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OncoKB: A Precision Oncology Knowledge Base

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Executive Summary

PURPOSE—With prospective clinical sequencing of tumors emerging as a mainstay in cancer care, there is an urgent need for a clinical support tool that distills the clinical implications associated with specific mutation events into a standardized and easily interpretable format. To this end, we developed OncoKB, an expert-guided precision oncology knowledge base.

METHODS—OncoKB annotates the biological and oncogenic effect and the prognostic and predictive significance of somatic molecular alterations. Potential treatment implications are stratified by the level of evidence that a specific molecular alteration is predictive of drug response based on US Food and Drug Administration (FDA) labeling, National Comprehensive Cancer Network (NCCN) guidelines, disease-focused expert group recommendations and the scientific literature.

RESULTS—To date, over 3000 unique mutations, fusions, and copy number alterations in 418 cancer-associated genes have been annotated. To test the utility of OncoKB, we annotated all genomic events in 5983 primary tumor samples in 19 cancer types. Forty-one percent of samples harbored at least one potentially actionable alteration, of which 7.5% were predictive of clinical benefit from a standard treatment. OncoKB annotations are available through a public web resource (http://oncokb.org/) and are also incorporated into the cBioPortal for Cancer Genomics to facilitate the interpretation of genomic alterations by physicians and researchers.

CONCLUSION—OncoKB, a comprehensive and curated precision oncology knowledge base, offers oncologists detailed, evidence-based information about individual somatic mutations and structural alterations present in patient tumors with the goal of supporting optimal treatment decisions.

Introduction

The last decade has witnessed accelerating growth in our understanding of the genomic landscape of common and rare cancer types, and prospective clinical sequencing of patient tumors is now increasingly recognized as a component of routine cancer care[1,2]. Tumor sequencing is also being applied more broadly as an investigational tool, with the goal of matching patients to treatments that target the mutations or downstream pathways that drive the growth and/or progression of individual tumors. With the shift from single analyte tests and small hotspot panels to larger gene panels and whole exome and genome platforms, interpreting the clinical significance of the increasing number of genomic alterations identified in individual tumors has become a challenge. Most somatic alterations identified by whole exome and large gene panel sequencing are likely passenger events with no influence on the patient's prognosis or response to therapy. A smaller subset are known or

suspected functionally significant mutations with no clear therapeutic implications, and the smallest subset consists of known driver mutations that are clinically actionable.

The information that discriminates whether an alteration is clinically actionable can reside in various silos, including US Food and Drug Administration (FDA) labeling, National Comprehensive Cancer Network (NCCN) guidelines, conference proceedings, disease-focused expert group recommendations and the scientific literature. Therefore, there is an urgent need for a clinical support tool that distills this information into a standardized and easily interpretable format that democratizes its access to clinicians of all knowledge levels and at all centers. Such a support tool would help clinicians to interpret genomic alterations detected in patient tumor samples and enable them to make optimal treatment decisions for each individual cancer patient.

Several knowledge base efforts exist including MyCancerGenome, CIViC [3], the IMP Knowledgebase [4], the JAX-Clinical Knowledgebase (CKB) [5], Cancer Genome Interpreter (CGI), CANDL [6], TumorPortal [7], Targeted Cancer Care, and the Personalized Cancer Medicine Knowledge Base [8]. Some of these databases are in their early stages of development, do not yet contain sufficient breadth or detail to be used in clinical decision support and vary in the methods by which data are collected, stored, or accessed. To address these limitations, we describe OncoKB, a comprehensive precision oncology knowledge base that offers oncologists detailed, evidence-based information about individual somatic mutations and structural alterations present in patient tumors to support optimal treatment decisions. OncoKB content is supervised by a dedicated panel of physicians and cancer biologists that review and edit biomarker-associated investigational therapeutic strategies (Data Supplement). Through a continuing dialogue with the scientific and medical community, OncoKB therefore integrates clinical best practices as defined by institution-wide, multi-disciplinary disease management teams (Fig. 1). OncoKB communicates information on the biomarker-guided use of FDA-approved therapies and investigational agents that are under evaluation in clinical trials and highlights negative clinical results to discourage off-label use of expensive targeted therapies that have been shown to be ineffective in specific mutational contexts.

The OncoKB Framework

OncoKB includes biological, clinical, and therapeutic information curated from multiple unstructured information resources, including guidelines and recommendations derived from FDA labeling, NCCN guidelines, other disease-specific expert and advocacy group recommendations, and the medical literature (Fig. 1). Recognizing that clinical implications vary substantially based on the specific alteration within a gene and tumor context, information in OncoKB is hierarchically organized by gene, alteration, tumor type, and clinical implications (Fig. 1). OncoKB information is publicly available through an interactive website (http://oncokb.org/) and incorporated into the cBioPortal for Cancer Genomics (http://cbioportal.org/)[9,10], where genomic alterations of patients are annotated with information from OncoKB and their biologic effects and clinical implications are summarized, facilitating the interpretation of complex genomic data by cancer researchers

and clinicians (Fig. 1). To date, OncoKB has annotated over 3000 alterations in 418 cancer-associated genes (Table 1).

Levels of Evidence

To communicate the clinical utility of individual mutant alleles consistently, a levels of evidence classification system was developed (Fig. 2), which takes into account the site of tumor origin, by recognizing that the effects of targeted inhibitors vary by tumor lineage, even in cancers that share the same mutant allele (Fig. 3). Potentially actionable alterations in a specific cancer type are assigned to one of four levels based on the strength of evidence that the mutation is a predictive biomarker of drug sensitivity to FDA-approved or investigational agents in a specific indication. OncoKB has currently annotated 3405 alterations in 418 genes (Table 1), including those in genes with standard therapeutic implications (either Level 1 or 2A) such as the *ALK, BRAF, EGFR* and *ERBB2* targetable kinases and genes that play a role in maintaining DNA integrity, such as *BRCA1* and *BRCA2*.

Level 1

Level 1 includes genes for which specific alterations have been recognized by the FDA as predictive of response to an FDA-approved drug in a particular disease context (Supplementary Table 1). Examples include BRAF V600E and either vemurafenib or dabrafenib as monotherapy or in combination with the MEK inhibitors cobimetinib or trametinib in melanoma; EGFR L858R and erlotinib, afatinib or gefitinib in non-small cell lung cancer (NSCLC); and mutations in exons 9 and 11 of *KIT* and imatinib, sunitinib and regorafenib in gastrointestinal stromal tumors (GIST). In total, 82 alterations in 12 genes are considered Level 1 (Supplementary Table 1). Recognizing that some alterations in what would be considered a Level 1 gene are intrinsically resistant to currently available FDA-approved drugs or fall outside of explicit FDA-approval, OncoKB assigns individual alterations to a level of evidence, as opposed to the entire gene. For example, BRAF K601E has been shown to be pharmacologically resistant to the RAF inhibitors vemurafenib and dabrafenib[11] and while compelling preclinical data associates this biomarker as being predictive of response to MEK inhibitors, the use of MEK inhibitors in patients with BRAF K601E mutant tumors remains investigational[12]. Therefore intrinsically RAF-inhibitor resistant *BRAF* mutations, while oncogenic, are not designated as Level 1 and BRAF K601E is assigned Level 3A based on emerging clinical data supporting this biomarker as predictive of response to the MEK inhibitor trametinib[13,14] (Fig. 3).

Levels 2A and 2B

Level 2A includes alterations that are not FDA-recognized biomarkers, but are considered standard care predictive biomarkers of response to an FDA-approved therapy in specific cancer types. These alterations are highlighted in expert panel guidelines such as the NCCN Compendium or ASCO Clinical Practice Guidelines. Several Level 2A associations involve rare cancer types (for example, *BRAF* V600E mutant histiocytosis) or small sub-populations of common cancers (for example, *BRAF* V600E mutant lung cancer) and therefore accrual to a prospective randomized phase III clinical study may not be feasible. However, administration of a targeted agent to patients whose tumors harbored the biomarker

demonstrated sufficient clinical activity to have changed standard practice. As the total number of patients affected is often small, an application for an FDA indication may never be filed, but disease experts consider the biomarker-drug association as a standard off-label use. In total, there are currently 11 genes with 85 alterations that are considered Level 2A (Supplementary Table 2).

As an example, *MET* exon 14 alterations are present in ~3% of non-small lung cancers (NSCLC)[15] and represent a distinct, molecularly-defined subpopulation of lung adenocarcinomas that are mutually exclusive with tumors harboring activating mutations in *EGFR* and *KRAS* and fusions of *ALK, ROS1* and *RET*[16–18]. An adequately powered randomized trial comparing the MET inhibitor crizotinib to other standard approaches such as chemotherapy or immunotherapy has not yet been performed in part due to the rarity of these alterations. However, durable complete or partial responses to crizotinib and cabozantinib in patients with *MET* exon 14 altered lung cancers have been reported[15,19]. While widely available due to its FDA-approval for use in *ALK*-fusion positive NSCLC, crizotinib is not explicitly FDA-approved in the setting of *MET* exon 14 altered NSCLC. Since the NCCN guidelines (version 3.2017 NSCLC, NSCL-H) consider off-label prescription of crizotinib a standard treatment approach for lung cancer patients with *MET* exon 14 alterations, they are classified as Level 2A (Fig. 2). Another example of a Level 2A alteration is BRAF V600E as a predictive biomarker for BRAF inhibitor sensitivity in NSCLC and malignant histiocytosis. While vemurafenib is not FDA-approved for use in patients with these specific BRAF V600E-mutant indications, the off-label use of vemurafenib is a well supported treatment option that is included in NCCN guidelines based on compelling clinical data[20–22] (Fig. 3).

Level 2B includes alterations that are standard predictive biomarkers of drug sensitivity in other tumor types but for which data in the tumor in question are either lacking or negative to date. As an example, BRAF V600E mutations have been identified in several cancer types, including urothelial carcinomas and germ cell tumors[23,24], for which there are currently no clinical response data reported in the literature. In these tumors, the use of RAF inhibitors in patients with BRAF mutant tumors remains investigational and BRAF V600E is therefore classified as a Level 2B (Fig. 3)[23]. In patients with BRAF V600E mutant colorectal cancer, BRAF inhibitors such as vemurafenib have been tested and have shown disappointing results[25]. Such negative data are referenced in OncoKB and argue against the use of RAF inhibitor monotherapy in patients with BRAF V600E colorectal cancers. Level 2B also takes into account that early results in investigational clinical trials with RAF inhibitors as part of combination regimens appear promising[26].

Levels 3A and 3B

Level 3A includes mutations that are candidate predictive biomarkers of drug response based on off-label use of FDA-approved drugs or investigational agents not yet FDA-approved for any indication. For the former, the evidence supporting the predictive value of the alteration is not considered sufficient to warrant a change in standard clinical practice and the use of the FDA-approved drug in this context would be considered investigational by disease experts. The OncoKB Level 3A classification only applies to tumor types in which clinical

activity has been reported, and the mutation/drug association is classified as Level 3B in all other tumor types. Fifty-five alterations in 25 genes are considered Level 3 (Supplementary Table 3).

A representative example of a Level 3 alteration is AKT1 E17K (Fig. 3). Promising clinical activity consistent with preclinical studies of this compound has been reported with the investigational pan-AKT inhibitor AZD5363 in patients with AKT1 E17K mutant breast, lung, cervical squamous and endometrial cancers[27–29]. On the basis of this emerging clinical data, AKT1 E17K is classified as a Level 3A mutation in breast, cervical, endometrial, ovarian, and lung cancers. Lacking disease-specific data, AKT1 E17K is classified as a Level 3B mutation in all other cancer types (Fig. 3). A more complex example of Level 3 alterations are ERBB2 missense mutations that are present in a minority of a broad range of human cancers[30] and often arise in patients without ERBB2 amplification or HER2 protein overexpression[31]. These ERBB2 mutants demonstrate varying degrees of sensitivity to HER2-selective kinase inhibitors such as lapatinib and neratinib[31] (Fig. 3). Since ERBB2 mutations are not recognized by the FDA as a predictive biomarker of response to ERBB2 targeted therapies and the clinical utility of such agents is promising[32] but not yet established, activating missense mutations in ERBB2 are classified as Level 3A in breast cancer and 3B in other cancer types (Fig. 3).

Level 4

Level 4 alterations are candidate predictive biomarkers of response to either FDA-approved or investigational agents based on compelling laboratory data and an absence of substantiating compelling clinical data. While anecdotal responses to targeted agents may have been demonstrated in individual patients whose tumor harbored a Level 4 alteration, the data is not sufficiently robust as to indicate that the presence of the mutation is associated with significantly greater activity compared to tumors lacking the alteration. For example, while studies in mouse- and patient-derived xenograft models have suggested that mTOR- or AKT-targeted inhibitors may be effective in PTEN-null tumors[33], the clinical data supporting PTEN loss as a predictive biomarker of response to PI3K, AKT or mTOR inhibitors in patients is limited and conflicting[34–39]. Therefore, classification of loss-of-function PTEN alterations as a Level 4 alteration indicates that patients with PTEN-deficient tumors would be rational candidates for a clinical trial of investigational PI3-kinase pathway inhibitors alone or in combination with other agents, but that the use of such agents outside the context of a clinical trial is not yet supported by the sum of the clinical data. Additional examples of Level 4 alterations include NF1 inactivating alterations, which may be predictive of response to MEK1/2 inhibitors[40], and EGFR exon 20 insertions in lung adenocarcinomas that respond poorly to erlotinib[41,42], but which may be sensitive to AP32788, an investigational inhibitor of EGFR and HER2[43] (Fig. 3). As of this publication, 47 alterations in 17 genes are considered Level 4 (Supplementary Table 4). As OncoKB levels are dynamic, mutations currently classified as Level 4 may be reclassified as Level 3 or higher if additional compelling clinical data emerges.

Levels of Resistance (Level R1–3)

OncoKB classifies mutations that have been shown to confer resistance to specific targeted therapies into one of three levels based upon the strength of the evidence that the mutation is predictive of treatment resistance (Fig. 2). Level R1 includes mutational events for which there is sufficient evidence to recommend routine testing for the mutation to identify with a high likelihood patients that will not respond to a standard therapy. Identification of R1 mutations would therefore lead to a recommendation that the associated therapy be withheld in patients whose tumors harbor the mutation. By definition, Level R1 mutations predict for resistance to FDA-approved drugs, and testing for such mutations is typically recommended by expert guidelines such as those published by the NCCN. Level R1 alterations include activating RAS mutations in colorectal cancer, which predict for resistance to the EGFR-targeted monoclonal antibodies cetuximab and panitumumab (NCCN Guidelines, Colon Cancer, Version 2.2016); EGFR T790M mutations in NSCLC, which predict for intrinsic and acquired resistance to the EGFR tyrosine kinase inhibitors (TKIs) erlotinib, afatinib and gefitinib (NCCN Guidelines, Non-Small Cell Lung Cancer, Version 4.2016); and PDGFRA D842V, which predicts for resistance to imatinib in patients with gastrointestinal stromal tumors (GIST) (NCCN Guidelines, Soft Tissue Sarcoma, Version 2.2016). Alterations classified as Level R2 and R3 have hypothetical therapeutic implications and include alterations that are predictive of drug resistance on the basis of clinical or biological data, respectively, but their use in guiding treatment decisions is considered investigational (Fig. 2). In some cases, alternative targeted therapies have been developed that specifically target an alteration predictive of resistance to first- or second-line targeted therapies. For example, EGFR T790M is Level R1 for erlotinib, gefitinib and afatinib, but is also classified as Level 1, as it predicts sensitivity to the EGFR inhibitor osimertinib, which was recently approved for use in patients with NSCLC that have progressed on first-line EGFR TKI therapy and whose tumors harbor the EGFR T790M mutation[44].

Functional alterations without compelling treatment implications

There are many genes that are critical mediators of tumorigenesis but for which compelling targeted therapeutic strategies have yet to be developed. For example, while multiple strategies to reverse the oncogenic effects of TP53 loss have been explored in laboratory studies and early phase clinical trials[45,46], the agents tested do not directly target TP53, their activity is typically not restricted to TP53 mutant models, and clinical trials testing these agents have either been aborted due to lack of efficacy or do not use TP53 status as a selection criterion[47]. Thus, while genomic alterations in TP53 are typically oncogenic, OncoKB does not consider them therapeutically actionable. In fact, over 90% of alterations in OncoKB have curated biological effects and are classified as oncogenic, but are not associated with actionability.

Actionable alterations across cancer types

While targeted inhibitors have been shown to improve clinical outcomes in melanoma and lung cancer among others[48], the broader clinical utility of large panel or whole exome testing remains undefined. To assess the potential clinical impact of prospective broad tumor genomic testing, we used the OncoKB levels of evidence classification to annotate

mutations, copy number alterations, and gene fusions in a publicly available data set of 5,983 primary tumor samples representing 19 cancer types profiled by whole exome and RNA sequencing by the Cancer Genome Atlas (TCGA)[1]. While over 90% of samples harbored at least one known oncogenic mutation, only 41% had one or more alterations for which compelling clinical data currently exists to justify the use of a standard or investigational agent (Levels 1–3B) (Fig. 4A). Overall, 7.5% of all samples harbored alterations that predict for response to a standard therapy in that disease context (Levels 1 and 2A) (Fig. 4A). Level 1 and 2A alterations were most common in melanoma (44%), ovarian cancer (21% - this includes 65 out of 312 samples that have either germline or somatic BRCA1 or BRCA2 inactivating mutations), soft tissue sarcomas (19% - based on CDK4 amplifications, which are predictive of response to palbociclib in well-differentiated and dedifferentiated liposarcomas but not in other soft tissue sarcomas), non-small cell lung (14%), esophagogastric (13%) and breast (12%) cancers. Low grade gliomas (LGG) and melanomas had the highest proportion of actionable alterations. However, whereas 44% of melanomas had mutations that predict for clinical benefit with standard therapies in melanoma patients, the vast majority of actionable alterations in LGG were associated with only investigational implications, with the most common mutation being IDH1 R132C (77% of LGG samples), a level 3B alteration based upon promising clinical data with the IDH1 inhibitor AG-120 in patients with acute myeloid leukemia (Fig 4A, B). In total, just over 10% of all samples had a Level 3A mutation as their highest actionable alteration, a cohort of patients for which enrollment onto a clinical trial would represent a compelling treatment option after standard treatments. Additionally, approximately 15% of samples had Level 3B alterations as their highest actionable event, alterations for which promising clinical data has been observed in an investigational setting in another cancer type.

On average, there were approximately three oncogenic mutations per sample, and the number of known oncogenic mutations per sample in tumor types was independent of overall mutation burden (Fig. 4C). The number of actionable and oncogenic mutations varied greatly across cancer types. For example, while renal cell cancers had on average one oncogenic mutation per sample, these were typically inactivating mutations in tumor suppressors such as VHL and PBRM1, which are not clinically actionable at this time. Therefore, 95% of renal cell cancers had no actionable alteration (Fig. 4D). In contrast, while thyroid cancers also on average had approximately one oncogenic mutation per sample (Fig. 4C), these were typically actionable alterations, such as RET fusions, BRAF and NRAS mutations. Therefore, 60% of thyroid samples had at least one actionable alteration (Fig. 4D). Breast, colorectal, and esophagogastric cancers were found to have a large fraction of samples with two or more actionable mutations (31%, 25%, and 22%, respectively), consistent with data demonstrating that these tumor types are driven by multiple oncogenic mutations in non-redundant pathways[49–52] (Fig. 4D), which may explain why targeted monotherapies have shown disappointing results to date in some of these cancer types[53,54].

Discussion

Since the introduction of imatinib for chronic myeloid leukemia over a decade ago[55,56], a growing number of drugs that target specific genetic alterations required for tumor initiation

and progression have been shown to significantly improve outcomes in molecularly defined populations of cancer patients[48,57,58]. While tumor genetic testing is now part of routine patient care in an increasing number of tumor types, interpretation of variants remains a critical challenge, and in major academic cancer centers a significant fraction of physicians report low confidence in their ability to make optimal treatment recommendations based on genomic information[59].

While multiple classification systems exist for the annotation of germline variants[60,61], efforts to define the clinical utility of somatic alterations have been limited to established biomarkers[62–64] and prior efforts have often classified actionability as a binary variable resulting in the grouping together of biomarkers that are FDA-recognized with those that are non-actionable but oncogenic. To this end, we assigned each mutation to one of four levels based upon the available clinical and laboratory data supporting the use of the mutation as a predictive biomarker. Standard therapeutic implications are classified as either Level 1 and 2A to recognize that not all mutation-drug associations employed in standard practice have been recognized by the FDA. Levels 2B, 3 and 4 include mutations for which the biomarker-drug association remains investigational and may be useful in guiding the use of an FDA-approved therapy in an off label setting or preferably prompting consideration for enrollment in appropriate clinical trials. The latter is particularly relevant as clinical trials are increasingly incorporating into their eligibility criteria the molecular profiles of patient tumors, including basket studies such as the Roche VE BASKET (NCT01524978), SUMMIT (NCT01953926) and LOXO NTRK trials (NCT02576431), and master or umbrella studies including NCI-MATCH (NCT02465060), ASCO TAPUR (NCT02693535), Genentech MyPathway (NCT02091141) and Novartis Signature (NCT02186821).

There is a long history of off-label use of cancer drugs in oncology[65–67], particularly in patients with rare cancer types for which randomized clinical trials may not be feasible. Access to drugs off-label is becoming increasingly difficult due to high drug costs and variations in reimbursement among individual insurance providers. Moreover, there are cases where off-label use of an FDA-approved drug is explicitly not warranted due to existing data that argue against the use of a targeted agent in a specific cancer type. For example, while the BRAF inhibitor vemurafenib is a standard treatment option for patients with BRAF V600E mutant melanoma or NSCLC, robust clinical reports from multiple independent centers demonstrate that patients with BRAF V600E mutant colorectal cancer do not respond to RAF-inhibitors, at least as monotherapy[25,68]. Similarly, while ERBB2 amplification predicts for the clinical utility of HER2-targeted therapies in breast and esophagogastric cancers, the clinical activity of trastuzumab in ERBB2-amplified lung cancers has been disappointing[69]. BRAF V600E and ERBB2 amplification are therefore Level 2B when detected in colorectal cancers. A Level 2B designation should encourage consideration of a clinical trial. In the case of BRAF V600E mutant colorectal cancers, somewhat more encouraging results have now been observed with BRAF-EGFR inhibitor combinations[26]; however, given that thus far this is a non-standard approach and expensive agents are involved, obtaining these drugs outside of a clinical trial in this scenario may be difficult.

Individual mutant alleles within a single gene may be functionally distinct, with different predictive value and therefore individual therapeutic implications. This complicates the development of clinical decision support tools, particularly in the context of often vague FDA-labeling and expert guidelines, such as that provided by the NCCN, which may not define at a granular level whether or not specific mutations within a gene are predictive of drug response. To address this complexity, OncoKB groups mutations in Level 1 genes such as EGFR and KIT according to 1) whether or not they are biologically active, 2) whether there is preclinical data suggesting that the allele is sensitive or resistant to the matched targeted agents and 3) whether there is clinical data suggesting clinical sensitivity or intrinsic resistance to the approved targeted therapy. Consequently, less common EGFR mutations such as L861Q and G719A are classified as Level 1, whereas EGFR exon 20 insertions are classified as Level 4. A limitation of this approach is that the response rate of erlotinib in patients with these mutations may prove to be lower relative to the more common EGFR TKI sensitizing alleles such as L858R once more clinical data with these rare alleles becomes available. We therefore anticipate further refinement of OncoKB classifications as additional clinical data emerges for rare targetable alleles. The challenge of rare drug sensitive variants in Level 1 genes also highlights the need for consortia efforts such as AACR Project GENIE, which should allow the collection of clinical response data for rare alleles to help guide the treatment of patients with these less common mutations.

While new laboratory and clinical data are continually generated, FDA-labels and professional guidelines are updated at irregular intervals. Thus, the level of evidence assigned to an annotated alteration may change, with immediate implications for patients with active disease. For example, while explicit FDA approval of crizotinib in ROS1-rearranged NSCLC did not occur until March 2016, off-label use of crizotinib in ROS1 fusion-positive patients had been considered standard-of-care by several expert groups for some time[70–72]. As another example, while KRAS was initially considered a Level 3A alteration in NSCLC based on promising data from the randomized phase 2 study that supplemented standard chemotherapy with a MEK inhibitor[73], the subsequent phase 3 trial showed no survival benefit with this combination[74], consistent with negative data associated with MEK inhibitor use in KRAS-mutant pancreatic and colorectal cancers[75,76]. Nonetheless anecdotal clinical data and compelling preclinical data supports the use of KRAS as a predictive biomarker of sensitivity to novel MEK and ERK inhibitors, alone or in combination with other agents[77–81]. OncoKB thus recognizes and re-assigns the level of evidence of mutational events, if appropriate, based on newer, more definitive and negative randomized clinical data which takes precedence over prior preliminary clinical findings.

To incorporate new clinical and research findings, we have made the OncoKB annotation available publically through oncokb.org and via the cBioPortal. Both systems include a comment feature to facilitate crowdsourcing curation of this knowledge base. User suggestions are evaluated by the scientific team and incorporated into OncoKB through periodic updates. OncoKB is also an active member in efforts to promote harmonization of variant annotation across existent knowledge bases, and is participating in both ClinGen and the Global Alliance for Genomic Health (GA4GH) via the Variant Interpretation Cancer Consortium (VICC).

In the future, we will also curate information about mutational signatures such as overall mutation burden and the possible link to immunotherapy, mutational clonality and the impact on drug sensitivity of co-occurrence of specific oncogenic and/or actionable mutations. Additional prospective and retrospective clinical studies will further define the fraction of cancer patients and the specific patient subsets that benefit from targeted therapies. Towards this goal, it is critical that clinical and scientific researchers establish standards and aggregate scientific knowledge for the benefit of clinicians and patients. A curated database such as OncoKB can play an important role in realizing the promise of precision medicine by helping physicians to identify potentially actionable variants to ensure that patients receive appropriate standard therapies or are directed to the most appropriate clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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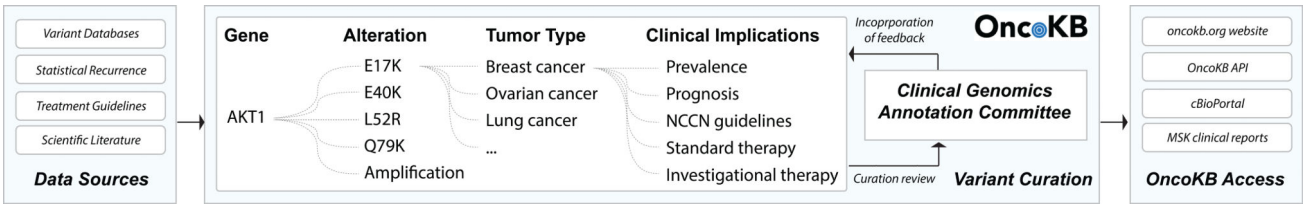

Figure 1. OncoKB Workflow

Data Sources: Alterations are identified by their recurrence (cancerhotspots.org), from public variant databases (cBioPortal, COSMIC, the MSK-IMPACT internal clinical sequencing cohort), and by prior knowledge available in the literature. Biological and clinical therapeutic implications of alterations are curated from several public resources including disease specific treatment guidelines, abstracts from major conference proceedings, such as ASCO, ESMO and AACR, and the scientific literature through PubMed. ***Variant Curation:*** This information is entered into the curation interface as structured data elements organized in a hierarchy of gene, alteration and tumor type. Within each tumor type, clinical implications including prevalence, prognostic implications, as well as standard or investigational therapeutic implications which are individually curated and stored. ***Clinical Genomics Annotation Committee:*** OncoKB annotation is vetted by selected clinicians and physician scientists across 22 disease management teams who make up the Clinical Genomics Annotation Committee (CGAC). Curation review occurs in the form of sample medical reports sent on a 3-monthly basis to CGAC members and monthly emails requesting feedback. CGAC recommendations and feedback are incorporated into OncoKB in real time. ***OncoKB Access:*** OncoKB data is available for public use through oncokb.org and the cBioPortal for Cancer Genomics and is used internally to annotate MSK clinical reports.

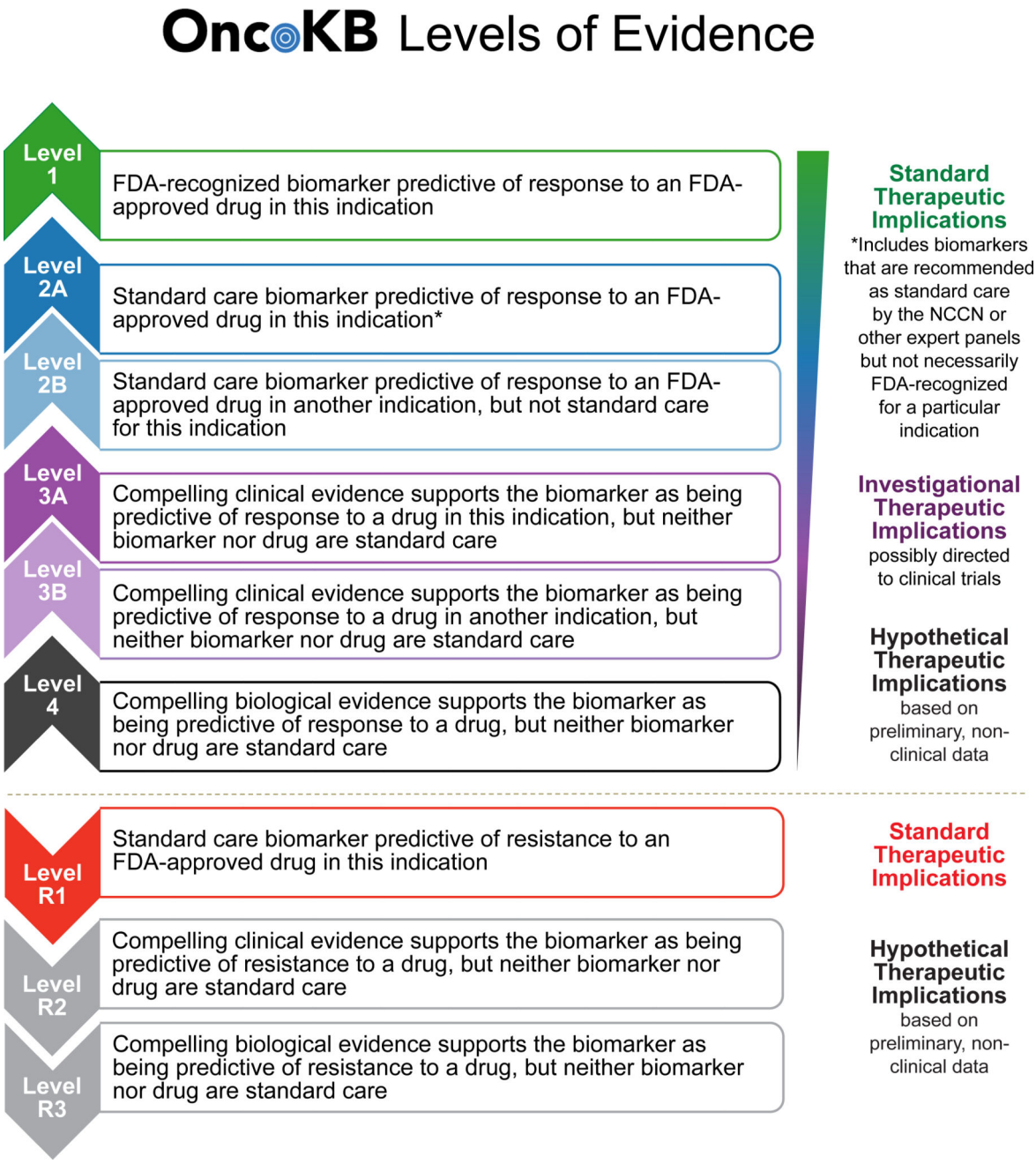

Figure 2. OncoKB Levels of Evidence

Individual mutational events are annotated by the level of evidence that supports the use of a certain drug in an indication harboring that mutation. ***Standard therapeutic implications*** include FDA-recognized biomarkers that are predictive of response to an FDA-approved drug in a specific indication (Level 1) and standard care biomarkers that are predictive of response to an FDA-approved drug in a specific indication (Level 2A). ***Investigational therapeutic implications*** include FDA-approved biomarkers predictive of response to an FDA-approved drug detected in an off-label indication (Level 2B), FDA or non-FDA-recognized biomarkers that are predictive of response to novel targeted agents that have

shown promising results in clinical trials (Level 3A), and non-FDA-recognized biomarkers that are predictive of response to novel targeted agents based on compelling biological data (Level 4).

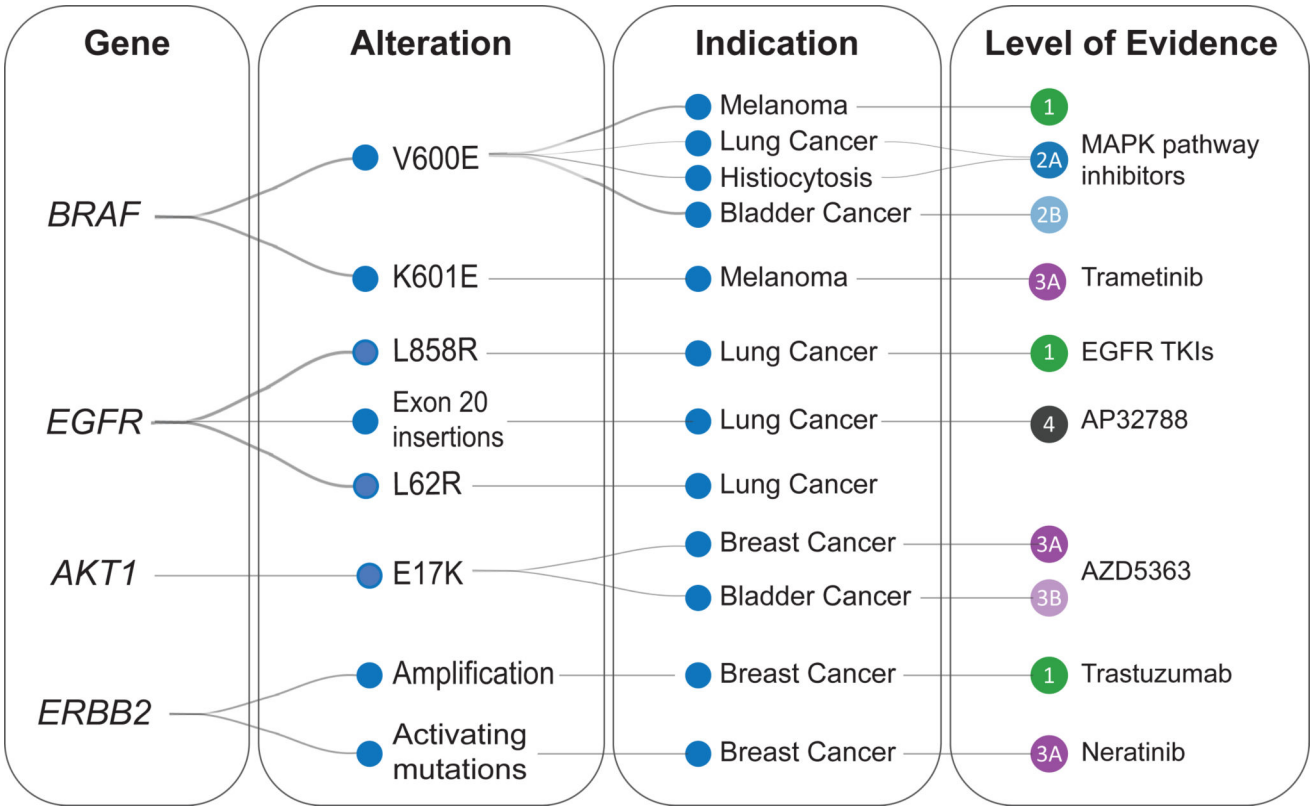

Figure 3. Examples for the OncoKB Levels of Evidence system

Information in OncoKB is organized hierarchically by gene, alteration, indication and level of evidence. Implicit in the designation of a level of evidence for each branch is whether the biomarker is FDA-recognized, standard care or investigational, and whether it is predictive of response to a drug that is FDA-approved or currently being tested in clinical trials. Examples shown here are BRAF, EGFR, AKT1 and ERBB2.

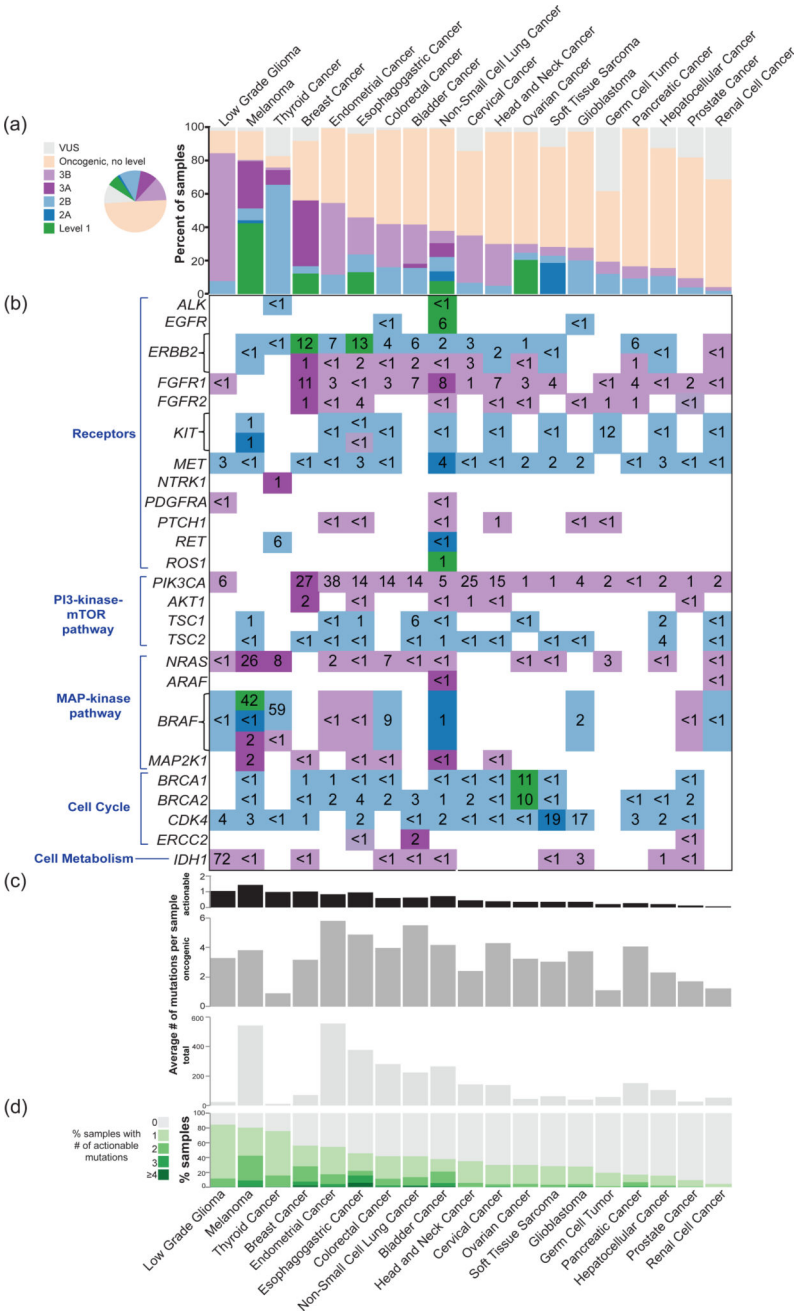

Figure 4. Frequencies of Levels of Evidence 1 to 3 assignments in TCGA cohorts

Patient samples from 19 cancer types (TCGA) are classified by the alteration carrying the highest level of evidence. **a)** ***Inset pie-chart:*** Fraction of samples across all cancer types that carry a mutation considered actionable according to the levels of evidence, oncogenic but not actionable or variants of unknown significance (VUS). ***Stacked bar-graph:*** Similar analysis as inset pie-chart. Tumor-type specific samples are analyzed by variants considered actionable, oncogenic but not actionable, or VUS. **b)** Highest level of evidence by tumor type and gene. Cell color: Level 1 - green, Level 2A - dark blue, Level 2B - light blue, Level 3A - dark purple, Level 3B - light purple. *Columns:* Sample tumor type, *Rows:* Gene

alteration present in sample, and number indicates the percentage of samples per tumor type harboring an alteration in each gene. **c)** Each patient sample was classified by the number of oncogenic alterations or the number of actionable alterations. Shown here is the mean number of actionable (black), oncogenic (dark grey) or total (grey) mutations per sample per tumor type. **d)** Each tumor type was evaluated for the percent of samples that carry 0, 1, 2, 3 or ≥4 actionable mutations per sample (indicated in shades of green).

Table 1

OncoKB Results

OncoKB Annotations Metrics	Genes	Alterations
Total Number	418	3405
FDA-approved (Level 1)	12	82
Standard care (Level 2A)	11	85
Emerging clinical evidence (Level 3A)	25	55
Biological evidence (Level 4)	16	38
Oncogenic without a level of evidence	375	3199