SPECIAL SERIES: NEXT-GENERATION SEQUENCING

review article

Operationalization of Next-Generation Sequencing and Decision Support for Precision Oncology

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abstract

Genomic testing has become a part of routine oncology care and plays critical roles in diagnosis, prognostic assessment, and treatment selection. Thus, in parallel, the variety of genomic testing providers and sequencing platforms has grown exponentially. Selection of the best-fit panel for each case can be daunting, with many factors to consider. Among them is whether alteration interpretation and therapy/clinical trial matching are included and/or sufficient. In this article, we review some common commercially available sequencing platforms for the genes and types of alterations tested, samples needed, and reporting content provided. We review publicly available resources for a do-it-yourself approach to alteration interpretation when it is not provided or when supplemental research is needed, along with resources to identify genomically matched treatment options that are approved and/or investigational. However, with both commercially provided interpretation and publicly available resources, there are still caveats and limitations that can stem from insufficient or ambiguous nomenclature as well as from the presentation of information. Use cases in which clinical decision making was affected are discussed. After treatment options are identified, it is important to assess the level of evidence for use within the patient's tumor type and molecular profile. However, numerous level-of-evidence scales have been published in recent years, so we provide a publicly available tool to facilitate interoperability. The level of evidence, along with other factors, such as allelic frequency and copy number, can be used to prioritize treatment options when multiple are identified.

INTRODUCTION

The high-throughput capacity of next-generation sequencing (NGS) and the rapid development of bioinformatics tools have transformed genomic testing and our understanding of cancer. As of May 2019, more than 30,000 genetic tests in the National Institutes of Health Genetic Testing Registry list NGS as their primary test method.[1,2] Within recent years, the US Food and Drug Administration (FDA) has approved three NGS-based gene panels: Oncomine Dx Target Test (Thermo Fisher Scientific, Waltham, MA), MSK-IMPACT (Memorial Sloan Kettering Cancer Center, New York, NY), and FoundationOne CDx (Foundation Medicine, Cambridge, MA).[3] There is no doubt that we are transitioning to an era during which comprehensive tumor profiling by NGS is routine oncology practice. A recent survey of 1,281 United States oncologists revealed that 75.6% of oncologists used NGS tests to guide treatment decisions in the past 12 months.[4] Usage of these tests ranged from decision support for patients with advanced refractory disease to clinical trial eligibility screening and off-label use of FDA-approved drugs. Despite the high adoption rate of NGS tests, studies suggest that many oncologists find it difficult or do not have adequate confidence to interpret NGS results,[4-6] which potentially hinders the clinical utility of the results. In this review, we discuss the overall workflow for a clinician to select a platform for genomic testing, interpret the significance of the results, and identify genomically informed treatment options.

OPTIONS FOR NGS

Many larger medical centers offer NGS testing in house for clinical decision making. However, institutions/oncology practices with smaller patient populations thought to benefit from NGS may need to rely on commercial vendors. Even for larger centers, these panels may have specific advantages. There is quite a bit of variability between these platforms. Thus, a summary of commercially available assays aimed at detection of somatic or germline alterations is listed in Table 1.

Clinical Laboratory Improvement Amendments (CLIA) or Good Clinical Laboratory Practice certification is required for any institutional or commercial vendor that offers NGS-based cancer diagnostic tests. In addition, when an NGS provider is selected, the following

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CONTEXT

Key Objective

How do we operationalize the use of next-generation sequencing and decision support resources to enable high-quality delivery of personalized cancer care to patients on a routine basis?

Knowledge Generated

We reviewed several common commercially available sequencing platforms and their reporting structure, along with publicly available resources for alteration interpretation and identification of genomically matched treatment options. In addition, we identified caveats and limitations that resulted from the lack of standardization of nomenclature and report formatting, and we debuted a public-facing Web site to harmonize level-of-evidence scales among several prevailing standards.

Relevance

Genomic testing plays a critical role in cancer care. However, navigation of the complex landscape of precision oncology is a significant undertaking, which remains a major challenge to clinicians. In this review, we discuss an operational workflow to help a clinician select a genomic testing platform, interpret the significance of results, and identify the genomically informed treatment options.

specific criteria should be considered. (1) Genes, codons, and alteration types covered by the panel: For example, the FoundationOne Liquid panel does not report gene deletions, and this may be important for tumor types in which loss of tumor suppressor genes, such as *PTEN* or *CDKN2A*, is common and may be therapeutically targeted.[7,8] (2) Sample available for testing: If insufficient tissue is available, liquid biopsies are an alternative and have the advantage of being minimally invasive, potentially more representative of the entire tumor mutational profile, and possibly preferred when serial testing after treatment is desired.[9] (3) Germline or somatic testing: Providers such as Ambry and Myriad offer germline testing with interpretation of variant pathogenicity and guidelines for relative cancer risk. Other assays report somatic or potentially somatic alterations. Notably, an alteration cannot be unequivocally determined to be somatic unless a normal sample is tested alongside; however, most providers who do not test normal tissue take measures to filter out common polymorphisms. (4) Tumor type: Many providers offer cancer type–specific assays, such as FoundationOne Heme, or assays that test for mutations known to predispose to a particular cancer type, such as the Colaris assay for hereditary colorectal and endometrial cancer (Myriad, Salt Lake City, UT). (5) Biomarkers measured: Some panels provide other analytics, such as transcriptome readouts, promoter methylation, and protein expression levels, that cover clinically relevant markers individualized to tumor type or other factors. Benefits include determination of whether a mutation in a tumor suppressor gene leads to loss of expression or detection of immunotherapy markers, such as programmed death ligand 1.[10] (6) Interpretation: Commercial vendors that offer interpretation of testing results, as well as do-it-yourself resources, are discussed next.

INTERPRETATION OF ALTERATIONS REPORTED FROM NGS

After NGS results are reported, interpretation of the functional and therapeutic significance of the alterations is essential to assess treatment options.[11,12] Several commercial vendors offer interpretation bundled with their sequencing panel workflow (Table 2). Germline testing providers, such as Myriad or Ambry, typically report gene and alteration type descriptions (eg, inactivating *BRCA1* mutations in general) in addition to alteration-specific (eg, *BRCA1* C24R) interpretation for functional significance and cancer risk. Conversely, providers who report somatic mutations vary considerably on the level of interpretation provided, which includes no interpretation, only gene or alteration type summaries, or variant-specific interpretation as well. However, most providers have become consistent in reporting gene-drug associations, FDA indications, potentially National Comprehensive Cancer Network (NCCN) guidelines, and limited clinical trial matching.

Although some oncologists may choose to conduct their own research for alterations detected on panels that lack sufficient interpretation, the demand of keeping up with ever-evolving data has led several large cancer centers to develop dedicated in-house alteration interpretation teams, such as the Precision Oncology Decision Support team at MD Anderson[13] and the Memorial Sloan Kettering OncoKB (Precision Oncology Knowledge Base) team.[14] For institutions without such options, in-house alteration interpretation can be performed with the aid of many publicly available resources (Table 3). These tools can assist by identifying exon or protein features (eg, domains, regions, motifs) in which the alteration is located or could be inferred to affect (eg, Ensembl, UniProt, TransVar). In silico prediction tools (eg, SIFT, Polyphen, FASMIC) provide an indication of the alteration's significance but should be used with strong caution, because methods may not agree and in silico results may not align with experimental observations. Resources that provide alteration frequency, germline/somatic status of previous detections, and potential calls about clinical significance (eg, dbSNP, COSMIC, cBIOPortal, ClinVar) can be used to infer the

TABLE 1. Commercial Next-Generation Sequencing Panels

Category	Foundation Medicine: FoundationOne CDx	Foundation Medicine: FoundationOne Heme	Tempus: Tempus xT Assay	Caris: MI Profile (excluding NY)	Foundation Medicine: FoundationOne Liquid	Guardant Health: Guardant360	Invitae: Invitae Multi-Cancer Panel	Myriad: myRisk Hereditary Cancer	Ambry: CancerNext
Genomic alterations by DNA-seq (No. of genes)	Mutation (324)	Mutation (406)	Mutation (596)	Mutation (592)	Mutation (35, entire CDS; 35, select exons)	Mutation (73, SNVs, some select exons; 23, indels)	Mutation (83)	Mutation (35)	Mutation (34)
	CNV (324)	CNV (406)	CNV (596)	CNV (450)	CNV (35)	CNV (18)	CNV (0)	CNV (0)	CNV (0)
	Rearrangement/ fusion (36)	Rearrangement/ fusion (31)	Fusion (21)	Fusion (0)	Rearrangement/ fusion (7)	Fusion (6)	Rearrangement/ fusion (0)	Fusion (0)	Fusion (0)
Genomic alterations by RNA-seq (No. of genes)	No	Fusions (265)	Yes: whole transcriptome	Yes: whole transcriptome for fusions and variant transcripts in select tumor types	No	No	No	No	No
Transcript expression levels by RNA-seq	No	No	Yes: whole transcriptome (research use only)	No	No	No	No	No	No
Tumor mutation burden	Yes	Yes	Yes	Yes	No	No	No	No	No
Microsatellite instability	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
IHC testing	PD-L1 as supplemental order	No	Supplemental orders available	Yes: tumor type–specific biomarkers	No	No	No	No	No
In situ hybridization	No	No	No	CISH for HER2, TOP2A, HPV in specific tumor types	No	No	No	No	No
Other biomarkers	No	No	No	*MGMT* methylation in gliomas	No	No	No	No	No
Tumor type	Solid tumor	Hematologic and sarcoma	Solid and hematologic	Solid tumor	Solid tumor	Solid tumor	solid and hematologic	Solid and hematologic	Solid and hematologic
Specimen type	Tissue-based tumor sample	Tissue-based tumor samples; peripheral whole blood; bone marrow aspirate	Tissue-based tumor sample and blood or saliva normal sample	Tissue-based tumor sample	Blood (cfDNA)	Blood (cfDNA)	Blood or saliva	Blood or saliva	Blood or saliva
Germline or somatic reporting	Presumed somatic (normal tissue not tested)	Presumed somatic (normal tissue not tested)	Somatic and germline	Presumed somatic (normal tissue not tested)	Somatic	Somatic	Germline	Germline	Germline
Resource	https://www.foundationmedicine.com/genomic-testing/foundation-one-cdx	https://www.foundationmedicine.com/genomic-testing/foundation-one-heme	https://www.tempus.com/genomic-sequencing/	https://www.carismolecularintelligence.com/wp-content/uploads/2017/03/Profile_Menu_Brochure.pdf	https://www.foundationmedicine.com/genomic-testing/foundation-one-liquid	http://www.guardant360.com/images/gene-list.png	https://www.invitae.com/en/physician/tests/01101/?cat=CAT000036#info-panel-assay_information	https://myriad.com/products-services/hereditary-cancers/myrisk-hereditary-cancer/	https://www.ambrygen.com/clinician/genetic-testing/1/oncology/cancernext-r

NOTE. Last updated May 2019.

Abbreviations: CDS, coding sequence; cfDNA: circulating free DNA; CISH, chromogenic in situ hybridization; CNV, copy number variations; HER2, human epidermal growth factor receptor 2; HPV, human papillomavirus: IHC, immunohistochemistry; indel, insertion/deletion; PD-L1, programmed death ligand 1; SNV, single-nucleotide variations; seq, sequencing; TOP2A, DNA topoisomerase II alpha.

TABLE 2. Summary of NGS Interpretation and Implication Content Provided by Commercial Vendors

Provider	Narrative Annotation of Gene or Alteration Type	Narrative Annotation of Specific Variant	Association With Drug Indications or Guidelines Identified	Gene-Drug Associations	Trial Matching
Ambry*	Yes	Yes (cancer risk)	No	No	No
Caris Life Sciences	Yes†	Yes† (pathogenicity and cancer risk but not therapeutic implications)	Yes†	Yes†	Yes†
Color*	Yes† (cancer risk)	No	No	No	No
Foundation Medicine	Yes†	Yes†	Yes†	Yes†	Yes†
GeneDX*	Yes	Yes (cancer risk)	No	No	No
Guardant Health	No	No	Yes	Yes	Yes
Invitae*	Yes	Yes (cancer risk)	No	No	No
Myriad*	Yes	Yes (cancer risk)	No	No	No
NeoGenomics	Yes	Yes	Yes	Yes	Yes
Perthera	Yes	No	Yes	Yes	Yes
Strata Oncology	No	No	Yes	Yes	Yes
Tempus	Yes†	No	Yes†	Yes†	Yes†
The Jackson laboratory	Yes	Yes	Yes	Yes	Yes

NOTE. These data are based on the most current information that our team has encountered; reporting formats may evolve.

Abbreviation: NGS, next-generation sequencing.

*Exclusively or predominantly germline testing.

†For alterations that do not reside within the Variants of Unknown Significance section of the report.

likelihood that an alteration is a driver event versus a benign polymorphism. Alteration-specific functional and therapeutic significance is provided within several publicly available knowledge bases, including personalizedcancertherapy.org, OncoKB, and JAX-CKB, which provide annotations with references that should be reviewed as the primary source. Finally, tools such as PubMed, Google Scholar, and American Association for Cancer Research or ASCO abstract search engines may be used for direct literature queries.

CAVEATS AND LIMITATIONS OF ALTERATION INTERPRETATION

Limitations of NGS testing

Despite the advantages of NGS, one must be cognizant of the inherent limitations. Specific details about tissue sample acquisition, preparation, preservation, and storage that can affect NGS outputs should be predetermined, when possible, and aligned with the specific NGS method and analysis to be used.[15] Alteration detection after NGS relies on computational data analysis, which is ever evolving and often varies between users. In most cases, current algorithms are best designed to identify somatic single nucleotide variants (SNVs); small insertions or deletions; and some structural variants/fusions, either relative to patient-matched DNA (when testing normal–tumor pairs) or to a standardized genome (when testing tumor only). In addition, copy number alterations (CNAs) may be estimated on the basis of the number of reads that covers the genetic region.[16] Each of these types of aberrations requires a different computational approach, and there are important caveats that should be considered. For example, the fraction of cancer cells within the sample can affect the detection of aberrations, especially SNVs with a low variant allele frequency (VAF) and gene loss CNAs. In addition, the detection of larger insertions or deletions and more complex SNVs can be computationally challenging; thus, methods and calling algorithms intended to assess specific aberrations of these types may be absent or quite variable. Recommendations focused on these issues in clinical NGS development and use are recently published.[16,17]

Insufficient Nomenclature

Sometimes insufficient information is provided within the sequencing report, which hinders interpretation of the functional and therapeutic significance of alterations. The Association for Molecular Pathology, ASCO, and College of American Pathologists issued a joint recommendation for CLIA-accredited laboratories that included guidelines to properly report alterations.[18] Here, we present two real case scenarios in which insufficient data elements hindered interpretation. In case 1, *PDGFRB* (platelet-derived growth factor receptor beta) rearrangement was reported without specific information on the type of rearrangement or fusion partner. Because *PDGFRB* fusions that retain the kinase domain and result in a gain of function may be therapeutically targeted,[19,20] the treating oncologist was considering PDGFR-targeted therapies as treatment options. Additional inquiry revealed a fusion between

TABLE 3. Summary of Publicly Available Resources for Alteration Interpretation

Resource Topic	Manager	URL
Alteration location, frequency, germline/somatic detection, and/or potential clinical significance calls		
dbSNP		https://www.ncbi.nlm.nih.gov/snp/
1,000 genomes project		http://www.internationalgenome.org/
UCSC genome browser		http://genome.ucsc.edu/cgi-bin/hgGateway
Ensembl		https://www.ensembl.org/
ExAC		http://exac.broadinstitute.org/
canSAR		https://cansar.icr.ac.uk/cansar/
ClinVar		https://www.ncbi.nlm.nih.gov/clinvar/
TCGA		https://portal.gdc.cancer.gov/
cBioPortal		http://www.cbioportal.org/
COSMIC		https://cancer.sanger.ac.uk/cosmic
UniProt		https://www.uniprot.org/
BIC database (no longer active)		https://research.nhgri.nih.gov/projects/bic/Member/index.shtml
BRCA exchange		https://brcaexchange.org/
IARC *TP53* database		http://p53.iarc.fr/
TransVar		www.transvar.net | https://bioinformatics.mdanderson.org/transvar/
VariantValidator		https://variantvalidator.org/
MOKCa		http://strubiol.icr.ac.uk/extra/mokca/index.html
In silico prediction tools		
Cancer-specific high-throughput annotation of somatic mutations		http://wiki.chasmsoftware.org/
Combined annotation dependent depletion		http://cadd.gs.washington.edu/
Functional analysis through hidden Markov models		http://fathmm.biocompute.org.uk/
Mutation assessor		http://mutationassessor.org/
Polymorphism phenotyping		http://genetics.bwh.harvard.edu/pph2/
PON-P2		http://structure.bmc.lu.se/PON-P2/
Protein analysis through evolutionary relationships		http://pantherdb.org/
Predicting functional effects of sequence variants		https://www.rostlab.org/services/SNAP/
Sorting intolerant from tolerant		http://sift.jcvi.org/
Annotate variation		http://annovar.openbioinformatics.org
FannsDB, consensus deleteriousness, and transformed functional impact for cancer		http://bbglab.irbbarcelona.org/fannsdb/
Variant effect predictor		https://useast.ensembl.org/info/docs/tools/vep/
Cancer-specific driver missense mutation annotation with optimized features		https://bioinformatics.mdanderson.org/public-software/candra/
FASMIC		https://ibl.mdanderson.org/fasmic/#!/

(Continued on following page)

TABLE 3. Summary of Publicly Available Resources for Alteration Interpretation (Continued)

Resource Topic	Manager	URL
Publicly available alteration functional significance and/or therapeutic implications interpretation		
Personalized cancer therapy	MD Anderson Cancer Center	www.personalizedcancertherapy.org | https://pct.mdanderson.org/
Precision oncology knowledge base	Memorial Sloan Kettering Cancer Center	https://oncokb.org/
Clinical knowledge base	The Jackson laboratory	https://ckb.jax.org/
Precision medicine knowledge base	Weill Cornell Medical College	https://pmkb.weill.cornell.edu/
Clinical interpretations of variants in cancer	Washington University in St Louis	https://civicdb.org/home
Database of evidence for precision oncology	Ding lab at Washington University in St Louis	http://depo-dinglab.ddns.net/
My cancer genome: genetically informed cancer medicine	Vanderbilt-Ingram Cancer Center	https://www.mycancergenome.org/
Medical genomics Japan variant database	Japan Agency for Medical Research and Development, external submitters	https://mgend.med.kyoto-u.ac.jp/
VarSome	Saphetor, a Swiss precision medicine company	https://varsome.com/
Cancer genome interpreter	Barcelona biomedical genomics lab	https://www.cancergenomeinterpreter.org/
Cancer driver log	Roychowdhury lab team at the Ohio State University	https://candl.osu.edu/
Pharmacogenomics knowledgebase	Stanford University	https://www.pharmgkb.org/
Drug gene interaction database	Washington University in St Louis	http://www.dgidb.org/
11-database collection	ARUP laboratories with Huntsman Cancer Institute at The University of Utah	http://www.arup.utah.edu/database/index.php
Literature search tools		
PubMed	NCBI	https://www.ncbi.nlm.nih.gov/pubmed/
Conference abstracts	AACR, ASCO, ASH, EORTC, others	*Various*
Google Scholar and Google	Google	https://scholar.google.com/; https://www.google.com/
MasterMind: comprehensive genomic search engine	Genomenon	https://mastermind.genomenon.com/
Mitelman database of chromosome aberrations and gene fusions in cancer	Mitelman F, Johansson B, Mertens F	https://cgap.nci.nih.gov/Chromosomes/Mitelman
GeneView: a comprehensive semantic search engine for PubMed	omicX	https://omictools.com/geneview-tool

Abbreviations: AACR, American Association for Cancer Research; ARUP, XXXX; ASH, American Society of Hematology; BIC, XXXX; COSMIC, Catalogue of Somatic Mutations in Cancer; dbSNP, XXXX; EORTC, European Organization for Research and Treatment of Cancer; FannsDB, XXXX; FASMIC, XXXX; IARC, International Agency for Research on Cancer; MOKCa, mutations, oncogenes, knowledge, and cancer; NCBI, National Center for Biotechnology Information; PON-P2, XXXX; UCSC, XXXX.

PDGFRB and *RB1* that did not retain the kinase domain of *PDGFRB*, so the mutation was not actionable for PDGFRB inhibitors and likely inactivated RB1. Loss of RB1 is relevant as it confers resistance to clinically available cyclin-dependent kinase 4/6 inhibitors.[2] In case 2, an NGS provider reported *BRCA1* truncation intron 18. Truncating mutations typically are described within coding regions, so this terse nomenclature delayed care recommendation until it was confirmed that this alteration was a genomic deletion that encompassed exons 19 to 23.

Ambiguous Nomenclature

Even sufficient protein nomenclature can lead to ambiguity without the inclusion of genomic coordinates and/or the reference transcript. For example, FGFR1:p.T726A maps to both chr8:g.38271680T>C and chr8:38271773T>C mutations, and chr7:g.116412023A>T maps to both

MET:p.Y1021F and MET:p.Y1003F, dependent on the transcripts used in each case. Thus, it is ideal that all nomenclatures reported and used within a knowledge base be normalized to genomic coordinates to support a query. When not provided, the multilevel variant annotator TransVar[21] can be used to convert all protein nomenclatures to genomic coordinates and vice versa. This tool was used to identify the correlative MET:p.Y1003 mutation, which multiple publications described as a gain-of-function alteration,[22-24] from a query of MET:p.Y1021F. The biologic significance and therapeutic implication of correlative alterations such as these should be judged on a case-by-case basis. If it is concluded that the functional and clinical consequences are identical, then the alterations should be associated with each other to enhance recall.

Potentially Misleading Representation

When a commercial vendor is used for interpretation, all evidence provided should be examined rather than taken as "front-page" recommendations at face value. In a case example, *BRCA2* E2981K was reported alongside FDA-approved poly (ADP-ribose) polymerase inhibitors as a therapy with clinical benefit on the front page of a clinical report. Although substantial evidence exists for targeting deleterious *BRCA2* mutations with poly (ADP-ribose) polymerase inhibitors,[25-27] this particular variant was of unknown functional significance, as described within its detailed description located later in the report. In another case, a patient with equivocal *FGF* ligand amplifications was not considered for an FGFR (fibroblast growth factor receptor) inhibitor clinical trial, because equivocal amplifications did not meet trial eligibility criteria. However, additional examination of the VUS (variant of unknown significance) section revealed *FGFR1* amplification, which was actionable for clinical trial accrual. Thus, an alteration's location within the report is not definitive for actionability.

SURVEYANCE OF TREATMENT OPTIONS

After actionable alterations are identified, the oncologist must next assess treatment options applicable to the patients' molecular and clinical profile, including FDA-approved therapies, expert panel recommendations (eg, NCCN), and/or clinical trials. However, not all CLIA-validated panels currently return this information or may do so in a limited fashion. We surveyed several commercial NGS vendors and summarized their coverage of the treatment options in Table 2. Most commercial vendors, as well as dedicated decision support teams, routinely use the following publicly available resources.

FDA-Approved Drugs

The FDA Web site Drugs@FDA[28] lists all drugs approved by the agency as well as links to the labels that detail their indications. Each indication must be analyzed thoroughly to determine whether it is indicated for a specific biomarker and for which cancer types. Metadata about the patient's alteration and the FDA-indicated biomarker must be considered to determine if the biomarkers match. For example, *EGFR* L747_A750delinsP matches to the erlotinib FDA indication for *EGFR* exon 19 deletions. If the cancer type(s) in the indication match the patient's (when disease hierarchy is considered), this treatment option is generally considered to be the one with the highest level of supporting evidence. However, if the cancer types differ, the use of the therapy for this indication would be considered off label.

Standard-of-Care Options

NCCN compiles clinical practice guidelines in oncology by cancer type and is considered an authoritative reference for standard-of-care options within the United States. Compendiums from the perspective of drugs and biologics and biomarkers are also made available for a fee that facilitates searches of the concepts that involve drugs and biomarkers.[29,30]

Clinical Trials

Within the United States, ClinicalTrials.gov is the most comprehensive registry of clinical trials. To identify relevant trials, users can search by a combination of cancer types, drugs, and/or biomarkers. However, searches like this will only return clinical trials with text that exactly matches the keywords. All of the aforementioned concepts may have synonymous terms that should be accounted for in the search. Furthermore, cancer types are hierarchical in nature, and molecular aberrations as drug targets are usually not explicitly stated in ClinicaTrials.gov—sometimes, not even specifically stated in the protocol—which may compromise recall. To provide more robust and streamlined support, some informatics infrastructure is critical. Zeng et al[31] have reviewed related work and presented a generalized framework that addresses the unique needs of precision oncology and aims to interpret NGS results.

PRIORITIZATION OF TREATMENT OPTIONS

In patients who undergo NGS, prioritization of treatment options is an important area to consider. Needs include the following: (1) prioritization of genomically targeted treatment options when more than one exists; (2) prioritization of targets when more than one actionable alteration exists; and (3) identification of optimal therapy, through comparison of expected efficacy of genomically matched therapy with expected outcome of standard-of-care options.

Level of Evidence

To prioritize treatment options when multiple actionable alterations/therapies are identified, a quantification of their level of evidence (LoE) is necessary. To that effect, several LoE standards have been proposed. Although each has its own merit and unique perspectives, the lack of interoperability strategy that allows for a direct comparison from one to another complicates their utility. Here, we propose an interoperability tool that analyzes all of the unique features of seven existing LoE standards: Precision Oncology Decision Support,[11] OncoKB,[14] Association for Molecular Pathology,[18] NCI-MATCH (National Cancer Institute-Molecular Analysis for

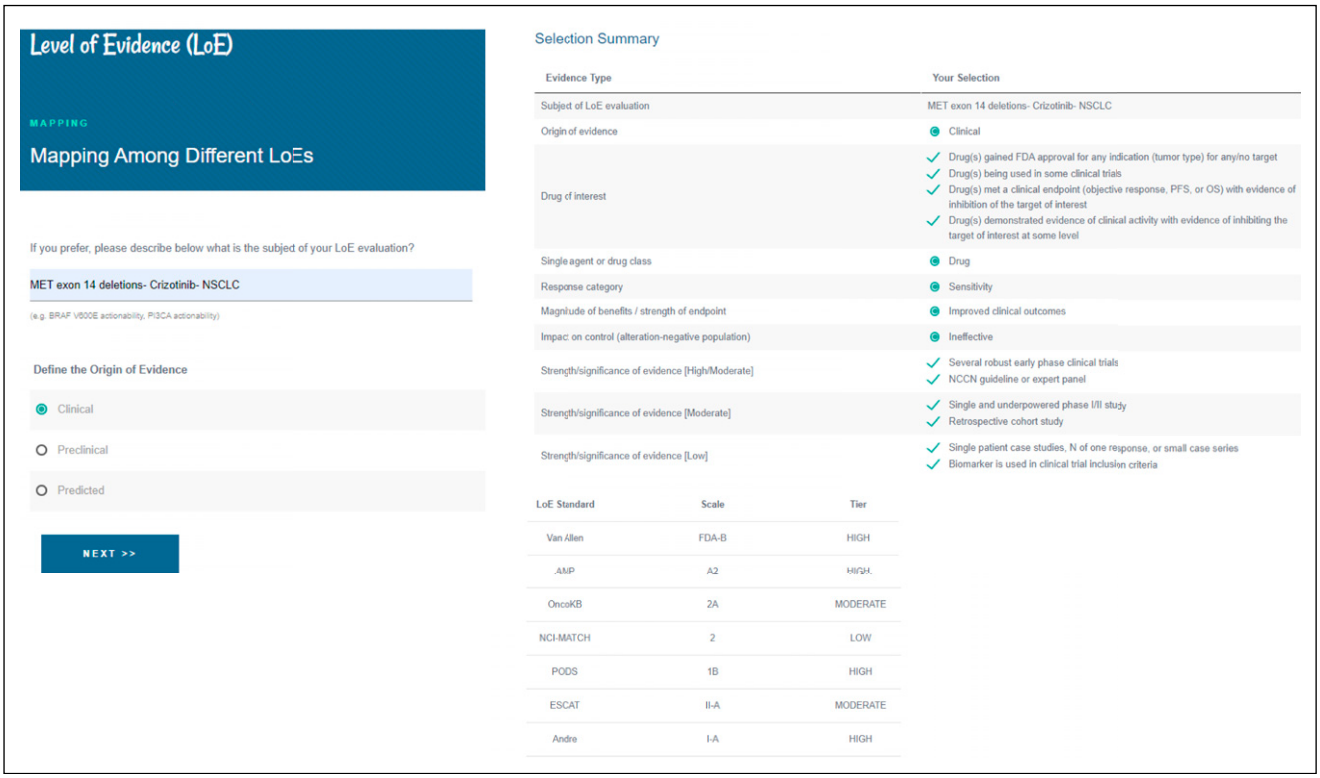

FIG 1. Level of Evidence (LoE) mapper. The LoE mapper provides an interface for a user to provide a description of the evidence found for use of a therapy within the context of a specified disease harboring a specific biomarker. On the basis of the evidence criteria selected, the LoE scale associated with seven standards is displayed along with a tier which we have defined within this article.

Therapy Choice),[32] Van Allen et al,[33] Andre et al,[34] and ESCAT (ESMO Scale for Clinical Actionability for Molecular Targets)[35] (Fig 1). To maximize interoperability, we extracted the features that contribute to the LoE assignment of each scheme and developed a Web application (https://pct.mdanderson.org/loe/) that interactively guides the users to provide necessary information of their sources to match the evidence with as many LoE schemes as possible. The data elements considered by the application, along with their permissible values in square brackets, are enumerated in Table 4. Table 5 lists the background of the data element by indicating the LoE schemes that use them for LoE assignment.

VAF and CNA Levels

VAF and CNA levels can help physicians choose treatment options. Priority may be given to the mutation with higher VAF or gene copy number, when a patient has multiple alterations that are otherwise equally targetable. In addition, VAF via sequencing of liquid biopsy is used as a noninvasive method to monitor response to treatment and disease progression. This may provide an earlier measure of patient prognosis compared with imaging-based methods.[36-38] When a matched normal sample is not available, the VAF can give insight about the potential that an alteration is a germline mutation.[18] Also, an extremely low VAF may warrant additional confirmation of the alteration with orthogonal technologies, such as Sanger sequencing. Low VAF would suggest that an alteration is subclonal, and subclonal alterations are likely inferior therapeutic targets.

Consideration of Alterations Within the Context of One Another

Multiple mutations also can exist in the same driver gene; thus, treatment options must be tailored according to the functions of all mutations. For example, a clinical study reported that first-generation epidermal growth factor receptor (EGFR) inhibitors are not effective in patients who have non–small-cell lung cancer with coexisting EGFR inhibitor–sensitive and –resistant mutations; conversely, the third-generation inhibitor osimertinib demonstrated benefit.[39]

Feedback and crosstalk between signaling pathways adds another layer of complexity in cancer treatment. In the scenario of concomitant driver mutations, multiple pathways may compensate each other and counteract the effect of a drug that targets one pathway. It is already known that *KRAS* mutations confer resistance to EGFR-targeted therapy in colon cancer. Recently, it also has been shown that *KRAS* mutations confer resistance to human epidermal growth factor receptor 2–targeted therapy (trastuzumab/pertuzumab) for *HER2*-amplified colon cancer.[40] It thus is likely that these principles of drug resistance are extrapolatable to other drug classes and to other mitogen-activated protein kinase pathway alterations. Additional data are needed to determine how to best incorporate these principles into clinical decision support.

TABLE 4. Data Elements Used Within the LoE Mapper

Data Element	Description	Permissible Values
Origin of evidence	Type of study	Clinical study
		Preclinical study
		Predicted evidence
Strength of evidence	Compilation of the criteria used in the level-of-evidence schemes that ascertain strength of evidence. To facilitate cross-comparison, we have grouped them into four major tiers: high, moderate, low, and sublow.	High: FDA-approved indication
		High: NCCN
		High: prospective biomarker selection
		High: prospective randomized
		High: prospective nonrandomized
		High: basket trials
		High: several robust early-phase trials
		Moderate: retrospective cohort study
		Moderate: prospective biomarker secondary objective
		Moderate: case-control study
		Moderate: single and underpowered phase I/II study
		Low: trial inclusion
		Low: case studies
		Low: preclinical
		Low: alteration with predicted functional impact as high tier
		Sublow: target predicted
Tumor type match	Describes whether the tumor type indicated in the evidence (if applicable) matches that of the tumor type of interest (eg, patient's tumor type)	Yes
		No
Drug credential	Indicates if the drug is approved for the biomarker of interest, generally FDA approved for any target or nontarget, and the level of activity demonstrated	FDA approved for the target
		FDA approved for any target
		Drug met a clinical end point with evidence of target inhibition
		Drug demonstrated evidence of clinical activity with evidence of target inhibition at some level
Drug or drug class	Indicates if the drug of interest a single agent or a class of drugs	Single agent
		Class of drugs
Response category	Describes the type of response the biomarker elicits on the drug	Sensitivity
		Resistance
Magnitude of benefits	The biomarker's effect on clinical outcome	Improved clinical outcome
		Unknown
Impact on control	Indicates if the drug selectively affects cells expressing the biomarker	Ineffective
		Unknown
Preclinical models	Indicate whether the preclinical models have been tested in all of human samples, cell lines, and animal models or just a subset of the aforementioned	Yes
		No
Related to known cancer gene	Indicate whether the gene the alteration belongs is known to be carcinogenic	Yes
		No

Abbreviations: FDA, US Food and Drug Administration; LoE, Level of Evidence; NCCN, National Comprehensive Cancer Network.

TABLE 5. Data Elements per Level-of-Evidence Scheme

Data Element	PODS[12]	OncoKB[15]	AMP[19]	NCI-MATCH[33]	Andre et al[35]	Van Allen et al[34]	ESCAT[36]
Origin of evidence	X	X	X	X	X	X	X
Strength of evidence	X	X	X	X	X	X	X
Tumor type match status	X	X	X		X	X	X
Drug credential				X		X	
Drug or drug class						X	
Response category		X					
Magnitude of benefits							X
Impact on control					X		
Preclinical models					X		
Related to known cancer gene					X		

Abbreviations: AMP, Association for Molecular Pathology; ESCAT, ESMO Scale for Clinical Actionability of Molecular Targets; NCI-MATCH, National Cancer Institute-Molecular Analysis for Therapy Choice; OncoKB, Precision Oncology Knowledge Base; PODS, Precision Oncology Decision Support.

There has been growing interest in targeting multiple driver alterations, when they exist, to broaden actionability and enhance efficacy. Although this may not be feasible in some cases because of expected toxicity, a pilot study by Sicklick et al[41] demonstrated that, in experienced clinical trial units, a combination therapy approach may indeed be feasible and that targeting a larger fraction of alterations may improve disease control rates, progression-free survival, and overall survival.

INCORPORATION OF NGS INTO ROUTINE CLINICAL PRACTICE: CURRENT CHALLENGES AND FUTURE DIRECTIONS

Although NGS is used increasingly, several clinical challenges to implementation of NGS as part of routine care remain. These include the broader context of when NGS should be ordered, especially in tumor types for which there are no drugs approved by the FDA on the basis of a genomic marker. Although some studies were not able to demonstrate an advantage of genomically matched therapy,[42] Kopetz et al[43] demonstrated with a larger panel that patients with actionable alterations who were treated with matched therapy had improved overall survival compared with patients who were not.

Debate also remains about the utility of fresh biopsies versus archival tissue for NGS, the value of repeat biopsies and NGS after intervening therapy, and the use of liquid biopsies for serial monitoring. However, there is increasing recognition of genomic evolution, mechanisms of acquired resistance, and emerging strategies to overcome these resistance mechanisms. Overall, it is likely that the most compelling alterations therapeutically may be truncal alterations; however, emerging alterations, such as *MET* amplifications after use of EGFR inhibitors in lung cancer, represent promising therapeutic opportunities.[44]

For NGS to truly affect outcomes, patients with actionable alterations need access to genomically matched therapies. Thus, it is critical for them have access to a large clinical trial portfolio so that compelling alterations can be acted upon either in the context of standard of care or via investigational therapies. For example, Dumbrava et al[45] recently demonstrated that patients with *HER2* amplification, even beyond breast and gastric cancer, had improved overall survival if they received human epidermal growth factor receptor 2–targeted therapy.

Finally, to date, most NGS-based decision making has been based on the principle of matching a single gene to a single targeted therapy. It is likely that combination therapies may enhance efficacy by deepening responses and enhancing their durability. Thus, future decision support efforts must be nimble in incorporation of multianalyte information as well as dynamic markers, such as pharmacodynamic markers, or in target inhibition and adaptive response to facilitate selection of optimal monotherapy or combination therapy in a longitudinal continuum.

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