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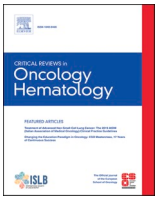

Standardized and simplified reporting of next-generation sequencing results in advanced non-small-cell lung cancer: Practical indications from an Italian multidisciplinary group

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A R T I C L E I N F O

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A B S T R A C T

Molecular biomarker testing is increasingly becoming standard of care for advanced non-small cell lung cancer (NSCLC). Tissue and liquid biopsy-based next-generation sequencing (NGS) is now highly recommended and has become an integral part of the routine management of advanced NSCLC patients. This highly sensitive approach can simultaneously and efficiently detect multiple biomarkers even in scant samples. However full optimization of NGS in clinical practice requires accurate reporting and interpretation of NGS findings. Indeed, as the number of NSCLC biomarkers continues to grow, clinical reporting of NGS data is becoming increasingly complex. In this scenario, achieving standardization, simplification, and improved readability of NGS reports is key to ensuring timely and appropriate treatment decisions. In an effort to address the complexity and lengthy reporting of NGS mutation results, an Italian group of 14 healthcare professionals involved in NSCLC management convened in 2023 to address the content, structure, and ease-of-use of NGS reporting practices and proposed a standard report template for clinical use This article presents the key discussion points addressed by the Italian working group and describes the essential elements of the report template.

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1. Introduction

The development of targeted drugs able to selectively inhibit oncogenic drivers in tumor cells has revolutionized the treatment of non-small lung cell carcinoma (NSCLC) (Penault-Llorca et al., 2022a,b; Roberts et al., 2023). Moreover, recently approved immune checkpoint inhibitors (ICIs) have shown a major clinical improvement in non-oncogene addicted NSCLC patients (Mamdani et al., 2022). Therefore, the use of molecular profiling of tumor DNA and RNA mutations by next-generation sequencing (NGS) to identify genomic alterations in clinically approved predictive biomarkers has become standard practice in the diagnosis and management of NSCLC (Penault-Llorca et al., 2022b; Hendricks et al., 2023). Indeed, current guidelines issued by international cancer organizations strongly recommend molecular testing of druggable predictive biomarkers for the clinical stratification of NSCLC patients (Planchard et al., 2020; Hendricks et al., 2023). As of today, predictive biomarker testing is required for a number of so called must-test genes, in particular *EGFR, KRAS, BRAF, ERBB2* hotspot mutations, *ALK*, *ROS1, RET, NTRK* aberrant transcripts, and *MET* exon 14 skipping mutations (Kerr et al., 2021). In addition, comprehensive testing panels are now available to identify patients eligible for early access to investigational clinical trials (Planchard et al., 2020; Vingiani et al., 2023).

The rapidly evolving landscape of diagnostic, predictive, and prognostic biomarkers for NSCLC has prompted the development of various molecular detection approaches. Among these are single gene sequencing, namely, Sanger, and high throughput sequencing, namely NGS. Despite being as sensitive as NGS, the former fails to comprehensively detect all actionable biomarkers in up to 25 % of cases. The reason is that Sanger sequencing requires a "technical selection" of tested biomarkers since tumor tissue from NSCLC patients is often insufficient. This translates into reducing NSCLC patients' therapeutic options (Frampton et al., 2013). By contrast, NGS enables simultaneous detection of several molecular alterations in different biomarkers even in scant cytological, histological and liquid biopsy specimens (Frampton et al., 2013). Moreover, thanks to its high technical sensitivity and specificity, this technique accurately detects low-frequency genomic alterations in clinically relevant cancer genes (Frampton et al., 2013; Aziz et al., 2015; Hynes et al., 2017; Rolfo et al., 2021; Krebs et al., 2022). Accordingly, NGS platforms are increasingly being used for the molecular testing of clinically approved biomarkers in routine NSCLC samples. Not surprisingly, NGS-based molecular profiling of advanced NSCLC patients is strongly recommended by scientific agencies including the European Society for Medical Oncology (ESMO) and the Association of Italian Medical Oncologists (AIOM) (Mosele et al., 2020; Hendricks et al., 2023).

However, the full application of NGS in routine clinical practice still remains challenging. One of the biggest challenges is the clinical interpretation of the results. This is an important issue to address as integrated, comprehensive, and easy-to-interpret clinical reports from NGS analysis constitute an indispensable tool to support and ensure appropriate treatment decisions (Penault-Llorca et al., 2022b). However, as a result of the growing number of mandatory biomarkers, interpreting NGS reports remains a major hurdle for clinical oncologists (Hynes et al., 2017). Despite considerable efforts made by leading consensus societies to improve NGS reporting, the standardization process is still lagging (Li et al., 2017; Schmid et al., 2022). To address unsolved issues in NGS reporting and data interpretation, 14 Italian expert healthcare professionals *from different institutions?* identified a number of key elements necessary to obtain a fully-integrated, comprehensive, and easily interpretable reporting format for NSCLC patients. The multidisciplinary team was made up of n = 9 molecular pathologists' members of The Italian Society of Pathology -SIAPEC-PMMP group, n = 7 oncologists, n = 1 cancer institute director, and n = 1 member of a patient advocacy organization. In this study, we set out to summarize the key discussion points and outcomes of the meeting. To this aim, we carried out a real-time online survey, comprising 11 questions designed by two scientific coordinators (SN and UM). This survey investigated the technical specifications of NGS-based molecular analysis to include in the clinical report. The specifications comprised the following elements: the types of NGS assays adopted; the names of patients, ordering clinicians, and pathologists; a description of specimens and, details of pre-analytical sample processing, identification of molecular signatures namely, nucleotide alterations and amino acid variations mentioning of therapeutic approaches and matched technical specifications; finally, identification of potential germline variants needing referral to genetic counseling. For each answer, a consensus agreement of 10 out of 18 (> 50.0 %) positive considerations was requested. Of note, all discussion points reached a positive agreement. The members of the working group were asked to express their agreement or disagreement by describing how the different items were handled in the report template. (Fig. 1) Here, we discuss the results of the survey and propose a report template to be used by clinical administrations for the management of advanced NSCLC patients.

2. Reporting the results of NGS-based profiling of advanced NSCLC

As indicated by the working group, an NGS report for clinical practice should be concise but thorough. Ideally, the main findings should be reported on a single page and should be easily interpreted by all users, particularly clinicians who lay out the treatment plan. In addition, a standard nomenclature approved by international agencies and straightforward access to any additional information should be guaranteed. In particular, the group of clinical oncologists involved in this study highlighted the importance of receiving a fully integrated clinical report able to clearly and concisely summarize the technical and molecular data. These general requirements have also been underlined by published international recommendations on cancer sequence variant reporting (Li et al., 2017; Schmid et al., 2022; Penault-Llorca et al., 2022b). Moreover, because NGS platforms use different sequencing technologies, they suggested that clinical reports also contain the technical specifications of the NGS platforms adopted. In particular, they suggested including the following technical details in the final section of the report**:** the type of gene panel and corresponding reference range (*i.e.,* the list of referral genomic alterations covered by the NGS panel), the limit of detection *(i.e.,* the minimum level of mutant allele fraction (MAF) detected by the NGS system), and the clinical cut-off values selected for the annotation of molecular alterations. An important point of the discussion was that all technical specifications should meet the readability criteria of a technical report integrated into the clinical final report. In this regard the working group considered including two key points. In particular, they suggested indicating (1) the molecular status (positive or negative) for each targeted alteration required by the guidelines in the principal part of the report and (2) any other NGS-detectable genes or variants in the appendix section. The group also deemed necessary to include demographic information (age, sex) and clinically informative specifications (histological diagnosis, tumor site, grading, stage, and smoking habit) in the dedicated clinical section of the report. Noteworthy, the committee also agreed that the report should include the names of the physicians who ordered the testing, generally oncologists, of the pathologists who managed the sample, and of the molecular biologists who performed the molecular test. Each patient should also be identified and traced with an anonymous internal code (ID), generally reported at the top of the specimen section. Other crucial aspects mentioned were the identification of sample data collection and turnaround time (TAT) of the laboratory. Regarding the latter, international guidelines recommend that diagnostic samples (from request to data reporting) should be processed and assessed within 10 working days (Cree et al., 2014). Such time window is often heavily impacted by several factors, mainly by the types of technical platforms used, the availability, or lack thereof, of dedicated personnel in

Page 1

REPORT

Tumor type		**Country code**	**Report date**
Lung adenocarcinoma		.....	.....
Patient	**Physicians**	**Specimen**	**NGS assay**
Name:	Ordering physician:	ID:	.....
Date of birth:	Pathologist:	Site:	
Sex:		Type:	
Diagnosis:	**Laboratory**	Date of collection:	
Medical record n.:	Certified: yes/no	Date of receipt:	
	Type of certification:	PD-L1 expression:	
	Date of certification:		
	Date and type of last quality control:		

Genomic signatures

Tumor mutational burden: .....

Microsatellite status: .....

Genomic variants	**MAF**	**ESCAT level**
.....		
.....		
.....		
Copy Number Variations	**CNV Ratio**	
.....		
.....		
.....		
Wild-type actionable alterations in disease relevant genes		
.....		
.....		
.....		
.....		
Inconclusive results		
.....		

Incidental findings

.....

[1] Information on the ESCAT levels is in the Appendix on page 2.

Fig. 1. Proposed template document for reporting NGS findings in advanced NSCL.

Page 2

APPENDIX

1) Description of NGS assay (entire gene panel)

2) Specimen processing

3) Definitions and clinical implications of genomic signatures

4) Complete list of detected alterations

45ESCAT levels

https://www.esmo.org/policy/esmo-scale-for-clinical-actionability-of-molecular-targets-escat

As this report is meant for use in clinical practice in Italy, the definition of the ESCAT levels will be translated into Italian.

6) Any other requested supplementary information

Fig. 1. (*continued*).

predictive molecular pathology laboratories, and the volume of molecular requests sustained by the center. Not surprisingly, referral institutions with high expertise in NGS systems have shown lower TAT (n = 8 working days) in their diagnostic activity (Pisapia et al., 2022). In general, when NGS testing is outsourced, the final report should indicate whether the facility that performed the analysis was certified by providing information regarding the type and date of certification, and the name of the agencies that issued the laboratory certification. In Italy, however, where laboratory certifications are not mandatory, external quality control programs monitor the overall quality and reliability of laboratory activities.

Recently, the clinical approval of complex genomic signatures, like microsatellite instability (MSI) and tumor mutational burden (TMB), have affected the clinical intelligibility of molecular records in the final report. However, considering the highly predictive and diagnostic nature of these genomic signatures, the members of the working committee deemed that it was necessary to include these types of molecular hallmarks in clinical reports. Indeed, both MSI and TMB can predict immunotherapy response to immune checkpoint inhibitors (ICIs); moreover, MSI is a diagnostic biomarker associated with cancer-predisposing syndromes (*e.g.,* Lynch syndrome) and may therefore identify patients who would potentially benefit from genetic counseling. For example, in some cases, especially those who fail to respond to ICIs, these complex biomarkers may be subjected to scrutiny by a Molecular Tumor Board (MTB) to generate more tailored therapeutic choices. In this scenario, comprehensive NGS profiles able to calculate these molecular signatures may offer an integrative molecular record that could acquire diagnostic, prognostic, and therapeutic implications for these patients (Louie et al., 2022). Generally, eligibility of NSCLC patient for ICIs is based on immunohistochemical detection of PD-L1. In accordance with this strategy, such assessment should be included in the sample section of the report. The working committee also decided to include mutant allele frequency (MAF) in the report. Such decision was based on the fact that MAF values help to discriminate between somatic and germline mutations in tumor cells when genomic DNA from non-tumor cells is unavailable. For instance, in inherited diseases, MAF values range from 50 % to 100 % (Li et al., 2017). Hence, this approach is key to providing a thorough picture of patients' genomic alterations.

As for the nomenclature, the working group unanimously agreed to apply the standard gene nomenclature recommended by the Human Genome Organization (HUGO) (http://www.genenames.org) and the Human Genome Variation Society (HGVS) (http://www.hgvs.org) (Li et al., 2017). Moreover, because of the rapidly evolving scenario of cancer molecular profiling, all members also suggested updating all molecular records routinely in accordance with the latest clinical interpretations provided by the websites of international societies.

Both wild-type and inconclusive molecular results were also considered essential elements to include on the first page of the report. The reason for including inconclusive molecular results was that technical reporting of wild-type and inconclusive results increases the readability of the report. Furthermore, a consensus was also reached to include in the Appendix technical glitches that may have yielded inadequate results. Some of these included over-fixation, low neoplastic cell percentage, technical errors in the processing phase, and decalcification procedures. Moreover, the committee agreed to include non-actionable alterations in clinically approved cancer related genes in the appendix section (part 4) where the entire list of molecular alterations detected by NGS is cited. Subsequently, non-actionable alterations found in clinically relevant genes should also be archived in internal databases (Marchiò et al., 2019). Regarding actionable mutations, each alteration should be linked with a corresponding level of clinical relevance according to previously cited classification systems. Such classification systems have been based on the clinical impact of evidence-based assessments (Hynes et al., 2017; Li et al., 2017; Schmid et al., 2022). Among these systems is the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT). This system classifies the clinical relevance of genomic alterations in six tiers on the basis of actionability (Mateo et al., 2018; https://www.esmo.org/policy/esmo-scale-for-clinical-actionability-of-molecular-targets-escat). Another useful knowledge-based database of actionable genomic variations is the public Precision

Oncology Knowledge Base (OncoKB), which is powered by the Memorial Sloan Kettering Cancer Center (Chackravarty et al., 2017; https://www.oncokb.org/). Finally, as not all NGS-report users may be familiar with the Italian ESCAT classification, additional information about this system (link or table) should be included in the Appendix of the NGS report.

Concerning the inclusion of available therapies targeting the genomic alteration identified during NGS-based molecular profiling, the working group, in accordance with Italian guidelines, [references] consensually decided to exclude this type of information from NGS reports. Although all members agreed that the ultimate goal of NGS tumor molecular profiling is the identification of targeted therapy, they all acknowledged that the indication of available therapies went beyond the responsibility of molecular pathologists and that such responsibility belonged to molecular tumor boards (MTBs).

Finally, the working group concurred that the ESCAT score system was to be included in the report. This classification system, developed by a working group of ESMO, provides both evidence-based information on the availability, or lack thereof, of clinically relevant targeted therapies for each actionable mutation (Level 1), and information on investigational drugs (Level 2). Finally, including ESCAT in the report was deemed crucial since many Italian laboratories performing NGS testing of NSCLC patients may not have access to the databases that provide updated information on newly approved targeted therapies and unapproved investigational targeted therapies.

3. NGS report template

Having discussed and unanimously agreed on all the essential data elements to include in an NGS report, the Italian working group generated a document template to report NGS findings. They laid out a two-page report template containing the following data elements **(**Fig. 1**).**

- Page 1 contained clinically significant genomic signature results and relevant technical and clinical specifications readable by all users, including patients.
- Page 2 consisted of an Appendix containing all supplementary information. It included technical details regarding the methodological aspects of the adopted assay, starting from nucleic acids extraction to NGS analysis. Additional pages could be added if needed.

Genomic signatures were considered the central message of the report and were reported on the first page of the report. Molecular results, including negative results of NSCLC-relevant alterations and inconclusive results were also placed in a prominent section on page 1 to improve readability. On the other hand, the ESCAT level for each alteration was placed in the Appendix section on page 2**,** as it was considered useful information to inform clinicians about the clinical relevance of the NGS findings. They also suggested translating the whole ESCAT classification system. As mentioned in the previous section, available targeted therapies were not detailed in the report template as they would be comprehensively evaluated by TMBs. Finally, incidental findings of clinically relevant molecular signatures detected by comprehensive NGS panels, were also included on page 1 in view of a possible renewal of their clinical implications.

Conceivably, the complexity of NGS reporting stems from the high volume of information required by personalized medicine to develop targeted therapies. This phenomenon is reflected in recent efforts to redefine the structural content of pathological and molecular reports. Previously, molecular pathology reports were free text, highly narrative, prone to omission of necessary data, and marred by inconsistent formatting. Over the past few decades, synoptic reporting has been the most important and widely used way to effectively generate, read, and digest complex molecular records integrated in an easily manageable clinical format. In particular, this reporting system allows to provide prespecified data in a specific format for surgical pathology reports. As a result, it not only ensures that all reports contain all necessary data elements, but also allows for scalable data capture, interoperability, and exchange. Currently, ongoing efforts are being made to create a national and international health care meaningful use of standardized cancer-related health records by using cancer registries and health information exchanges for storing and accessing data. Ideally, data should be fed dynamically and seamlessly into and out of these data exchanges by using lean and streamlined automated processes. In this context, synoptic reporting should also be adopted for molecular reporting, as it could incorporate the huge amount of genomic data (Big Data) and circumvent major issues related to information overload and data exploitation. These reports should be in JASON or XML format, making sure that the data are computer identifiable, retrievable, and processable by using a standardized lexical data set. In this regard, the Italian Society of Pathology (SIAPEC) is currently working on the creation of a standard data set for cancer pathology reporting. The ultimate goal is to gradually shift from free-text narrative reports (level 1) to synoptic reports (level 3) to fully structured reporting. Structured reports should include discrete data embedded in laboratory information systems (LIS) and structured messaging/data exchange standards (level 6) (Renshaw et al., 2018; Torous et al., 2021).

4. Conclusions and perspectives

Precision medicine for the treatment of advanced NSCLC is offering patients a valuable option beyond non-selective chemotherapy. Consequently, molecular tumor profiling by NGS, is currently integrated within the management of NSCLC patients, as recommended by the international and Italian guidelines. The wealth of data generated by NGS analyses, especially when broad gene panels are used, can be highly challenging to interpret especially for pathologists, who are responsible for summarizing the NGS results in a clinical report, and for report recipients, including oncologists, general practitioners, and patients. As of today, there are still a number of unmet needs that ought to be addressed to simplify NGS reporting. Among these are standardization and simplification of reports, accurate and ~~a~~ consistent nomenclature, and classification of results on the basis of their clinical relevance. Moreover, the progressively increasing knowledge of NSCLC biology requires consistent updates on biomarker analysis. Accordingly, we believe that educational programs aimed at increasing the awareness of these tools among healthcare professionals involved in NSCLC care are definitely warranted. Realizing that reporting, let alone interpreting, NGS molecular data can be a daunting task, an Italian working group designed a concise and thorough report template to assist clinicians in reporting NGS-derived molecular data from NSCLC patients. In this paper, we highlighted the main discussion points and final statements on the essential elements to include in NGS reporting. In particular, this template was designed and developed to include all essential background information and NGS results on a single page (page 1), additional technical information in an Appendix section (page 2). Moreover, since the available validated resources are used to standardize reporting and ensure a correct interpretation of NGS findings, we focused on the optimization of clinical reports for the handling of NGS data in NSCLC patients. Through this activity, we hope to render the interpretation of NGS reports less challenging for clinicians involved in NSCLC clinical management and to improve patients' overall clinical outcomes.

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Conceptualisation: all the authors. Validation: all authors. Formal analysis: all authors. Investigation: all authors. Data curation: all

authors. Writing - original draft preparation: UM, FP. Writing - review and editing: all authors. Visualisation: all authors. Supervision: UM, SN, GT. Project administration: UM, SN and GT.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Umberto Malapelle has received personal fees (as consultant and/or speaker bureau) from Boehringer Ingelheim, Roche, MSD, Amgen, Thermo Fisher Scientific, Eli Lilly, Diaceutics, GSK, Merck and AstraZeneca, Janssen, Diatech, Novartis and Hedera unrelated to the current work. Fabio Pagni received personal fees (as consultant and/or speaker bureau) from, MSD, GSK, Merck and AstraZeneca, unrelated to the current work. Elena Guerini Rocco has relevant relationship (advisory fees, honoraria, travel accommodation and expenses, grants and non-financial support) with AstraZeneca, Exact Sciences, GlaxoSmithKline (GSK), Novartis, Roche, Thermo Fisher Scientific unrelated to the current work. Giulia Pasello has received personal fees (as consultant and/or speaker bureau) from Amgen; Astrazeneca; BMS; Lilly; MSD; Roche; Jansenn Research support: Astrazeneca; Roche unrelated to the current work. Giuseppe Perrone has received personal fees (as consultant and/or speaker bureau) from AstraZeneca, Amgen, Boehringer Ingelheim, Daiichi-Sankyo, Diatech Pharmacogenomics, Eli Lilly, Incyte, GSK, Janssen, Merck Serono, MSD, Novartis, Roche unrelated to the current work. Francesco Pepe has received personal fees (as consultant and/or speaker bureau) from Menarini Stemline unrelated to the current work. Giancarlo Pruneri has received personal fees (as consultant and/or speaker bureau) from Lilly, Roche Foundation One, Bayer, Novartis unrelated to the current work. Giancarlo Troncone reports personal fees (as speaker bureau or advisor) from Roche, MSD, Pfizer and Bayer, unrelated to the current work. Silvia Novello reports personal fees (as speaker bureau or advisor) from Eli Lilly, MSD, Roche, BMS, Takeda, Pfizer, Astra Zeneca and Boehringer Ingelheim, unrelated to the current work. No relevant conflict of interest declared by Alessandro Delle Donne, Filippo Fraggetta, Simona Vatrano, Sandro Pignata, Carmine Pinto, Antonio Russo, Hector J Soto Parra, Stefania Vallone, Antonio Marchetti related to the current work. No relevant conflict of interest declared by Alessandro Delle Donne, Filippo Fraggetta, Simona Vatrano, Sandro Pignata, Carmine Pinto, Antonio Russo, Hector J Soto Parra, Stefania Vallone, Antonio Marchetti related to the current work.

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APPENDIX A

1) NGS assay description (entire gene panel).

2) Specimen processing.

3) Definitions and clinical implications of genomic signatures.

4) ESCAT levels.

https://www.esmo.org/policy/esmo-scale-for-clinical-actionability-of-molecular-targets-escat.

As this report is meant for use in clinical practice in Italy, the definition of the ESCAT levels will be translated into Italian.

5) Any other supplementary information required.

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