCOR and BCORL1 mutations in myelodysplastic syndromes and related disorders. Blood 2013;122:3169–77.
24. Shern JF, Chen L, Chmielecki J, Wei JS, Patidar R, Rosenberg M, et al. Comprehensive genomic analysis of rhabdomyosarcoma reveals a landscape of alterations affecting a common genetic axis in fusion-positive and fusion-negative tumors. Cancer Discov 2014;4:216–31.
25. Bridge JA, Kanamori M, Ma Z, Pickering D, Hill DA, Lydiatt W, et al. Fusion of the ALK gene to the clathrin heavy chain gene, CLTC, in inflammatory myofibroblastic tumor. Am J Pathol 2001;159:411–5.
26. Kelly LM, Barila G, Liu P, Evdokimova VN, Trivedi S, Panebianco F, et al. Identification of the transforming STRN-ALK fusion as a potential therapeutic target in the aggressive forms of thyroid cancer. Proc Natl Acad Sci U S A 2014;111:4233–8.
27. Thomas GA, Bunnell H, Cook HA, Williams ED, Nerovnya A, Cherstvoy ED, et al. High prevalence of RET/PTC rearrangements in Ukrainian and Belarussian post-Chernobyl thyroid papillary carcinomas: a strong correlation between RET/PTC3 and the solid-follicular variant. J Clin Endocrinol Metab 1999;84:4232–8.
28. Falchook GS, Ordonez NG, Bastida CC, Stephens PJ, Miller VA, Gaido L, et al. Effect of the RET inhibitor vandetanib in a patient with RET fusion-positive metastatic non-small-cell lung cancer. J Clin Oncol 2016;34:e141–4.
29. Ross JS, Wang K, Chmielecki J, Gay L, Johnson A, Chudnovsky J, et al. The distribution of *BRAF* gene fusions in solid tumors and response to targeted therapy. Int J Cancer 2016;138:881–90.
30. Botton T, Yeh I, Nelson T, Vemula SS, Sparatta A, Garrido MC, et al. Recurrent BRAF kinase fusions in melanocytic tumors offer an opportunity for targeted therapy. Pigment Cell Melanoma Res 2013;26:845–51.
31. Ou SH, Chalmers ZR, Azada MC, Ross JS, Stephens PJ, Ali SM, et al. Identification of a novel TMEM106B-ROS1 fusion variant in lung adenocarcinoma by comprehensive genomic profiling. Lung Cancer 2015; 88:352–4.
32. Charest A, Lane K, McMahon K, Park J, Preisinger E, Conroy H, et al. Fusion of FIG to the receptor tyrosine kinase ROS in a glioblastoma with an interstitial del(6)(q21q21). Genes Chromosom Cancer 2003;37:58–71.
33. Suehara Y, Arcila M, Wang L, Hasanovic A, Ang D, Ito T, et al. Identification of KIF5B-RET and GOPC-ROS1 fusions in lung adenocarcinomas through a comprehensive mRNA-based screen for tyrosine kinase fusions. Clin Cancer Res 2012;18:6599–608.
34. Peraldo Neia C, Cavalloni G, Balsamo A, Venesio T, Napoli F, Sassi F, et al. Screening for the FIG-ROS1 fusion in biliary tract carcinomas by nested PCR. Genes Chromosomes Cancer 2014;53:1033–40.
35. Gotlib J, Cools J, Malone JM, Schrier SL, Gilliland DG, Coutré SE. The FIP1L1-PDGFRalpha fusion tyrosine kinase in hypereosinophilic

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syndrome and chronic eosinophilic leukemia: implications for diagnosis, classification, and management. Blood 2004;103:2879–91.
36. Chmielecki J, Ross JS, Wang K, Frampton GM, Palmer GA, Ali SM, et al. Oncogenic alterations in ERBB2/HER2 represent potential therapeutic targets across tumors from diverse anatomic sites of origin. Oncologist 2015;20:7–12.
37. Schrock AB, Frampton GM, Herndon D, Greenbowe JR, Wang K, Lipson D, et al. Comprehensive genomic profiling identifies frequent drug-sensitive EGFR exon 19 deletions in NSCLC not identified by prior molecular testing. Clin Cancer Res 2016;22:3281–5.
38. Ye K, Wang J, Jayasinghe R, Lameijer EW, McMichael JF, Ning J, et al. Systematic discovery of complex insertions and deletions in human cancers. Nat Med 2016;22:97–104.
39. Allegra CJ, Rumble RB, Hamilton SR, Mangu PB, Roach N, Hantel A, et al. Extended RAS gene mutation testing in metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy: American Society of Clinical Oncology Provisional Clinical Opinion Update 2015. J Clin Oncol 2016;34:179–85.
40. Tsukuda K, Tanino M, Soga H, Shimizu N, Shimizu K. A novel activating mutation of the K-ras gene in human primary colon adenocarcinoma. Biochem Biophys Res Commun 2000;278:653–8.
41. Gremer L, Merbitz-Zahradnik T, Dvorsky R, Cirstea IC, Kratz CP, Zenker M, et al. Germline KRAS mutations cause aberrant biochemical and physical properties leading to developmental disorders. Hum Mutat 2011;32: 33–43.
42. Tebbutt N, Pedersen MW, Johns TG. Targeting the ERBB family in cancer: couples therapy. Nat Rev Cancer 2013;13:663–73.
43. Ojesina AI, Lichtenstein L, Freeman SS, Pedamallu CS, Imaz-Rosshandler I, Pugh TJ, et al. Landscape of genomic alterations in cervical carcinomas. Nature 2014;506:371–5.
44. Dickson MA, Tap WD, Keohan ML, D'Angelo SP, Gounder MM, Antonescu CR, et al. Phase II Trial of the CDK4 inhibitor PD0332991 in patients with advanced CDK4-amplified well-differentiated or dedifferentiated liposarcoma. J Clin Oncol 2013;31:2024–8.
45. Cadoo KA, Gucalp A, Traina TA. Palbociclib: an evidence-based review of its potential in the treatment of breast cancer. Breast Cancer 2014;6: 123–33.
46. Ciznadija D, Liu Y, Pyonteck SM, Holland EC, Koff A. Cyclin D1 and Cdk4 mediate development of neurologically destructive oligodendroglioma. Cancer Res 2011;71:6174–83.
47. Chi AS, Batchelor TT, Kwak EL, Clark JW, Wang DL, Wilner KD, et al. Rapid radiographic and clinical improvement after treatment of a MET-amplified recurrent glioblastoma with a mesenchymal-epithelial transition inhibitor. J Clin Oncol 2012;30:e30–3.
48. Kim MS, Jeong EG, Yoo NJ, Lee SH. Mutational analysis of oncogenic AKT E17K mutation in common solid cancers and acute leukaemias. Br J Cancer 2008;98:1533–5.
49. Malapelle U, Pisapia P, Sgariglia R, Vigliar E, Biglietto M, Carlomagno C, et al. Less frequently mutated genes in colorectal cancer: evidences from next-generation sequencing of 653 routine cases. J Clin Pathol 2016; 69:767–71.
50. Hechtman JF, Sadowska J, Huse JT, Borsu L, Yaeger R, Shia J, et al. AKT1 E17K in colorectal carcinoma is associated with BRAF V600E but not MSI-H status: a clinicopathologic comparison to PIK3CA helical and kinase domain mutants. Mol Cancer Res 2015;13:1003–8.
51. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol 2014;15:852–61.

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