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REVIEW

How to read a next-generation sequencing report—what oncologists need to know

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Next-generation sequencing (NGS) of tumor cell-derived DNA/RNA to screen for targetable genomic alterations is now widely available and has become part of routine practice in oncology. NGS testing strategies depend on cancer type, disease stage and the impact of results on treatment selection. The European Society for Medical Oncology (ESMO) has recently published recommendations for the use of NGS in patients with advanced cancer. We complement the ESMO recommendations with a practical review of how oncologists should read and interpret NGS reports. A concise and straightforward NGS report contains details of the tumor sample, the technology used and highlights not only the most important and potentially actionable results, but also other pathogenic alterations detected. Variants of unknown significance should also be listed. Interpretation of NGS reports should be a joint effort between molecular pathologists, tumor biologists and clinicians. Rather than relying and acting on the information provided by the NGS report, oncologists need to obtain a basic level of understanding to read and interpret NGS results. Comprehensive annotated databases are available for clinicians to review the information detailed in the NGS report. Molecular tumor boards do not only stimulate debate and exchange, but may also help to interpret challenging reports and to ensure continuing medical education.

Key words: next-generation sequencing (NGS), NGS-report, molecular targets, tumor genomic profiling, ESMO scale of clinical actionability for molecular targets (ESCAT), minimal requirement

INTRODUCTION

Next-generation sequencing (NGS) technology has revolutionized our ability to search for cancer-related genomic alterations in tumor cells and therefore our understanding of tumor biology and consequently has facilitated targeted drug development. A decade ago, NGS was mainly a research tool in academic centers, but facilitated by decreasing costs and overall increased availability, it has recently been widely implemented in the routine diagnostic workflows at many institutions. Multiple challenges related to the optimal use of NGS technology, however, remain. Interpretation of complex molecular datasets generated by large gene panels remains a challenge for physicians, underscoring the need for education and expert support frameworks.[1-3] Furthermore, out of >400 established growth promoting genomic alterations, only a few are predictive for approved targeted anticancer drugs,[4,5] questioning the benefit of large gene panels outside academic centers that offer access to clinical trials. For selected genomic alterations, the use of targeted therapies may be possible, even though the given drug may not be licensed in this indication (off-label use).[6] Ideally, off-label use of cancer drugs, particularly for rare genomic alterations or orphan diseases, is carried out in a way that allows learning through structured collection and sharing of the data.[7]

Overall, the proportion of patients with targetable molecular alterations is increasing[4] and what are considered variants of unknown significance (VUS) today may be reclassified and can become relevant targets in the future and therefore possibly relevant information for individual patients. There is an ongoing controversy to what extent large gene panels should be implemented as standard of care outside of research settings and how to deal with the vast amount of data obtained by mutational profiling.[4,8-10]

In order to offer some guidance, The European Society for Medical Oncology (ESMO) has published the ESMO scale of

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Table 1. European Society for Medical Oncology (ESMO) clinical actionability of molecular targets

Tier I	Alteration-drug match is associated with improved outcome in clinical trials
Tier II	Alteration-drug match is associated with antitumor activity, but magnitude of benefit is unknown
Tier III	Alteration-drug match is suspected to improve outcome based on clinical trial data in other tumor types(s) or with similar molecular alteration
Tier IV	Preclinical evidence of actionability
Tier V	Alteration-drug match is associated with objective response, but without clinically meaningful benefit
Tier X	Lack of evidence for actionability

clinical actionability for molecular targets (ESCAT, Table 1), a clinical benefit-centered system attributing six levels of clinical evidence based on implications of the respective alteration on patient management.[11] More recently, ESMO has published detailed recommendations for the use of NGS in oncology daily practice.[12]

This review will focus on how to read and interpret an NGS report with data on mutational profiling, which is generally generated by pathologists and molecular biologists. It will summarize types of alterations detected by NGS, minimal requirements of NGS reports and relevant databases that support information on clinical relevance of alterations. Importantly this review reflects our opinion and is not intended to cover all aspects in detail or to serve as a guideline.

THE ROLE OF THE ONCOLOGIST

In many cancer centers, testing in cases of newly diagnosed non-squamous non-small-cell lung cancer (NSCLC) for relevant genomic alterations including *EGFR*, *ALK* and *ROS1* has become standard of care to expedite initial workup, mostly with PCR (*EGFR*) and FISH (*ALK; ROS1*) or small targeted NGS panels including a few other common mutations. In most cases the treating oncologist orders molecular testing either at initial diagnosis or at the time of later disease progression, to evaluate potential molecular alterations contributing to treatment resistance (sequential or repetitive testing). Therefore, the decision of molecular testing and the respective time point(s) and how broad it should be carried out is generally determined by the oncologist as well as by availability and reimbursement. The main aims of molecular testing irrespective of testing modality applied are to identify patients in whom a specific alteration would result in access to an approved and reimbursed targeted agent with proven benefit available in the general community, or to identify patients for molecularly selected, biomarker-driven clinical trials.[13] NGS technology cannot only be applied for targeted hotspot panels or larger panels of up to 500 genes, but also for whole-genome sequencing (WGS) and whole-exome sequencing (WES). The decision on the desired coverage of possible alterations depends on the clinical context and available reimbursement.

Based on the recently published ESMO recommendations, routine use of multigene NGS testing in daily clinical practice is recommended only in patients with advanced non-squamous NSCLC, prostate and ovarian cancer and cholangiocarcinoma to detect ESCAT level I alterations in patients for which a matched targeted drug has been validated in clinical trials, whereas for other cancer types, single very focused molecular testing with PCR or ISH for specific alterations should remain the standard outside of academic centers.[11,12] If NGS testing is carried out, targeted small panels including the relevant alterations are recommended, and larger NGS panels should only be chosen if they do not add relevant costs,[12] as there is no evidence that their use is associated with improved patient outcome at present.[9] So far, moderate cost-effectiveness of panel testing has been shown for NSCLC where multiple alterations with targeted treatment available are known[14]; however, it remains to be demonstrated for other cancer types. A problem of larger NGS panels is not only the lack of evidence for the therapeutic use of potential findings, but also the fact that a frequently triggered off-label use of potential targeted drugs and their benefit or lack of benefit are not systematically collected and made available to the oncology community. This would be important to optimize future treatment. As an example of such an approach, we would like to mention The Drug Rediscovery protocol, which facilitates the defined use of approved drugs beyond their labels in rare subgroups of cancer, identifies early signals of activity in these subgroups, accelerates the clinical translation of new insights into the use of anticancer drugs outside of their approved label and creates a publicly available repository of knowledge for future decision making.[15]

In a large retrospective analysis of 5688 NSCLC patients treated in a community setting in the USA, the majority of patients were only assessed for *EGFR* and *ALK* alterations and only 875 patients actually received broader molecular testing using multigene panels of at least 30 genes before the start of third-line treatment. In the majority of patients, at least one molecular alteration was identified, however, mainly (at the timepoint of this study) non-targetable mutations such as *TP53* (55.1%), *KRAS* (34.2%) and *STK11* (12.2%). In consequence, only 14.3% of this patient population were eventually treated with a targeted therapy: 9.8% received routine EGFR/ALK targeted treatments and only 4.5% of patients received another targeted agent based on a detected molecular alteration (mainly for *BRAFV600E, MET* and *ERBB3* alterations), whereas 85.1% only received standard-of-care non-targeted treatments, mainly chemotherapy. No difference in 12-months mortality rate was seen depending on the extent of initial molecular testing.[16] In an analysis of NSCLC patients treated at the Memorial Sloan Kettering Cancer Center, however, of 860 patients profiled with a large multigene panel, 14.4% patients did receive a matched targeted therapy, which was not standard of care.[17] This reflects the limited gain in treatment opportunities based on NGS results if test results are not discussed within a multiprofessional and dedicated team helping to interpret results and possibly facilitating inclusion of patients with possible targets into molecularly selected clinical trials.[3,10,16] Therefore, a molecular tumor

board where the optimal testing strategy depending on patients and treatment or clinical trial opportunities, but also specific NGS findings, can be discussed is of utmost importance. In addition, genomic alterations detected by tumor testing like e.g. BRCA1/2 mutations may be a sign of a hereditary tumor syndrome. It is important that these findings are recognized and followed up and that these patients are offered adequate genetic counseling and possibly also germline testing.

With the tumor agnostic approval of checkpoint inhibitors in patients with defective mismatch repair protein status or high tumor mutational burden [Food and Drug Administration (FDA) approval, tumor agnostic, European Medicines Agency: only colorectal cancer] and neurotrophic tyrosine receptor kinase (NTRK) inhibitors in case of *NTRK* fusion, tumor mutational profiling has become more important, particularly for patients lacking standard treatment options. Both events [mismatch repair-deficient/microsatellite instability-high (dMMR/MSI-high), high tumor mutational burden, *NTRK* fusion] are rare in unselected cancer patients, but the benefit of targeted therapies on the basis of these molecular alterations is potentially high.

In summary

In most tumor entities apart from NSCLC and outside of an academic context, benefit of broad NGS testing is limited and not cost-effective. Therefore a careful evaluation of a reasonable testing strategy is advised. If broad NGS testing is being carried out, discussion at a molecular tumor board is crucial to transform the cost of molecular testing into potential benefit for the patient, especially if alterations with no matching approved targeted drug are detected.

THE ROLE OF THE PATHOLOGIST AND MOLECULAR BIOLOGIST AND PRE-ANALYTICAL ASPECTS OF CLINICAL NGS

The pathologist carries an important role in tailoring targeted NGS panels in collaboration with the oncologist to define genes and genomic alterations to be interrogated. The list of targets with clinical significance and utility is determined by the availability of approved drugs and access to clinical (open) trials. The design of clinical NGS assays should also consider the sample types, the minimum amount of input DNA/RNA, and turnaround times. In contrast to WES and WGS, targeted NGS enables higher levels of sequencing depth and thereby higher sensitivity to obtain conclusive results, even for samples with low tumor cell content.

In clinical routine, NGS is most often carried out on tumor material derived from formalin-fixed, paraffin-embedded (FFPE) tissues and cytological samples (body fluids, fine-needle aspirates) that can contain varying numbers of tumor cells. Pathological evaluation of tumor specimens before molecular testing is essential and includes confirmation of the tumor diagnosis, assessment of sample adequacy and estimation of tumor cell content. Morphology-guided dissection of tumor cell areas/tumor cells is carried out to enrich for tumor DNA/RNA. Pre-analytical processing (formalin fixation, paraffin embedding) of samples needs to be compatible with NGS-based testing. Initial histopathological analysis of small biopsy samples should be conducted in a tissue-sparing way to maintain enough tumor tissue for subsequent molecular diagnostics. Tumor cell content of samples is relevant for the interpretation of NGS results.[18,19] The limit of detection of a given NGS assay may be different for the various types of genomic alterations, and low tumor cell content can lead to false-negative results.[20]

NGS uses high throughput, parallel-sequencing technology to detect variants on DNA and/or RNA level. Major steps of the NGS workflow include DNA/RNA extraction from tumor cells, target enrichment, library preparation, massive parallel sequencing, bioinformatics analysis and variant annotation and interpretation.[18] Although the technology allows for WGS and WES, current clinical NGS applications focus on the analysis of targeted gene panels that include actionable targets in oncological diseases. For targeted sequencing, selected regions of interest are enriched from genomic DNA and RNA using amplicon or hybridization capture.[21] Both enrichment approaches can detect the various types of genomic alterations with clinical significance, including single nucleotide variants (SNVs), small insertions or deletions (indels), copy number variants (CNVs), gene fusions and other structural variants. In addition to the detection of specific genomic alterations in individual genes of interest, sequencing of large gene panels, WES and WGS can deliver complex biomarkers such as tumor mutational burden[21] as well as mutational signatures linked to specific defects in DNA repair mechanism (e.g. mismatch repair, homologous recombination) and tumor etiologies.

Assay validation is an essential step during test development. It evaluates performance parameters including assay accuracy, precision, limits of detection, reference range, analytical sensitivity and specificity. Validation of NGS assays is carried out using molecular standards, reference samples or clinical samples with a known set of engineered and endogenous mutations present at specific variant allele frequencies (VAFs) quantified by an alternative validated method (e.g. droplet digital PCR or Sanger sequencing). Validation aims to achieve a high level of accuracy ($>$95%) for clinically relevant variants. Validation also provides information on allelic frequency cut-off values that are important for the interpretation of sequencing results.

It is recommended that molecular diagnostics laboratories adhere to international quality standards, such as ISO15189 or equivalent. Guidelines and recommendations regarding clinical laboratory standards for NGS have been published by the American College of Medical Genetics and Genomics.[19] Quality is maintained by regular participation in external quality assurance (EQA) programs.

In summary

As an initial step, quality and quantity of available tumor samples and feasibility of NGS on material that needs to be

evaluated, ideally at the respective disease tumor board and rebiopsy, should be considered provided tumor material is deemed inadequate by the pathologist. Assay validation is crucial to ensure adequate quality of molecular testing and the oncologist requesting NGS testing in a patient should be aware of how the applied NGS assay was validated and whether the laboratory he is working with is regularly participating in EQA programs to assure high quality of molecular analyses.

THE NGS REPORT: RECOMMENDATION FOR MINIMAL REQUIREMENTS

Molecular pathology reports should present results in a clear and concise manner to enable clinicians to select the best treatment options for patients (Table 2). Contents which in our view are essential to appear in a molecular pathology report are shown in Table 3 and guidelines for reporting have been provided by clinical and pathological organizations.[22-25] Essential information about the patient, the specimen, the requesting physician, the laboratory and the applied method should be provided.

The results section is the key part of the report. Genetic alterations should be reported using standard gene and sequence variation nomenclature (http://www.genenames.org; http://www.hgvs.org).[26] In addition to standard nomenclature, however, other terms for genetic alterations may be used in agreement with the treating physicians, in particular if it improves understanding. SNVs and indels should be reported using p. and c. annotation (e.g. *EGFR* p.Leu858Arg and c.2573T>G). Although the three letter code for amino acids is recommended, it is acceptable to use the one letter code (e.g. *BRA*F p.V600E instead of *BRAF* p.Val600Glu), also because many physicians are more familiar with it. For variants, the genomic coordinates, genome build and transcript reference sequence (e.g. NM_004333.4) should be provided. Allele fraction (VAF) along with tumor cell content should also be included in the report. This allows the results to be tested for plausibility and may give some indication of a possible germline mutation. According to recent recommendations, gene fusions should be reported listing both fused gene partners, including exons, separated by a double colon (e.g. *EML4::ALK* fusion).[27] CNVs generated from NGS tests should be reported in table format as copy number gain or loss.

The report should include in detail what has actually been tested. Optionally also negative results, in particular if they have been requested, can be reported in a disease-specific manner (e.g. *KRAS, NRAS* and *BRAF* status for colorectal cancer). Methodologic details should be provided in a separate section and include description of the test, sequencing instrument, assay performance characteristics (including limit of detection and minimal depth of sequencing coverage), critical quality metrics for the assay run and analysis pipeline. The report should also include the sequencing coverage cut-off for the NGS assay used. All genes and/or hot spots not meeting the minimal required sequencing coverage criteria should be declared in the report.

Table 2. Vocabulary of NGS reports (classification pathogenic, likely pathogenic, benign, VUS, etc.). Sequence Variant Nomenclature (hgvs.org)

Term	Explanation
ACMG/AMP classes	5-Pathogenic 4-Likely pathogenic (95% for cancer) 3-Uncertain significance—a VUS 2-Likely benign (95% for cancer) 1-Benign
Alleles	A series of variants in a protein encoded by one chromosome.
Copy number variation (CNV)	Structural variant, variations in the number of copies of a particular DNA segment.
Deletion (del)	A sequence change where, compared with a reference sequence, one or more nucleotides are not present (deleted).
Deletion-insertion (delins)	A sequence change where, compared with a reference sequence, one or more nucleotides are replaced by one or more other nucleotides and which is not a substitution, inversion or conversion.
Duplication (dup)	A sequence change where, compared with a reference sequence, a copy of one or more nucleotides is inserted directly 3′ of the original copy of that sequence.
Extension (ext)	A sequence change extending the reference amino acid sequence at the N- or C-terminal end with one or more amino acids.
Frameshift (fs)	A sequence change between the translation initiation (start) and termination (stop) codon where, compared with a reference sequence, translation shifts to another reading frame.
Insertion (ins)	A sequence change where, compared with the reference sequence, one or more nucleotides are inserted and where the insertion is not a copy of a sequence immediately 5′.
Mutation	'Mutation' is used to indicate 'a change', current guidelines recommend using neutral terms like 'variant'.
SNV	Single nucleotide change in DNA sequence.
Substitution (>)	A sequence change where, compared with a reference sequence, one nucleotide is replaced by one other nucleotide.
VAF	Variant allele frequency. The percentage of sequence reads observed matching a specific DNA variant divided by the overall coverage at that locus.
VUS	Variant of unknown significance

ACMG, American College of Medical Genetics; AMP, Association for Molecular Pathology.

Several classification systems have been developed to value the clinical significance (actionability) of genomic alterations.[11,24,28] The classification recommended by major clinical and pathological associations in the USA uses a four-tiered system and classifies variants according to their clinical impact integrating FDA approval for specific therapy.[24] According to the ESMO-recommended ESCAT guidelines, all detected genetic alterations should ideally be classified into:

Table 3. The NGS report: minimal content requirements

Laboratory/patient/sample identifier	• Patient's name, date of birth, sex • ID number • Date specimen collected • Date specimen received • Date results reported • Ordering physician • Laboratory name
Specimen used for NGS testing	• Specimen type (FFPE, frozen tissue, liquid biopsy) • in case of tumor tissue: o Tissue information with diagnosis o Tumor cell content
Results	• Results with test name • Range • Use standard gene nomenclature • For variants also VAF (variant allele frequency)
Methodology/procedure	• Target description • Specimen-enrichment method • Limit of detection • Additional assay limitations
Procedure	• Type of procedure (e.g. NGS) • Defined target (e.g. name of target tested such as gene, locus, or genetic defect; use HUGO-approved gene nomenclature, HGVS nomenclature) • Analytic interpretative comment • Clinical interpretative comment • Pathologist/designee signature • LDT reporting language • ASR language
Comments	• Significance of the result in general or in relation to this patient • Correlate with prior test results • Recommend additional measures (further testing, genetic counseling) • Condition of specimen that may limit adequacy of testing • Pertinent assay performance characteristics or interfering substances • Cite peer-reviewed medical literature or reliable web sites on the assay and its clinical utility • Document intradepartmental consultation • Document to whom preliminary results, verbal results, or critical values were reported and when • Incorporate information specifically requested on requisition • Answer specific questions posed by the requesting clinician • Reason specimen rejected or not processed to completion • If the report is an amended or addendum report, describe the changes or updates • Describe discrepancies between preliminary and final reports • Name of testing laboratory, if transmitting or summarizing a referral laboratory's results

ASR, analyte specific reagents; FFPE, formalin-fixed, paraffin-embedded; HGVS, Human Genome Variation Society; HUGO, Human Genome Organisation; LDT, laboratory-developed test; NGS, next-generation sequencing.

- Tier I, alteration-drug match is associated with improved outcome in clinical trials.
- Tier II, alteration-drug match is associated with anti-tumor activity, but magnitude of benefit is unknown.
- Tier III, alteration-drug match is suspected to improve outcome based on clinical trial data in other tumor types(s) or with similar molecular alteration.
- Tier IV, preclinical evidence of actionability.
- Tier V, alteration-drug match is associated with objective response, but without clinically meaningful benefit.
- Tier X: lack of evidence for actionability.

Tiers I to V should be reported in descending order of clinical importance. Currently, tumor testing often does not include analysis of matched germline samples due to regulatory, time and/or money constraints. Therefore, identification of germline variants can often only be suspected. Nevertheless, it is recommended to indicate that certain variants may be associated with inherited diseases. Finally, variants interpreted as benign/likely benign should not be mentioned in the report.

Treatment suggestions within the molecular pathology report may be provided depending on the country's regulations, local circumstances and agreement with the oncologists. In addition, specific clinical trials may be mentioned with general statements about availability or citation of published trials. The discussion of the findings and the resulting recommendations regarding the treatment strategy and possible clinical studies at a molecular tumor board is of central importance in our view. In addition to the oncologists, presence of the molecular pathologists at the molecular tumor board is important in order to discuss the findings in detail. The documentation of this discussion and the recommendations should complement the NGS report. A geneticist should also be present at the molecular tumor board in order to identify genomic alterations with a potential germline background that needs to be followed up.

BIOLOGICAL AND CLINICAL CLASSIFICATION OF GENETIC VARIANTS

Clinicians ordering NGS testing usually receive a report as described previously, with details of the methodology used, the results of the NGS testing and also an interpretation of the NGS results including clinical actionability. Since the field of genomic medicine is rapidly expanding, not all clinicians are familiar and confident to interpret the results.[2]

The publication of an increasing number of large-scale genome sequencing projects for a broad range of hematological and solid malignancies led to a wealth of genomic information consolidated in many publicly available databases Table 4.

For the annotation of somatic variants, there are numerous cancer databases, such as the Cancer Gene Census (http://cancer.sanger.ac.uk/cancergenome/projects/census/), the Catalogue of Somatic Mutations in Cancer (COSMIC; http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) and The Cancer Genome Atlas (TCGA; http://cancergenome.nih.gov/). The assessment of the functional relevance of a genomic alteration found depends largely on how frequently this alteration has already been found and described in the setting of tumors. These tumor-specific databases provide information regarding the incidence and prevalence of certain sequence variants in different tumor entities and are often linked to other

Table 4. Databases with genomic data and where to check for relevance of alterations

Database	Comments
Cancer Genome Atlas (TCGA)	Large database including cancer-associated genomic alterations of >20 000 cancer patients
International Cancer Genome Consortium (ICGC)	Global initiative to build a large database of genomic alterations in the most common tumor types
OncoKB	Memorial Sloan Kettering Cancer Centre precision oncology database including link to FDA levels of evidence
MyCancerGenome	Large database including cancer-associated genomic alterations of almost 100 000 tumor samples
CIViC	Clinical interpretation of variants in cancer, open access open source, community driven
COSMIC	Large catalogue of somatic cancer mutations including data from >37 000 genomes
ClinVar	Freely accessible, public archive of reports of the relationships among human variations and phenotypes, with supporting evidence
Online Mendelian Inheritance in Man (OMIM)	Comprehensive, authoritative compendium of human genes and genetic phenotypes
VarSome	Variant knowledge community, data aggregator and variant data discovery tool
Breast Cancer Information Core (BIC) Database	Large *BRCA1* and *BRCA2* gene mutation database
ARUP BRCA1 and *BRCA2* mutation databases	Provides information on *BRCA1* and *BRCA2* gene mutations and their impact on risk of developing breast cancer, ovarian cancer and certain other cancers. Two types of databases are provided. One is a list of mutations curated from critical review of literature and family studies. The other provides *in silico* prediction of risk to help understand variants of unknown significance

FDA, Food and Drug Administration.

databases and published literature, especially regarding potential targeted therapies. It is important to note that data on the prevalence and distribution of sequence variants in different tumors based on these databases may be interpreted with caution, as the sources of submitted variants vary (e.g. exploratory or discovery studies) and the clinical literature has generally not been curated in a uniform manner.

The greatest challenge in clinical practice is the interpretation of genomic alterations in a biological context with regard to their functionality and possible significance in terms of therapy. In principle, for a comprehensive annotation and clinical application, an assessment on the following four levels is desired: (i) gene; (ii) specific variant; (iii) sensitivity or resistance to a drug or group of drugs; (iv) tumor-specific context. Most available databases usually highlight partial aspects thereof. Whereas some focus on the association of genomic variant and tumor, others focus on the relationship between genomic alteration and target or drug. Originally designed primarily for germline variant annotation and preclinical research, these databases are of limited relevance to clinical applicability. The Therapeutic Targets Database (http://bidd.nus.edu.sg/group/cjttd/), Drug Bank (http://www.drugbank.ca/), the Pharmacogenomics Knowledge Base (https://www.pharmgkb.org/) and the Drug Gene Interaction database (http://dgidb.genome.wustl.edu/), for example, are useful curation resources, all of which have certain limitations.[29]

More recent approaches focus more on the aspect of application as a decision tool, including My Cancer Genome (Vanderbilt-Ingram Cancer Center: My cancer genome. https://www.mycancergenome.org), CIViC[30] (https://civic.genome.wustl.edu), the Precision Medicine Knowledge Base[31] (https://pmkb.weill.cornell.edu), The Jackson Laboratory Clinical Knowledgebase[32] (https://www.jax.org/clinical-genomics/ckb), Cancer Genome Interpreter[33] (https://cancergenomeinterpreter.org), Cancer Driver Log[34] (http://candl.osu.edu); Tumor Portal[5] (http://tumorportal.org), Targeted Cancer Care (Massachusetts General Hospital: Targeted cancer care. https://targetedcancercare.massgeneral.org), Personalized Cancer Therapy[35] (https://pct.mdanderson.org) and OncoKB[28] (https://www.oncokb.org). The methodology behind the construction of each database varies, and not all databases contain sufficient clinical information to be used as a clinical decision support tool. Importantly, not all databases include a comment feature to facilitate crowdsourcing curation of this knowledge base.

The difficulty of adequately classifying somatic variants found by NGS with respect to oncogenicity has recently been addressed by the Clinical Genome Resource (ClinGen) Somatic Cancer Clinical Domain Working Group and ClinGen Germline/Somatic Variant Subcommittee, the Cancer Genomics Consortium and the Variant Interpretation for Cancer Consortium who have developed a standard operating procedure for the classification of oncogenicity of somatic variants to improve consistency in somatic variant classification (Horak PMID: 35101336).

In summary

We note that the different databases have advantages and disadvantages. When using a database, it is always important to understand the content of the database and how the data are aggregated to avoid over-interpretation of annotation results. In daily clinical practice, however, it is impossible to keep an informed oversight over the diverse advantages but also shortcomings of individual databases. Therefore, minimal requirement for the oncologist may be to know which databases the respective collaborating pathology department is relying on and stating these clearly on the NGS report would be helpful in this respect. In addition, multidisciplinary discussions of results at the molecular tumor board are crucial to facilitate correct interpretation, especially of VUS.

CLINICAL INTERPRETATION OF NGS REPORTS—CHALLENGES AND CONTROVERSIES

In patients undergoing NGS testing, prioritization of targeted treatment options is an *important* area to consider and generates specific needs, including: (i) prioritization of targeted treatment options in case more than one actionable genomic alteration exists and (ii) identification of the most

promising targeted anticancer treatment when considering standard-of-care systemic treatment options as an alternative. To prioritize treatment options when more than one or several actionable genetic alterations are identified, a quantification of their level-of-evidence is crucial. With this regard, several algorithms have been proposed, including, but not limited to: Precision Oncology Decision Support,[35] OncoKB,[28] Association for Molecular Pathology,[24] NCI-MATCH (National Cancer Institute-Molecular Analysis for Therapy Choice),[36] Van Allen et al.,[37] Andre et al.[38] and ESCAT by ESMO.[11] Tools to facilitate interoperability and incorporate covariates such as allelic frequency and copy number among others have also been published.[39]

If multiple genetic alterations are detected in the same driver gene, treatment options must be tailored according to potential functional consequences of all respective mutations. Evidently, crosstalk between signaling pathways adds another layer of complexity, and multiple pathways may compensate each other and abolish targeted anticancer drug effects in cases of multiple driver mutations. There has been growing interest in targeting multiple driver alterations to broaden actionability and enhance treatment activity. Finally, current genomics knowledge is clearly ahead of our ability to therapeutically target tumors, given that many mutations identified by NGS are either linked to unapproved drugs or cannot be targeted by currently available treatments.

In summary

Current information retrieved by broad NGS testing often exceeds requirements needed to make informed treatment decisions for a respective patient, as only a minority of alterations detected match with an approved targeted drug or at least a drug with early data for efficacy in the respective alteration in a respective cancer type. Therefore, the value of very broad NGS testing outside an academic setting with access to early phase trials is questionable and should be critically evaluated by the treating oncologist with the individual patient in mind: would that patient qualify for an early-phase trial (Eastern Cooperative Oncology Group, comorbidities, laboratory parameters)? Would that patient be willing to travel to an academic institution to participate in such a trial?

OUTLOOK AND CONCLUSIONS

Tumor mutation profiling using NGS has become a standard for many patients with advanced cancer either upfront for first-line treatment selection or later in the disease trajectory. Many academic centers have established their own NGS platform and in parallel, many commercial providers are now available. The interpretation of the NGS data on the bioinformatics level is challenging and clinicians generally get an extracted report, summarizing the most important and potentially clinically relevant results including a classification of the relevance of the observed genomic alterations. In the literature, standards of NGS reporting have been discussed extensively. Points to be raised are the simple and straightforward summary of the relevant findings, the handling of VUS, interaction if multiple genomic alterations have been detected, the facilitation of relating to specific drugs with antitumor activity in a given alteration or the link to trials including patients with these alterations.[40] It is important, that the

Table 5. Sample report

Patient XY, DOB 01.01.1950, male
Date of report: 07.12.2021
Ordered by: Doctor X

Specimen used for NGS testing
Sample ID: B2020.24987
Sample received: 01.01.2020
Specimen type: Biopsy specimen
Diagnosis: Poorly differentiated lung adenocarcinoma
Tumor cell content: 70%

Gene	Variant	Reference sequence	VAF (%)	OncoKB level/ ESCAT	Drug
EGFR	*P.*L858R (c.2573T>G)	NM_005228.4	69	1/Tier I	Afatinib, dacomatinib, erlotinib, gefitinib, osimertinib
EGFR	*P.*T790M (c.2369C>T)	NM_005228.4	38	1/Tier I	Osimertinib
				R1/Tier I	Afatinib, erlotinib, gefitinib
MET	Amplification (copy number: 17)			2/Tier I	Crizotimib

Methodology
Test material: Tumor DNA/RNA
Gene panel: Oncomine™
Comprehensive Assay v3 (ThermoFisher) (see detailed list gene).
Instrument: Ion Torrent S5 platform (ThermoFisher).
Data analysis: Ion Reporter Software (Filter: Oncomine Variants, 5% CI, CNV ploidy ≥ gain of 2 over normal).
Reference genome: GRCh37 (hg19).
Databases used for variant annotation: dsSNP, 1000 Genomes, ClinVar, COSMIC, OncoKB. Reporting: Limited to genomic alterations with level 1, 2, or R1 evidence according to OncoKB and ESCAT Therapeutic Levels of Evidence V2 classification at the time of reporting.
Gene list
Sequence variants (hotspot regions): AKT1, AKT2, AKT3, ALK, AR, ARAF, AXL, BRAF, BTK, CBL, CCND1, CDK4, CDK6, CHEK2, CSF1R, CTNNB1, DDR2, EGFR, ERBB2 (HER2), ERBB3, ERBB4, ERCC2, ESR1, EZH2, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, FOXL2, GATA2, GNA11, GNAQ, H3F3A, HIST1H3B, HNF1A, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KIT, KNSTRN, KRAS, MAGOH, MAP2K1, MAP2K2, MAP2K4, MAPK1, MAX, MDM2, MED12, MET, MTOR, MYC, MYCN, MYD88, NFE2L2, NRAS, NTRK1, NTRK2, PDGFRA, PDGFRB, PIK3CA, PIK3CB, PPP2R1A, PTPN11, RAC1, RAF1, RET, RHEB, RHOA, ROS1, SF3B1, SMAD4, SMO, SPOP, SRC, STAT3, TERT, TOP1, U2AF1, XPO1.
Sequence variants (all coding regions): ARID1A, ATM, ATR, ATRX, BAP1, BRCA1, BRCA2, CDK12, CDKN1B, CDKN2A, CDKN2B, CHEK1, CREBBP, FANCA, FANCD2, FANCI, FBXW7, MLH1, MRE11A, MSH2, MSH6, NBN, NF1, NF2, NOTCH1, NOTCH2, NOTCH3, PALB2, PIK3R1, PMS2, POLE, PTCH1, PTEN, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RB1, RNF43, SETD2, SLX4, SMARCA4, SMARCB1, STK11, TP53, TSC1, TSC2.
Copy number alterations (amplification): AKT1, AKT2, AKT3, ALK, AR, AXL, BRAF, CCND1, CCND2, CCND3, CCNE1, CDK2, CDK4, CDK6, CDKN2A, CDKN2B, EGFR, ERBB2 (HER2), ESR1, FGF19, FGF3, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, IGF1R, KIT, KRAS, MDM2, MDM4, MET, MYC, MYCL, MYCN, NTRK1, NTRK2, NTRK3, PDGFRA, PDGFRB, PIK3CA, PIK3CB, PPARG, RICTOR, TERT, TSC1, TSC2.
Fusion transcripts: AKT2, ALK, AR, AXL, BRAF, BRCA1, BRCA2, CDKN2A, EGFR, ERBB2, ERBB4, ERG, ESR1, ETV1, ETV4, ETV5, FGFR1, FGFR2, FGFR3, FGR, FLT3, JAK2, KRAS, MDM4, MET, MYB, MYBL1, NF1, NOTCH1, NOTCH2, NRG1, NTRK1, NTRK2, NTRK3, NUTM1, PDGFRA, PDGFRB, PIK3CA, PRKACA, PRKACB, PPARG, PTEN, RAD51B, RAF1, RB1, RELA, RET, ROS1, RSPO2, RSPO3, TERT.

CI, confidence interval; CNV, copy number variant; COSMIC, Catalogue of Somatic Mutations in Cancer; dsSNP, ; ESCAT, ESMO scale of clinical actionability for molecular targets; NGS, next-generation sequencing; VAF, variant allele frequency.

NGS report also details other factors (see Table 3) that may be important for the clinical interpretation; please also see an example for a possible sample report in Table 5. For the interpretation of the NGS report, the oncologists need to obtain a basic level of understanding of the terminology used in the report. In addition, every oncologist should be able to check the significance of a reported alteration in commonly available databases and even more importantly should be aware of which databases were consulted by the provider he is working with (see Table 4). In addition, the detection of a pathogenic alteration such as for example BRCA1 should be recognized not only for the clinical implications, but also as a potential sign of a hereditary cancer syndrome that needs genetic counseling and/or germline genetic testing.

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