Precision medicine requires precision in clinical interpretation and reporting with NAVIFY® Mutation Profiler

Introduction

The power unleashed by being able to interrogate tens to hundreds of genes to identify the genetic makeup of a cancer through the advent of next-generation sequencing is, as it turns out, just a first step in realizing precision medicine. The greater challenge lies in translating variant calls into actionable knowledge with respect to what patient management options may now be available to a treating oncologist based on the vast amount of clinical evidence. To make this task even more daunting, each year there is an accelerated expansion of known molecular biomarkers, available targeted therapies, and emerging treatment resistance mechanisms in various cancer types.

With this new individualized medicine paradigm, each patient's unique molecular profile necessitates a tailored clinical test report and corresponding action plan. The information required to qualify any potential variant with respect to its pathogenicity in cancer and subsequently the level of evidence supporting its potential clinical actionability is large and growing. And yet the resulting clinical report must be concise, offering interpretations that are tailored to the patient's mutations, appropriate to the context of the patient's cancer, based on timely knowledge, and succinct.

In this white paper, we provide more details about the knowledge base that underlies NAVIFY Mutation Profiler and NAVIFY Therapy Matcher app and enables labs to deliver such concise, actionable clinical reports.

NAVIFY Mutation Profiler and NAVIFY Therapy Matcher are not yet commercially available in the United States.

The requisite knowledge is vast

Version 89 of the COSMIC cancer database includes over 7.4 million coding mutations, gene fusions and copy number variants identified across 1.4 million samples and over 40 different cancer types.[1] And while many variants are expected to be encountered repeatedly by a clinical lab, the long tail of variants observed (see Figure 2) means many variants will also be encountered anew by the lab, especially as panel sizes grow. Considering this, maintaining an internal curation effort that prompts a fresh literature search with each novel variant the lab encounters may not prove feasible for most clinical labs.

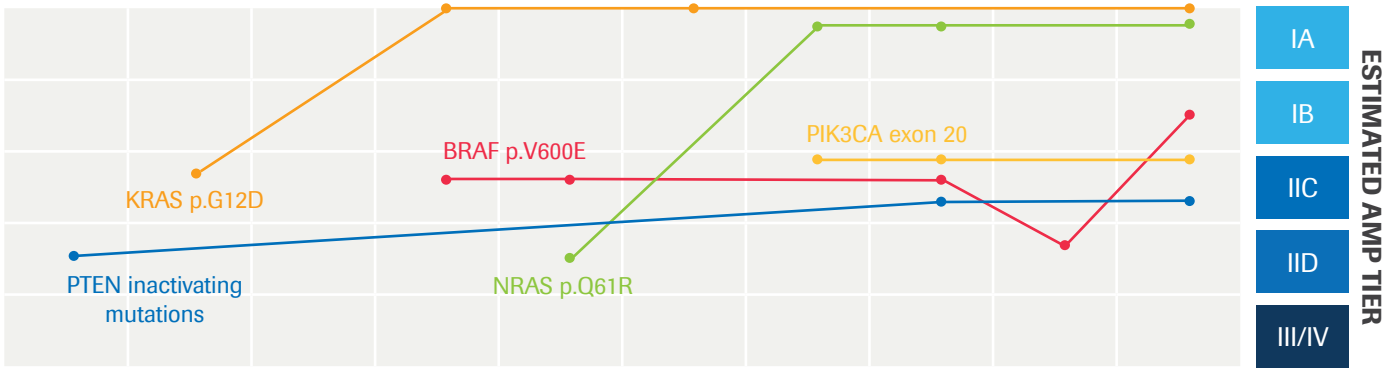
Knowledge is evolving

Labs attempting to build internally curated knowledge bases are also challenged by the dynamic state of knowledge in the oncology field. Take the example of the evolving understanding of how alterations in the RAS/RAF pathway have impacted the clinical utility of anti-EGFR therapy in colorectal cancer (Figure 1). The understanding of the actionability associated with variants within specific genes in this pathway has changed continuously over the last 20 years. This highlights the importance of revisiting information about variants, even when a variant has already been curated by the lab. In light of the challenge facing clinical labs to maintain a progressively growing body of knowledge, Roche has developed NAVIFY Mutation Profiler, a clinical decision support solution that includes content, specifically, the classification and curation of thousands of the most commonly encountered variants in the most commonly tested cancers, greater than 12,500 variants and 4 cancer types as of launch. Furthermore, Roche is committed to expanding curation to support additional genes and cancers.

Complementing this are clinical lab community-driven web-based resources, such as CIViC, that provide a shared repository of variant curation (corresponding to over 2000 variants as of January 2019[2]). These efforts can be considered complementary as the curated variant sets are distinct, but overlapping. As clinical labs trend towards larger testing volumes, a larger selection of panels, a greater breadth of genes being tested and more variant types such as gene fusions and copy number variants, the availability of a curation resource such as that provided with NAVIFY Mutation Profiler empowers the lab to support higher volumes and more complex testing without the need to proportionately grow an internal team of clinical curation scientists.

The dynamic state of knowledge for biomarkers in colorectal cancer

Figure 1. The change in the understanding of clinical significance for five biomarkers is shown. In many cases, clinical significance increases steadily based on advancing knowledge. Notes: The figure below is not exhaustive in terms of referencing every study forming the basis of estimated clinical significance shown in terms of Association for Molecular Pathology (AMP) tiers[3]; AMP Tier IA is geography-specific and is marked here as such, if Tier IA in either US or EU. Abbreviations used: metastatic colorectal cancer, mCRC; objective response rate, ORR.



2004

2004: PTEN inactivating mutations were detected in 17% of CRC patients[4]

2004: Saltz et al (2004)[5] show that **cetuximab** plus irinotecan therapy achieves a 22.5% major ORR

2004: FDA and EMA approve **cetuximab** for chemorefractory mCRC. However, only 10% of mCRC patients benefit from this therapy

2005

2005: KRAS mutations tested for prediction of clinical response to anti-EGFR treatment, but statistical significance not achieved.[6] However, authors show higher EGFR copy number correlates with response.[7] The latter finding is not borne out in subsequent studies.[13]

2006

2006: KRAS mutation is predictive of non-response to cetuximab in mCRC.[8] Only exon 2 is examined, and only mutations **codon 12 and 13** are observed among 13 patients that were KRAS mutation positive

2006: FDA approves **panitumumab** for mCRC

2007

2007: BRAF v600E mutation negatively associated with response to anti-EGFR therapies[9]

2007: EMA approves panitumumab in **KRAS-exon-2-wildtype** mCRC only

2007: KRAS mutation is predictive of non-response to cetuximab plus chemotherapy[10,11]

2008

2008: EMA narrows cetuximab to **KRAS-exon2-WT** mCRC

2008: Based on a retrospective study, **BRAF V600E** is a negative predictor of response to anti-EGFR therapy[12]

2009

2009: FDA restricts cetuximab and panitumumab to **KRAS-exon2-WT** mCRC

2010

2010: PIK3CA exon 20 and **NRAS mutation** statuses are both predictive of lack of response to cetuximab + chemotherapy in chemorefractory mCRC[13]

2014

2014: NCCN recommends **extended RAS testing**

2014: Based on meta-analysis, mutations in KRAS exon 3/4, NRAS, BRAF, **PIK3CA** and **PTEN** predict non-response to anti-EGFR therapies[14]

2015

2015: One meta-analysis of **BRAF-mutated** CRC patients shows a lack of clinical benefit with anti-EGFR therapy,[15] while a second meta-analysis is unable to demonstrate statistical significance[16]

2015: A meta-analysis of 6,000 patients confirms predictive value of **expanded RAS testing**[17]

2015: FDA restricts cetuximab/ panitumumab labels to **RAS-WT** CRC patients

2016

2016: ESMO medical guidelines recommend **BRAF testing** at time of RAS testing for prognostic value. BRAF-mutated patients should be treated with a chemotherapy or chemotherapy combination rather than with EGFR mAb therapy

With so many variants, which to curate?

While the number of variants the clinical lab community could potentially encounter is potentially limitless, the frequency with which genes are mutated, the frequency of mutations observed within a gene and even the frequency with which cancers are tested by NGS all follow long-tailed distributions. These observations provide a means by which to prioritize variant curation and should effectively minimize the time a lab should have to spend classifying and curating variants (see Figure 2). Additionally, since not curating a clinically significant but rare variant for a given cancer has worse potential implications than curating a moderately common variant of low to no clinical significance, variants with the highest clinical significance, those variants with an associated approved therapy based on drug labels or medical guidelines, are given the highest priority even when they occur at lower frequencies. As of product launch, NAVIFY Mutation Profiler will include more than 12,500 variants curated across four cancer types: non-small cell lung cancer, colorectal cancer, melanoma and breast cancer. With each release, curated content will be available to cover additional cancer types and greater numbers of variants.

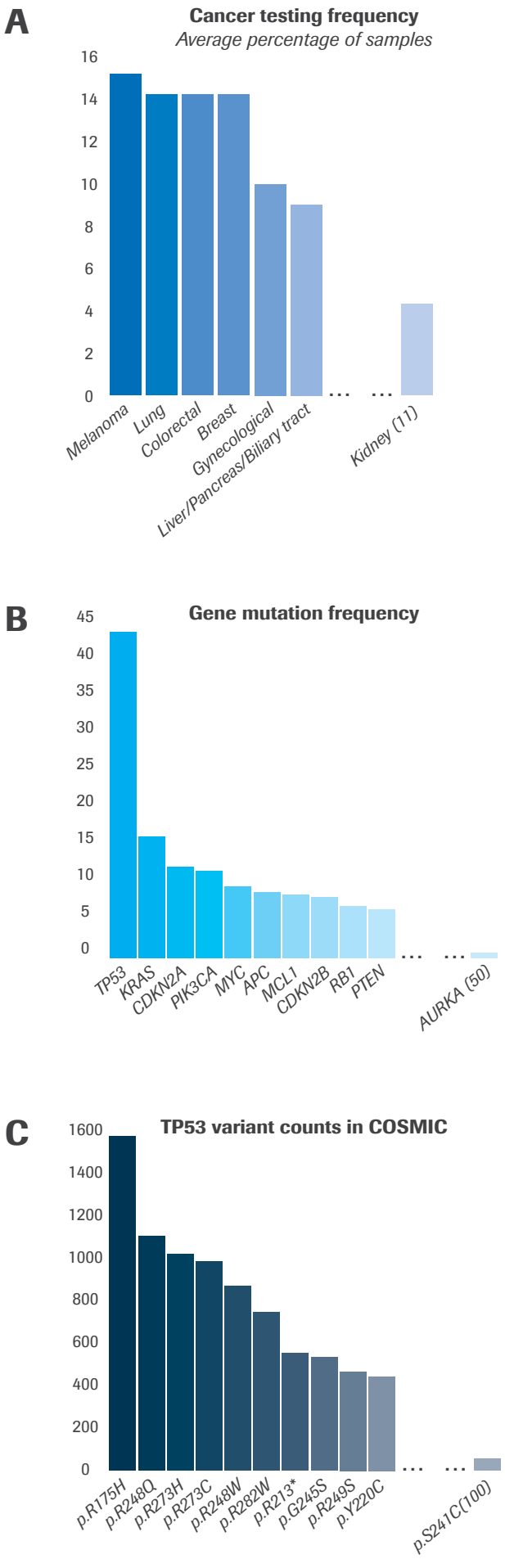


Figure 2. Cancer Types Tested, Genes Mutated and Variants Detected Follow a Long-Tailed Distribution. A. The average percentage solid tumor types tested based on analysis of clinical testing by 4 labs and French ProfiLER study[18,19,20,21,22,23]; The top 6 and the 11th most tested cancer types are shown for cancer types identified by 2 or more labs; B. Gene mutations frequencies based on genes mutated among 2201 solid tumor FFPE samples tested by Foundation Medicine. The ten most mutated and 50th most mutated genes are shown. Figure adapted from Frampton et al (2013).[17] The counts for different single point TP53 gene mutations as observed in the COSMIC version 87 database across multiple cancer types.

The How: Building evidence and classifying variants based on evidence

Most of the knowledge that informs variant classification resides in medical literature, guidelines and drug labels as written text embedded in larger documents. Yet a knowledge base implies that data is structured. Furthermore, when variants are classified based on evidence supporting predictive, prognostic or diagnostic significance, an ideal data structure can facilitate the classification process. To this end, the Roche team of clinical curation scientists monitors and queries medical literature and other relevant sources, recording evidence supporting the clinical significance of variants in a manner similar to that published by CIViC,[2] that of the accumulation of units of evidence in the form of evidence statements. One or multiple evidence statements may be derived from each document that is critically reviewed. Based on the review of a set of evidence statements mapping to a given variant in a specific cancer context - referred to as a biomarker profile - variants are tiered with respect to clinical significance to the classification scheme recommended by the Association for Molecular Pathology.[3] This work is performed internally and enables the Roche curation team to track knowledge with respect to each variant and its relevance for treatment, prognosis or in refining diagnosis as it accrues. However, with the intent to provide a fit-for-purpose workflow solution for clinical labs, the curation team then summarizes available evidence into concise paragraphs in a form that is both ready for review by clinical lab members, as well as suitable for inclusion in clinical reports that oncologists can consume. (See Figure 6 for the curation and review process that content is subject to before content release.) When clinical labs are pressed to process cases with a short turnaround time, these preconfigured summaries that convey the biological and functional relevance of both gene and variant, as well as the clinical evidence pertaining to both, have the potential to save the lab significant time without sacrificing quality in terms of accuracy or timeliness. Furthermore, within the NAVIFY Mutation Profiler user interface, the clinical lab member reviewing variants, can click on sources for the underlying evidence via embedded hyperlinks to PubMed where original publications can be accessed.

Figure 3. Curated content includes multiple summaries to aid interpretation. **A.** A variant clinical summary (such as for PIK3CA H1047R in breast cancer, shown here) provides a concise review of clinical significance of the biomarker profile, with relevant citations included. **B.** A gene biological and functional summary recaps the corresponding protein's role in cancer. **C.** The variant functional summary focuses on the precise impact of the variant of concern on the protein.

A **Variant clinical summary**

In a phase 1 trial, four patients with breast cancer harboring PIK3CA H1047R demonstrated a confirmed partial response when treated with the investigational PI3K inhibitor taselisib (PMID: 28331003). In preclinical studies, breast cancer cell lines harboring PIK3CA H1047R demonstrated resistance to trastuzumab and lapatinib alone and in combination (PMID: 26920887)(PMID: 19010894); however, one study showed sensitivity to lapatinib (PMID: 26627007). In other preclinical studies, breast cancer cell lines harboring PIK3CA H1047R demonstrated sensitivity to neratinib, trametinib and metformin (PMID: 26627007)(PMID: 23986086). Additional preclinical studies have determined PIK3CA H1047R in breast cancer is associated with sensitivity to investigational HER2 antibodies and PI3K, AKT and mTOR inhibitors (PMID: 26920887)(PMID: 21325073)(PMID: 21558396)(PMID: 26627007) (PMID: 26469692)(PMID: 26237138)(PMID: 28539475)(PMID: 27699769)(PMID: 27186432)(PMID: 23986086)

B **Gene biological summary**

{PIK3CA encodes the p110a catalytic subunit of the heterodimeric PI3K complex (PMID: 18794884). Activated receptor tyrosine kinases recruit PI3K and activate the PI3K/AKT/mTOR pathway to regulate growth, proliferation, autophagy, and survival (PMID: 18767981). Important domains in PIK3CA include the helical domain (residues 525-696), the kinase domain (residues 697-1068), the adaptor-binding domain (residues 1-108), the RAS-binding domain (residues 191-291), and the C2 domain (residues 328-480) (PMID: 18079394). (Uniprot.org).

C **Variant functional summary**

PIK3CA H1047R is a hotspot mutation that lies within the kinase domain of the PIK3CA protein (UniProt.org). This mutation results in increased phosphorylation of AKT and MEK1/2, growth factor-independent cell survival, and is transforming in cell culture (PMID: 26627007).

AMP variant classification overview

The classification scheme for variants in germline disease proposed by the American College of Medical Genetics (ACMG) is significantly simpler[22] than that proposed more recently by AMP which worked with multiple organizations, including ACMG, to determine recommendations for classifying somatic variants in cancer. ACMG proposes classifying variants in germline disease as one of *pathogenic, likely pathogenic,* v*ariant of uncertain significance*, *likely benign* or *benign* with respect to a given disease. In contrast, the AMP classification scheme recommends that pathogenic and likely pathogenic variants be stratified more finely into four tiers based on the underlying evidence supporting the clinical actionability of the variant.

Variants are tiered as specified in AMP guidelines. Specifically, variants which are included on a drug label for a given cancer, qualify at AMP Tier IA for that cancer. Alternatively, variants for which associated recommendations are found in regional medical guidelines that apply also qualify as Tier IA. In situations where robust clinical evidence supports the predictive, diagnostic or prognostic significance of a variant, but where the variant is not yet referenced in medical guidelines, the variant is tiered to Tier IB. Tier IIC variants correspond to variants that are either Tier IA in an alternate cancer, which serve as inclusion criteria in clinical trial(s) or for which evidence is emerging in support of clinical significance. Finally, Tier IID variants may be variants with conflicting or only preclinical evidence supporting clinical significance.

Region-specificity of guidelines

It follows from this that a Tier IA variant in one region may be classified to a lower tier in another region for lack of inclusion in the applicable drug label or medical guidelines. Roche content is curated so that labs see classifications that apply to their specified region.

The AMP classification scheme adds meaningful value for the oncologist in that it provides an immediate rank by priority when variants are listed in a clinical report. At the same time, however, the greater due diligence required for this finer stratification of variants places a higher burden on labs that may not be sufficiently staffed to directly take on the task of tracking drug approvals, reading updated cancer guidelines, and even reviewing research articles that, for instance, may elevate a potentially clinically significant variant (Tier II) from a lower level (IID) to a higher level (IIC) of potential clinical significance.

Figure 4. Tier IA variants are geography-dependent. Variants that are Tier IA in one region, such as the NTRK1 fusion, may correspond to a lower tier in other regions due to local drug agency labels and applicable regional medical guidelines.

AMP Tiers

AMP Tier	Geographic Regions	Disease	Biomarker
Tier I - Level A	United States of America	**colorectal cancer**	**NTRK1 fusion**
Tier I - Level B	European Union, Canada, United Kingdom, Switzerland		

Drug Indications

Drug	Response	Evidence Level	Approved by	Recommended by
larotrectinib	Sensitive	Approved	FDA	

Variant Groups

Many medical reference documents (research articles, published clinical trials results, drug labels, medical guidelines) refer to evidence that supports a set of variants (i.e. "EGFR exon 20 insertions") rather than individual variants (i.e. "EGFR p.V769_D770insGSV"). To this end, some curation is applied at the level of variant groups. Variant group membership is determined either case-by-case or by a set of strict and unambiguous rules aligned with understanding within the field of what defines membership to a group. In general, curators adopt a conservative approach, using more narrow definitions in order to avoid over-interpretation of less well-studied variants.

Nevertheless, with the use of variant groups, a larger umbrella of variants will inherit curated content, provided they meet rules for variant group membership. For example, exon 19 EGFR deletions comprise a large variety of changes, but seem to have a common effect on EGFR activity. As long as a variant meets the criteria for membership to the group (i.e. in-frame, confined to exon 19, etc), the variant, even if novel, inherits the curated content for the variant group.

Figure 5. Roche Classification Scheme. NAVIFY Mutation Profiler uses the Association for Molecular Pathology (AMP) classification scheme[3] to categorize somatic sequence variations based on the level of publicly available evidence supporting their clinical significance in cancer therapeutics, diagnosis, and/or prognosis. The following table outlines the classification rules used *outside* of the United States.

Tier	**Classification rules** (outside the United States)
IA	Biomarkers that predict response or resistance **and** are approved by drug agency (EMA/HCSC/Swissmedic) or recommended by medical guidelines based on the region specified. Biomarker that has prognostic or diagnostic clinical significance based on medical guidelines for the specified geographic region.
IB	Biomarkers that predict response or resistance to therapies for the tumor type based on well-powered studies (clinical trials) with consensus from experts in the field. Biomarkers that achieved high-clinical prognostic and diagnostic significance based on well-powered studies.
IIC	Biomarkers that predict response or resistance based on multiple small-published studies with some emerging consensus. Biomarkers that predict response or resistance to therapies approved by a drug agency or recommended by medical guidelines societies for a different type of tumor. Biomarkers that serve as inclusion criteria for clinical trials.
IID	Biomarkers that show plausible therapeutic significance based on at least one case report. Biomarkers with possible clinical significance based on preclinical studies.
III	Variants of unknown significance. Not observed at a significant allele frequency across the general population or subpopulations or not well-represented in cancer databases. No published functional evidence of cancer association.
IV	Observed at significant allele frequencies in the general population or specific subpopulations **and** no published evidence of cancer association.

AMP rules enable partial classification of variants in uncurated cancers

While NAVIFY Mutation Profiler includes comprehensive curation for four cancer types, some AMP rules for classification enable classification to apply to non-curated cancers. For instance, in the case of one rule, a variant that is Tier IA in one cancer type is minimally a Tier IIC in alternate cancer types. In such cases, users can view the content that supports the Tier IA designation in the other tumor type, and the variant will be classified as Tier IIC.

As a consequence of supporting variant groups and usage of AMP rules, users will find that curated content and classification is applied to more than the 12,500 variants and beyond just the four comprehensively curated cancer types. This corresponds to more than 50,000 biomarker profiles.

Variant combinations are curated as well

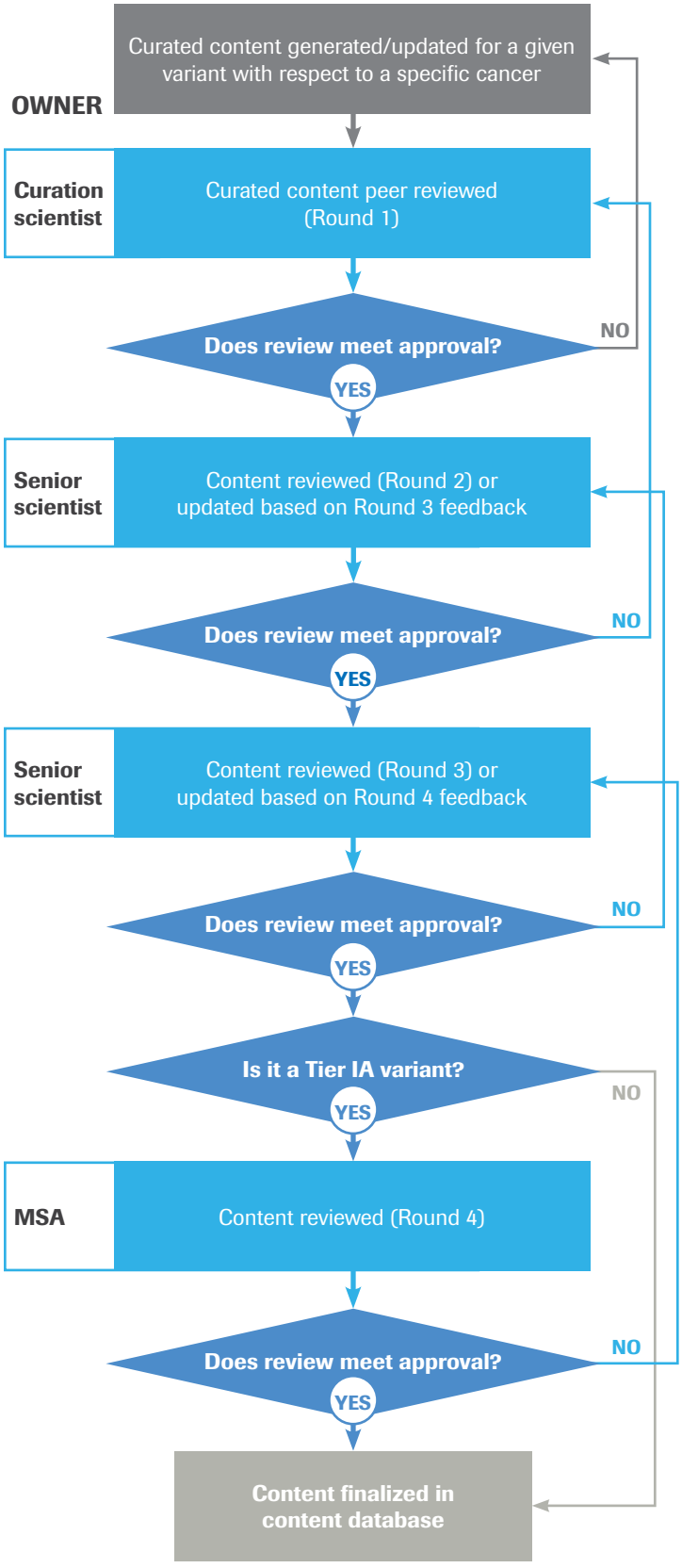
Since cancer is not the result of a single gene mutation, but rather of multiple gene mutations, the very real possibility that patient management options may vary for patients based on combinations of variants is being steadily uncovered as these discrete patient subpopulations are identified and investigated with respect to outcomes. In addition, as more targeted therapies are becoming available, so are the resistance mechanisms, which are often driven by variants arising either on the same gene target (example: L858R and T790M combination mutation in EGFR as a biomarker for Tarceva resistance), or in a different gene (example: RAS mutations that confer resistance toward EGFR-based therapies in colorectal cancer). To this end, the knowledge base can and does support curation for variant combinations. Here, too, evidence statements are accrued, and combinations that correspond to a AMP Tier IID or higher are classified. This represents yet another means by which clinical labs are able to advance the precision medicine paradigm and better serve their ordering oncologists and patients.

Curation of content follows a standard operating procedure

The curation team populating and revising the knowledge base that comes with NAVIFY Mutation Profiler consists of scientists with years of experience in multiple domains - basic research, clinical work, prior work in curation, work in oncology and work in additional disease areas. That collective experience is leveraged when variant classification and text summarizations are reviewed via a multiple-round-review process that involves a first review by a peer, followed by two rounds of review by a senior scientist. The content associated with variants ascribed Tier IA status is subject to additional review by an expert in Medical and Scientific Affairs.

The team responsible for curation receives proactive notification of all cancer-associated drug approvals and medical guidelines that could serve as the basis for a Tier IA variant classification. Publications of large well-powered clinical trials that may portend higher clinical significance for a set of somatic variants are reviewed with highest priority. And finally, variants are reviewed periodically for updated knowledge.

Figure 6. A multi-round review process ensures content accuracy. Biological and clinical summaries at the variant- and gene-level are composed, then sent for review by a peer curation scientist. Subsequently, the summaries are subject to two levels of review by senior curation scientists. Summaries for Tier IA variants, are subject to additional review by a Medical and Scientific Affairs (MSA) representative.



What about unclassified and uncurated variants?

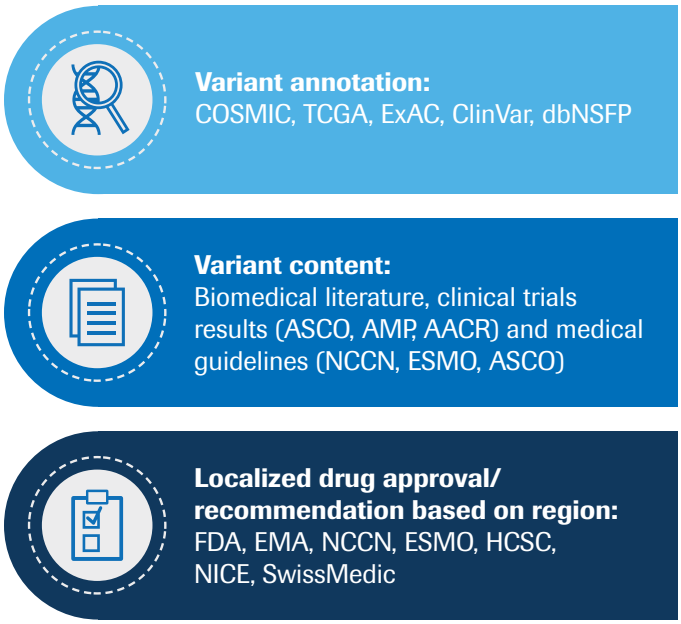
Except for labs running very limited hotspot panels, clinical labs will still encounter unclassified variants. In such cases for SNVs and indels, NAVIFY Mutation Profiler includes annotations and data from public sources to inform if the variant may have a pathogenic role in cancer. Users can observe variant representation, if it is represented among COSMIC or TCGA database samples, both in the matching cancer type if at least one COSMIC/TCGA database sample contains the variant, and in the two or three additional cancers in which the variant is most prevalent. COSMIC version 89, for instance, includes cancer representation for over 6 million coding mutations.[1] The solution will also include pathogenicity prediction scores for protein-coding SNVs and splice site SNVs. Users may also want to query one of the open-access databases, such as CIViC[2], that are offered by multiple institutions based on internal or community-based curation efforts, as well as search the internet and medical literature sources for additional information. Working with such cases in NAVIFY Mutation Profiler, users are able to classify a variant and customize a written clinical brief, effectively, complementing Roche content with lab-generated content for the lab's reuse in future cases of the same cancer type.

Conclusion

Early adopters undertaking somatic testing for cancer have had to dedicate staff to curate each new variant encountered or to revise variant interpretations as new knowledge became available. However, not every lab has the resources to undertake this continual curation effort. Thus, NAVIFY Mutation Profiler offers a complete clinical decision support solution for labs by offering a product that is both software and curated content – a resource replete with curations for over 12,500 variants, 4 cancer types, and 50,000 biomarker profiles generated based on a rigorous and continual process of review of new drug approvals, updated medical guidelines and a vast medical literature.

With NAVIFY Mutation Profiler, Roche expands its NAVIFY Decision Support portfolio, moving clinical labs one step closer to realizing the promise of personalized healthcare.

Figure 7: Roche Knowledgebase Leverages commonly referenced medical & scientific resources and synthesizes the information to help inform on clinical actionability.



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