

## Review

## The evolving landscape of biomarker testing for non-small cell lung cancer in Europe

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## ABSTRACT

The discovery of oncogenic driver mutations rendering non-small cell lung cancer (NSCLC) targetable by small-molecule inhibitors, and the development of immunotherapies, have revolutionised NSCLC treatment. Today, instead of non-selective chemotherapies, all patients with advanced NSCLC eligible for treatment (and increasing numbers with earlier, less extensive disease) require fast and comprehensive screening of biomarkers for first-line patient selection for targeted therapy, chemotherapy, or immunotherapy (with or without chemotherapy). To avoid unnecessary re-biopsies, biomarker screening before first-line treatment should also include markers that are actionable from second-line onwards; PD-L1 expression testing is also mandatory before initiating treatment.

Population differences exist in the frequency of oncogenic driver mutations: *EGFR* mutations are more frequent in Asia than Europe, whereas the converse is true for *KRAS* mutations. In addition to approved first-line therapies, a number of emerging therapies are being investigated in clinical trials. Guidelines for biomarker testing vary by country, with the number of actionable targets and the requirement for extensive molecular screening strategies expected to increase. To meet diagnostic demands, rapid screening technologies for single-driver mutations have been implemented. Improvements in DNA- and RNA-based next-generation sequencing

**Abbreviations:** ALK, anaplastic lymphoma kinase; AMP, Association for Molecular Pathology; ASCO, American Society of Clinical Oncology; BRAF, B-Raf proto-oncogene; CAP, College of American Pathologists; ctDNA, circulating tumour cell DNA; ddPCR, digital droplet PCR; EGFR, epidermal growth factor receptor; EMQN, European Molecular Genetics Quality Network; EQA, external quality assessment; ERBB2, Erb-B2 receptor tyrosine kinase 2; ESCAT, ESMO Scale of Clinical Actionability of Molecular Targets; ESMO, European Society for Medical Oncology; FDA, Food and Drug Administration; FGFR, fibroblast growth factor receptor; FISH, fluorescence *in situ* hybridisation; FNA, fine needle aspiration; HER, human epidermal growth factor receptor; IASLC, International Association for the Study of Lung Cancer; ICC, immunocytochemistry; IHC, immunohistochemistry; ISH, *in situ* hybridisation; KRAS, Kirsten rat sarcoma viral oncogene homolog; MEK, mitogen-activated protein kinase kinase; MET, hepatocyte growth factor receptor; MTB, molecular tumour board; NCCN, National Comprehensive Cancer Network; NEQAS, National External Quality Assessment Service; NGS, next-generation sequencing; NRG1, neuregulin-1; NSCLC, non-small cell lung cancer; NTRK, neurotrophic tyrosine receptor kinase; OncoKB, Oncology Knowledge Base; PCR, polymerase chain reaction; PD-1, programmed cell death protein 1; PD-L1, programmed cell death protein ligand 1; qPCR, quantitative PCR; QuIP, Quality Initiative for Pathology; RET, rearranged during transfection; ROS1, ROS proto-oncogene 1; ROSE, rapid on-site evaluation; RT-PCR, real-time polymerase chain reaction; SGT, single-gene testing; SOC, standard of care; SOP, standard operating procedures; TAT, turnaround time; TMB, tumour mutational burden; VAF, variant allele frequency.

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technologies enable analysis of a group of genes in one assay; however, turnaround times remain relatively long. Consequently, rapid screening technologies are being implemented alongside next-generation sequencing.

Further challenges in the evolving landscape of biomarker testing in NSCLC are actionable primary and secondary resistance mechanisms to targeted therapies. Therefore, comprehensive testing on re-biopsies, collected at the time of disease progression, in combination with testing of circulating tumour DNA may provide important information to guide second- or third-line therapies. Furthermore, longitudinal biomarker testing can provide insights into tumour evolution and heterogeneity during the course of the disease. We summarise best practice strategies for Europe in the changing landscape of biomarker testing at diagnosis and during treatment.

## 1. Introduction

Lung cancer remains the leading cause of cancer mortality worldwide [1]. Across Europe in 2018, there were an estimated 388,000 lung cancer-related deaths, which was higher than those related to colorectal cancer and breast cancer combined [2]. Non-small cell lung cancer (NSCLC) accounts for ~84 % of all lung cancer cases [3], imposing a substantial social and financial burden in Europe [4,5].

The treatment landscape for NSCLC is rapidly evolving and, following the principles of precision medicine, is becoming increasingly biomarker driven with new targeted therapies used in concert with companion molecular diagnostics [6,7]. ‘Precision medicine’ refers to the use of therapeutics that are more likely to confer benefit to a subgroup of patients whose cancer shows certain molecular or morpho-phenotypical characteristics [8,9]. NSCLC is associated with several additive oncogenic driver alterations, e.g. epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), ROS proto-oncogene 1 (*ROS1*), B-Raf proto-oncogene (*BRAF*) and neurotrophic tyrosine receptor kinase (*NTRK*), which are relevant for selecting the most beneficial regimen [8,10,11]. New NSCLC biomarkers continue to emerge, including rearranged during transfection (*RET*), hepatocyte growth factor receptor (*MET*) exon 14 (ex14) skipping mutations and *MET* fusions, receptor tyrosine-protein kinase erbB-2 (*ERBB2/HER2*), Kirsten rat sarcoma viral oncogene homolog (*KRAS*) exon 2 G12C, neuregulin (*NRG1*), fibroblast growth factor receptor (*FGFR*) fusions and activating point mutations, and *EGFR* exon 20 insertions. Additionally, programmed death receptor ligand 1 (PD-L1) and tumour mutational burden (TMB) can predict a favourable response to immune checkpoint inhibitors in NSCLC [12,13].

The growing number of licensed and emerging therapies targeted to NSCLC genetic alterations, combined with continuously evolving molecular biology technologies, has resulted in rapid change. This is challenging for formulating and implementing guidelines for biomarker testing. Therefore, despite serving as the foundation for NSCLC precision medicine, molecular testing rates remain suboptimal [14].

In this review, we outline the rapidly evolving diagnosis and treatment landscape for NSCLC in Europe, which has become increasingly biomarker driven over the last decade. We provide a comprehensive overview of established and emerging NSCLC biomarkers in Europe, including state-of-the-art scientific knowledge around biomarker testing and current best practice recommendations for biomarker testing. Emerging developments and implications for future practice are also discussed.

## 2. Overview of current and emerging biomarkers for NSCLC

Somatic alterations in NSCLC can lead to oncogenic activation through several mechanisms, including point mutations, insertions/deletions and rearrangements [10]. Broadly, actionable mutations guiding targeted therapy can be classified according to gene rearrangements (e.g. *ALK*, *ROS1*, *RET*, *NTRK*, *FGFR1/2/3*, *NRG1*) or variants including point mutations, insertions/deletions and amplifications (e.g. *EGFR*, *BRAF*, mitogen-activated protein kinase kinase [*MEK*], *KRAS*, *MET*, *ERBB2/HER2*). We use the term ‘targeted therapies’ in this article for therapies targeting actionable mutations, whereas ‘immunotherapies’

target immune checkpoint proteins (programmed cell death protein 1/programmed cell death ligand 1 [PD-L1] or cytotoxic T-lymphocyte-associated protein 4). TMB (the number of non-synonymous mutations per coding area of a genome) has also been shown to predict objective response rates and improvement in progression-free survival to immunotherapy [13,15–17]. Overall, outcomes for patients with actionable oncogenic driver mutations when receiving targeted therapy tend to be improved versus those without actionable mutations [18]. Molecular testing to detect these ‘actionable targets’ therefore plays a key role in the diagnostic work-up for NSCLC patients to guide therapy choices and improve outcomes. Table 1 [6,13,19–43] provides an overview of current and emerging biomarkers in NSCLC in Europe, their frequency and associated approved targeted therapies.

## 3. Guidelines for biomarker testing for NSCLC

### 3.1. International guidelines

Rates of oncogenic driver mutations in different populations with NSCLC can vary; for example, populations in Asia have higher rates of *EGFR* mutations and lower rates of *KRAS* mutations than those in Europe [44,45]. Hence, guidelines may have different priorities according to the region represented.

European Society for Medical Oncology (ESMO) NSCLC guidelines state that molecular subtyping is necessary for therapeutic decision making and should be performed whenever possible [6]. For patients with advanced NSCLC, ESMO recommend systematic testing of *EGFR* and *BRAF* mutations, analysis of *ALK*, *ROS1* and *NTRK* rearrangements, and determination of PD-L1 expression [6]. ESMO also recommends the routine use of next-generation sequencing (NGS) in advanced non-squamous NSCLC, and that large multi-gene panels could be used if additional costs are considered acceptable compared versus small panels [46]. Generally, there is consensus across international guidelines around the need for *EGFR*, *BRAF*, *ALK* and *ROS1* testing in advanced NSCLC and all of these have approved first-line targeted therapies in Europe (Fig. 1A) [6,24,28,47,48]. Similarly, ESMO, National Comprehensive Cancer Network (NCCN) and Pan-Asian NSCLC guidelines [6, 24,47] recommend PD-L1 testing, and expanded panel testing for *NTRK* is recommended by the NCCN [24]; both biomarkers have approved therapies in Europe. Expanded panel testing recommendations for the emerging biomarkers *KRAS*, *MET*, *RET* and *ERBB2/HER2* alterations are currently only included in NCCN, American Society of Clinical Oncology (ASCO), and College of American Pathologists (CAP)/International Association for the Study of Lung Cancer (IASLC)/Association for Molecular Pathology (AMP) guidelines (Fig. 1B) [24,28,48].

### 3.2. National guidelines

International guidelines often form the basis of regional/national guidelines. While most European pathologists/oncologists refer to ESMO/NCCN guidelines, national guidelines tailor these for compatibility with local healthcare models/resources [49–59]. National guidelines are thus reflective of ‘best practice’ in individual countries. Accordingly, testing algorithms vary across Europe (Fig. 2 [49–59]). All national guidelines recommend testing for *EGFR*, *ALK*, *ROS1* and PD-L1,

and most recommend *BRAF* and *NTRK*. The biomarkers *KRAS*, *MET*, *RET* and *ERBB2/HER2* are recommended in the Netherlands and Sweden. Additional molecular testing may be performed on request or for research/clinical trial enrolment purposes, using NGS, or in subsequent testing rounds after determination of more commonly assessed biomarkers using rapid techniques (e.g. single-gene tests/low multiplex assays, immunohistochemistry, fluorescence *in situ* hybridisation [FISH]). TMB is not routinely assessed in most European countries.

A challenge in NSCLC is keeping pace with rapidly evolving molecular testing technologies for the growing number of targeted agents. Developing new guidelines and reaching consensus takes time, resulting in a lag behind scientific developments. Countries where NGS is recommended and reimbursed may find it easier to add new biomarker genes to an NGS panel, in contrast to countries where only single-gene testing for named biomarkers is reimbursed.

Although a large number of biomarkers are recommended by guidelines in some countries, this does not necessarily translate into their uptake in everyday clinical practice: there are barriers to uptake,

not least reimbursement challenges (see Section 4).

## 4. Challenges/barriers to biomarker testing for NSCLC

### 4.1. Uptake of biomarker testing in Europe

Across Europe, there is considerable variability in uptake of biomarker testing technologies [60–64]. For example, the proportion of patients with advanced non-squamous NSCLC who received a molecular test varied between 65 % and 85 % across Germany, Italy and Spain (2011–2016) [64]. For advanced non-squamous NSCLC, *EGFR* testing rates ranged from  $\geq 65$  % in Central/Eastern European countries (2014) to 79 % in Switzerland (2014) [60,62]. Molecular testing typically increases with time: a Swiss observational study highlighted an increase from 32 % in 2009 to 79 % in 2014 for *EGFR* [60]. Similarly, in five countries (France/Germany/Italy/Spain/UK), *EGFR* testing increased from 71 % to 81 % during 2014–2017 [65]. This was also observed with *KRAS* testing, which increased from 38 % to 59 % in those countries over

**Table 1**

Established and emerging biomarkers for NSCLC in Europe.

Predictive biomarkers [6]	Estimated frequency in NSCLC adenocarcinoma <sup>e</sup> [19,43]	Guideline recommended testing technologies [6,20]	EMA-approved targeted therapy (first-line) <sup>f</sup> [6,21]
<i>EGFR</i> mutations <sup>a</sup>	15 % <sup>f</sup>	Any appropriate, validated technology, subject to external quality assurance	Afatinib, dacomitinib, erlotinib, gefitinib, osimertinib
<i>ALK</i> rearrangements <sup>a</sup>	5 %	FISH (historical standard); IHC (validated against FISH); NGS <sup>h</sup>	Alectinib, brigatinib, ceritinib, crizotinib, lorlatinib
<i>ROS1</i> rearrangements <sup>a</sup>	2 %	FISH (trial-validated standard); IHC to select for confirmatory FISH; NGS <sup>i</sup>	Crizotinib, entrectinib
<i>NTRK</i> rearrangements <sup>a</sup>	<1 %	IHC, FISH, RT-PCR, NGS	Entrectinib, larotrectinib
<i>BRAF</i> mutations <sup>b</sup>	2 %	Any appropriate, validated technology, subject to external quality assurance	Dabrafenib/trametinib
PD-L1 expression levels <sup>c</sup>	33 %: $\geq 50$ % TPS 30 %: 1–49 % TPS 37 %: <1 % TPS	IHC	Immune checkpoint inhibitors
Emerging biomarkers <sup>d</sup> [22–26]	Estimated frequency in NSCLC adenocarcinoma [19,25,27]	Potential testing technology [28]	Targeted therapies under investigation [6,13,29–41]
<i>RET</i> rearrangements	2 %	FISH, RT-PCR, NGS	Alectinib, cabozantinib, lenvatinib, nintedanib, ponatinib, pralsetinib <sup>j</sup> , regorafenib, selipergatinib <sup>j</sup> , sorafenib, sunitinib, vandetanib
<i>MET</i> mutations	3 %	IHC, FISH, NGS	Cabozantinib, capmatinib <sup>k</sup> , crizotinib, MGD265, tepotinib <sup>j</sup>
<i>ERBB2/HER2</i> mutations	2 %	NGS	Ado-trastuzumab emtansine, afatinib, dacomitinib, fam-trastuzumab deruxtecan-nxki <sup>j</sup> , trastuzumab
<i>KRAS</i> mutations	25–33 % <sup>g</sup>	RT-PCR, pyrosequencing, NGS	Direct <i>KRAS</i> <sup>G12C</sup> inhibitors: adagrasib (MRTX 849), sotorasib (AMG510), GDC-6036
<i>NRG1</i> rearrangements	<1 %	NGS <sup>h</sup>	Afatinib
FGFR1	Data not available	NGS <sup>h</sup>	BGJ398, rogaratinib
TMB	Data not available	Whole exome sequencing	Immune checkpoint inhibitors

ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene; EGFR, epidermal growth factor receptor; EMA, European Medicines Agency; ERBB2, Erb-B2 receptor tyrosine kinase 2; FDA, Food and Drug Administration; FGFR1, fibroblast growth factor receptor-1; FISH, fluorescence *in situ* hybridisation; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; KRAS, Kirsten rat sarcoma viral oncogene homolog; MEK, mitogen-activated protein kinase kinase; MET, hepatocyte growth factor receptor; NGS, next-generation sequencing; NRG1, neuregulin-1; NSCLC, non-small cell lung cancer; NTRK, neurotrophic tyrosine receptor kinase; PD-L1, programmed cell death ligand 1; RET, rearranged during transfection; ROS1, ROS proto-oncogene 1; RT-PCR, real-time polymerase chain reaction; TMB, tumour mutational burden; TPS, tumour proportion score.

<sup>a</sup> Predicts response to targeted therapy with tyrosine kinase inhibitors.

<sup>b</sup> Predicts response to BRAF with/without MEK inhibitors.

<sup>c</sup> Predicts response to immunotherapy.

<sup>d</sup> Under investigation as predictive biomarkers with the goal of identifying appropriate therapies for patients.

<sup>e</sup> No specific driver known in over one-third of cases [19].

<sup>f</sup> Exon 19 deletions, exon 21 L858R mutations, and exon 20 insertions comprise approximately 10 %, 6%, and 2.5 % of all mutations [42].

<sup>g</sup> Exon 2 G12C mutations comprise approximately 13 % of all mutations [27].

<sup>h</sup> Emerging technology.

<sup>i</sup> As of October 2020.

<sup>j</sup> FDA approved.

<sup>k</sup> Approved in Japan.























<sup>l</sup> FDA Breakthrough Therapy designation.

the same 3-year period [65]. In Sweden, *EGFR* testing rates in patients with advanced non-squamous NSCLC increased from 49 % in 2011–2012 [63] to 84 % in 2019 [66], and the coverage of emerging targets, including *KRAS*, was likely similar due to a nationwide implementation of NGS. For some of the newer predictive biomarkers (e.g. *BRAF* and *NTRK*), which are recommended by national guidelines in some European countries, testing rates may be lower than those for the more established predictive markers (*EGFR*, *ALK*, and *ROS1*). For example, in Germany between 2015 and 2019, *BRAF* testing rates were 53.0 % versus 72.5 %, 74.5 % and 66.1 % for *EGFR*, *ALK*, and *ROS1*, respectively [67]. As seen with the recent addition of a recommendation for *NTRK* testing in the ESMO guidelines [6], the biomarker landscape in European countries is rapidly evolving. However, there are inevitable delays in rolling out these changes in routine clinical practice.


























#### 4.2. Resource and organisational barriers


In a resource-limited healthcare environment, oncologists and pulmonologists must determine which biomarkers and test types to prioritise for patients with lung cancer. Bureaucratic and organisational considerations, encompassing drug approval by regulatory authorities (including local formulary approval), regulatory approval of tests, availability of tests (particularly for emerging biomarkers) and reimbursement/insurance coverage, complicate decision making. Reimbursement is a key determinant of drug and testing availability in Europe, and discrepancies exist between Western and Central/Eastern Europe regarding availability of targeted therapies [68] and molecular testing [62]. Limited reimbursement was identified as a significant barrier to molecular testing in Central/Eastern Europe, serving to


**A**


Predictive biomarkers	ESMO guidelines	NCCN guidelines	CAP/IASLC/AMP guidelines	ASCO guidelines	Pan-Asian guidelines
<i>EGFR</i>					
<i>ALK</i>					
<i>ROS1</i>					
<i>BRAF</i>					
PD-L1					
<i>NTRK</i>					


**B**

Emerging biomarkers	ESMO guidelines	NCCN guidelines	CAP/IASLC/AMP guidelines	ASCO guidelines	Pan-Asian guidelines
<i>KRAS</i>					
<i>MET</i>					
<i>RET</i>					
<i>ERBB2/HER2</i>					
TMB					

 Testing recommended

 Expanded panel testing recommended

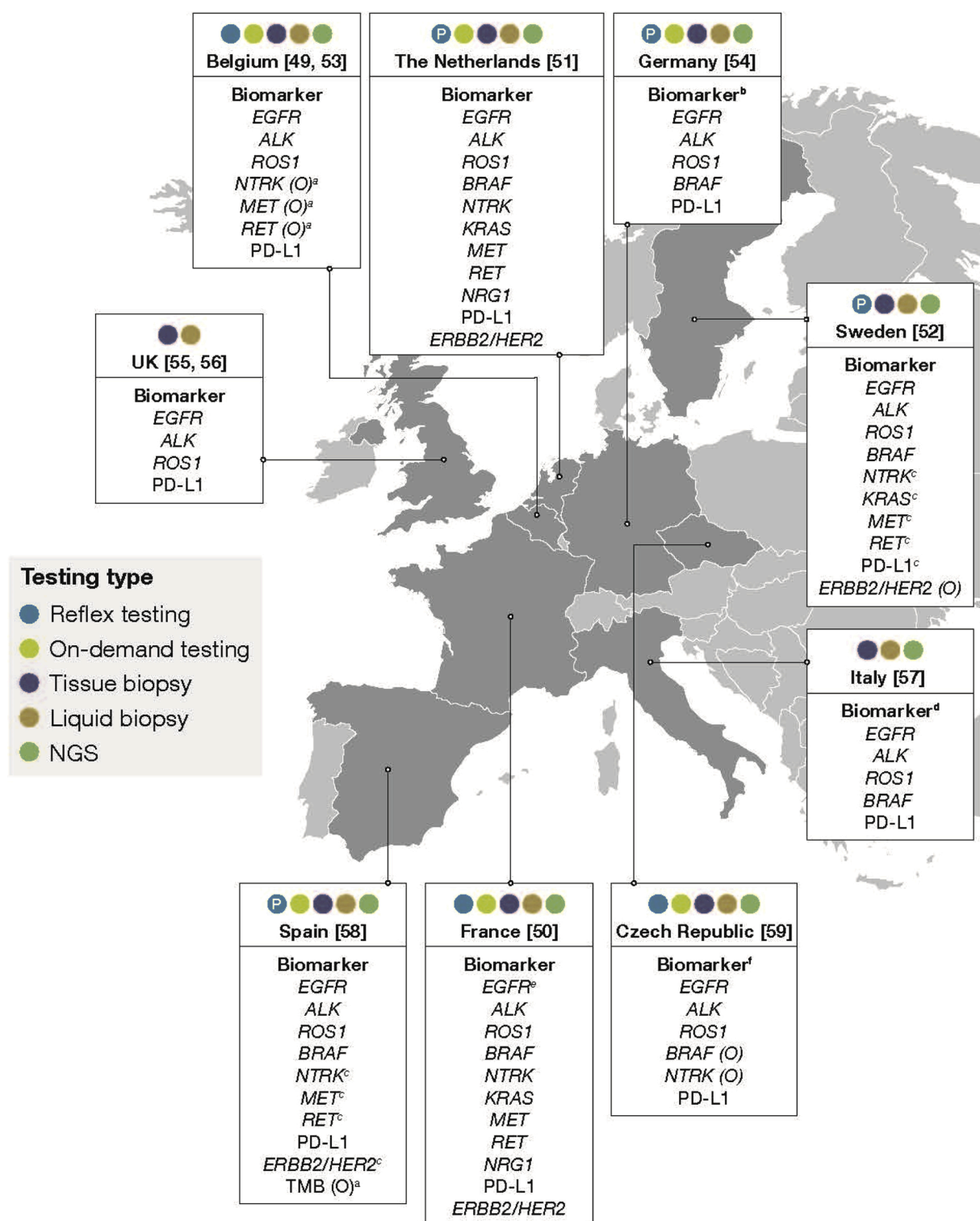
 Single gene or expanded panel testing recommended

 No guideline recommendations to date

**Fig. 1.** Guideline recommendations for (A) predictive biomarkers and (B) emerging biomarkers, to guide selection of precision therapies [6,24,28,47,48].

ALK, anaplastic lymphoma kinase; AMP, Association for Molecular Pathology; ASCO, American Society of Clinical Oncology; BRAF, B-Raf proto-oncogene; CAP, College of American Pathologists; EGFR, epidermal growth factor receptor; ESMO, European Society for Medical Oncology; HER, human epidermal growth factor receptor; IASLC, International Association for the Study of Lung Cancer; KRAS, Kirsten rat sarcoma viral oncogene homolog; MET, hepatocyte growth factor receptor; NCCN, National Comprehensive Cancer Network; NTRK, neurotrophic tyrosine receptor kinase; PD-L1, programmed cell death ligand 1; RET, rearranged during transfection; ROS1, ROS proto-oncogene 1; TMB, tumour mutational burden.





**Fig. 2.** Summary of country-specific guidelines for biomarker testing of advanced or recurrent NSCLC [49–59].

ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; KRAS, Kirsten rat sarcoma viral oncogene homolog; MET, hepatocyte growth factor receptor; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; NRG1, neuregulin-1; NTRK, neurotrophic tyrosine receptor kinase; O, optional; P, preferred; PD-L1, programmed cell death ligand 1; RET, rearranged during transfection; ROS1, ROS proto-oncogene 1; TMB, tumour mutational burden.

<sup>a</sup>Consider other molecular tests, depending on clinic or drug availability.

<sup>b</sup>NTRK, KRAS, MET, RET and ERBB2/HER2 will be included in the current revision.

<sup>c</sup>The use of these biomarkers as individual tests is currently not indicated; instead, it is advised to include in extended panels performed either initially in all advanced NSCLCs or when previous EGFR/ALK/ROS1/BRAF testing is negative.

<sup>d</sup>Liquid biopsy testing recommended if the patient cannot undergo biopsy or if tissue molecular analysis results were uninformative.

<sup>e</sup>Liquid biopsy for EGFR assessment only when tissue biopsy is not available.

<sup>f</sup>On-demand testing for cases not fulfilling the reflex criteria (e.g. for squamous carcinomas with some suggestive clinical features – young age, non-smokers, etc.).

impede the use of reflex testing and favouring on-demand testing [69, 70]. A further layer of complexity is provided by country differences in reimbursement for liquid biopsy versus tissue testing strategies. Furthermore (e.g. in parts of the UK's National Health Service), testing for molecular targets that are part of clinical trials is not supported.

Low-frequency biomarkers such as *NTRK* fusions pose particular economic challenges. A conservative testing approach (e.g. screening by immunohistochemistry followed by sequencing of positive cases) may be cost-effective [20]. However, given the often limited quantity of tumour tissue for patients with advanced-stage NSCLC, NGS panel multi-gene testing may prove valuable [71]. Indeed, for testing more than four targets in parallel, NGS is usually more cost-effective than single-gene testing. Furthermore, this approach may facilitate an increase in life-years gained for patients with advanced-stage NSCLC [72]. A model in the USA compared the value of an NGS panel (*EGFR*, *ALK*, *ROS1*, *BRAF*, *RET*, *MET*, and *NTRK*) with single-gene testing (*EGFR* and *ALK*) in NSCLC; a 10 % increase in NGS use over single-gene testing resulted in an additional 2630 life-years gained, with a saving of \$49–109 per life-year gained.

#### 4.3. Practical challenges (turnaround time, test complexity, reflex testing)

Turnaround time (TAT) can be an important barrier to molecular testing: oncologists/pulmonologists and patients may be reluctant to delay treatment initiation, given the potential risk for clinical deterioration [73,74]. To minimise TAT, molecular testing should ideally be carried out in the same centre where the patient was pathologically diagnosed, and using standard operating procedures (SOPs). However, on-site testing is dependent on local and regional efficiencies and is only feasible where adequate patient throughput means that appropriate equipment/expertise are available (including training on interpretation of test results). The complexity of test technologies may influence local availability. ASCO/CAP/IASLC/AMP guidelines recommend a TAT of 10 working days between sample receipt and reporting of molecular test results [28]; a recent European Expert Group suggests a general timeframe of 5 working days for molecular test results [75]. Delays in TAT can lead to less efficient use of targeted therapies; for example, initiating immunotherapy before mutation test results are received can mean *EGFR* tyrosine kinase inhibitors are used as second-line versus first-line agents [76,77].

Reflex testing, where molecular testing is ordered by pathologists immediately after histological diagnosis of advanced non-squamous NSCLC, can reduce the time to treatment initiation versus on-demand testing, where tests are ordered by treating physicians. Reflex testing is particularly relevant for biomarkers essential for immediate decision making in an aggressive disease like NSCLC [78]. Reflex testing may facilitate the optimal use of tissue (and save tissue if all relevant biomarkers are analysed simultaneously); it can also increase testing rates over on-demand testing [79]. However, testing algorithms need to reflect the complexities of clinical management, and pathologists may not have access to sufficient clinical information to guide their evaluation. CAP/IASLC/AMP recommend that pathologist-directed reflex testing is reasonable if the testing programme includes open communication between pathologists and the oncology team [28]. Reflex testing could increase costs if used inappropriately, and may be constrained by reimbursement considerations (i.e. requiring oncologist recommendation). Reflex testing with NGS covers a broader set of biomarkers, but potentially longer TAT, and it is currently difficult to achieve 10 working days even in ideal circumstances. Another solution is a two-stage approach: polymerase chain reaction for a rapid answer on key mutations for single genes, followed by a broader NGS panel. This approach could, however, underutilise tissue and monetary resources.

#### 4.4. Decentralised versus centralised biomarker testing

In the absence of local facilities, many pathologists in Europe

outsource testing to independent laboratories, or to regional specialist centres within the public health care system. Centralised testing offers efficiency (pooling of resources) and standardisation via SOPs (which may be less well developed in local laboratories). In contrast, centralised biomarker testing logistics (e.g. sample transfer) can negatively impact timelines. Moreover, pre-analytical conditions, which can vary from one laboratory to another, may influence centralised testing. While central laboratories generally meet TAT recommendations from receipt of sample, there can be delays between request and delivery of the sample. Additionally, the requirement for a minimum number of samples in batch testing may cause delays at local laboratories that receive few samples.

The use of centralised molecular testing laboratories varies greatly across Europe. For example, some countries such as Italy still predominantly perform in-house biomarker testing. Other countries such as the Czech Republic utilise a mixed approach, whereby a national network of 10 larger laboratories has been established to perform molecular testing; thus, small departments are outsourcing and larger laboratories are testing in-house. Finally, some countries, such as Germany, Sweden, the Netherlands and England, have set up centralised national networks for biomarker testing as part of an effort to increase testing rates. With the increasing use of NGS, it is expected that molecular testing will become more centralised in the future to facilitate the high throughput analysis of material and management of data. Overall, centralised high throughput analysis may also improve the quality of testing and reduce costs through improved efficiency.

#### 4.5. Tissue biopsy constraints

Historically, a key barrier to molecular testing in NSCLC was the amount of tumour tissue in biopsy samples. Improvements in tissue collection and management mean that the availability of biopsy tissue is less often a limiting factor. Proactive management of small biopsies may maximise molecular testing [80]. Initial NGS approaches required greater tissue input, but improving technology and the large number of targets for molecular testing have moved the balance in favour of NGS rather than standalone tests [80,81].

For optimal molecular testing, sampling regimens and biopsies (tissue or liquid) would ideally account for any clonal evolution leading to intra-tumour heterogeneity (genomic/biological variations within a tumour) and inter-tumour heterogeneity (genomic/biological variation in multiple small primary tumours or multiple metastatic nodules from the same primary tumour) [82], although this has not yet been achieved. Together, intra- and inter-tumour heterogeneity are key factors contributing to therapeutic failure and drug resistance; therapeutic strategies for targeting resistance mechanisms will be important for improving clinical outcomes in the future [82–84]. Serial sampling of tumour genomes from liquid biopsies is increasingly used to monitor clonal evolution, and may help identify targetable mutations arising in the tumour to guide second-line therapy [85].

#### 4.6. Technical considerations

Choice of test can be influenced by diagnostic sensitivities, run time, differences in performance of an assay from different suppliers (e.g. sensitivities, reference ranges and cut-offs), and preference for commercial kits versus laboratory-developed tests [86,87]. Table 2 summarises available molecular testing technologies in terms of their detection capabilities, sensitivity and TAT [14,88]. Further considerations are that tumour cell enrichment for DNA extraction is necessary in samples where their proportion is below the recommended threshold [80]. Cut-off/scoring systems can also vary between technologies, particularly for emerging biomarkers without commercial tests [28].

**Table 2**

Characteristics of common assays for biomarker testing.

Molecular technology	Variant types				Sensitivity (%)	Turnaround time
	Point mutations	Small deletions, insertions	Copy number alterations	Rearrangements		
Sizing assays	+/-	✓				2–3 days
PCR and Sanger sequencing	✓	✓			20–50	3–4 days
PCR and pyrosequencing	✓	+/-			20–50	3–4 days
PCR and mass spectrometry	✓	+/-			1–10	3–4 days
PCR and single-base extension	✓				1–10	3–4 days
qPCR and digital PCR	✓	✓		✓	0.00001	2–3 days
Allele-specific PCR	✓					1–2 days
FISH			+/-	✓	<1	2–3 days
NGS: targeted amplicon capture	✓	✓			1–10	7–10 days
NGS: targeted hybridisation capture	✓	✓	✓	+/-1	1–5	15–20 days
NGS: whole exome	✓	✓	✓	+/-1	Variable	Weeks
NGS: whole genome	✓	✓	✓	✓	Variable	Weeks

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FISH, fluorescence *in situ* hybridisation; NGS, next-generation sequencing; PCR, polymerase chain reaction; qPCR, quantitative PCR.

Note: FISH cannot assess complex copy number alterations because of low multiplexing capabilities [88].

#### 4.7. Reporting and interpretation

Accurate reporting of biomarker test results is crucial; it should be completed promptly and allow easy retrieval of biomarker status for future reference. The European Committee for Standardization has set out key reporting criteria for medical laboratories, to reduce lack of clarity (ISO 15189) [89]; these are summarised in Table 3 [89], along with some additional considerations for reporting results. Guidance on the format of typical clinical reports has previously been described [90, 91].

As the use of NGS increases, oncologists/pulmonologists need appropriate training to interpret results for clinical care. A recent survey

suggested that oncologists were most confident in using single-gene tests and least confident in using whole-genome or whole-exome sequencing to guide patient care. In adjusted models, training in genomics predicted higher confidence with the tests [92]. Developing a real-world knowledge database could help to address some interpretation issues among clinicians.

#### 4.8. Classification of molecular alterations

To facilitate implementation of precision medicine through introduction of new biomarkers into clinical practice, the reporting and interpretation of genomics data must be standardised. Several groups

**Table 3**

Reporting criteria for medical laboratories, adapted from ISO 15189 and additional considerations for biomarker testing.

Minimum ISO 15189 criteria [89]		Additional considerations for biomarker testing
General	<ul style="list-style-type: none"> <li>Results should be reported accurately, clearly, unambiguously and in accordance with specific procedural instructions</li> <li>The laboratory should define the format and medium of the report and the manner in which it is to be communicated</li> <li>The laboratory should have a procedure to ensure the correctness of transcription of laboratory results</li> <li>The laboratory should have a process for notifying the requester when an examination is delayed</li> </ul>	<ul style="list-style-type: none"> <li>Molecular test data should be reported in the context of the histo/cytopathology findings so that clinical relevance is assured</li> <li>Provide the report within 5–10 working days</li> <li>A tabulated format is recommended for multiplexed analyses of NGS results</li> <li>Test results should be discussed with the MTB</li> </ul>
Report attributes	<ul style="list-style-type: none"> <li>Comment on sample quality that might compromise examination results</li> <li>Comment on sample suitability with respect to acceptance/rejection criteria</li> <li>Contain critical results</li> <li>Interpret comments on results</li> </ul>	<ul style="list-style-type: none"> <li>Include a statement around the probability of the cancer responding to (or resisting) a specific target therapy<sup>a</sup></li> </ul>
Report content	<p>The report should include:</p> <ul style="list-style-type: none"> <li>A clear, unambiguous identification of the examination including, where appropriate, the examination procedure</li> <li>Identification of the laboratory that issued the report</li> <li>Identification of all examinations that have been performed by a referral laboratory</li> <li>Type of primary sample and date of collection</li> <li>Measurement procedure<sup>b</sup></li> <li>Examination results reported in SI units, units traceable to SI units, or other applicable units</li> <li>Biological reference intervals, clinical decision values, or diagrams/nomograms supporting clinical decision values<sup>b</sup></li> <li>Interpretation of results, where appropriate</li> <li>Identification of examinations undertaken as part of a research or development programme</li> </ul>	<p>The report should include:</p> <ul style="list-style-type: none"> <li>A description of the material used for analysis including pre-analytical parameters such as cold ischaemia time, fixative and fixation time, tumour cell enrichment method and final content of tumour cells and/or amount of DNA</li> <li>The analytical technology used, details of tests used, known limitations of tests and corresponding positive/negative predictive values if published</li> </ul>

NGS, next-generation sequencing; MTB, molecular tumour board.

<sup>a</sup> Where applicable; countries may vary with respect to treatment guidance.

<sup>b</sup> Where applicable.

have proposed classification schemes that assign clinical utility to the molecular alterations used for selecting targeted therapies [93–98]. The ESMO Scale of Clinical Actionability of molecular Targets (ESCAT) provides evidence-based criteria to prioritise markers and to select patients for targeted therapies [98]. ESCAT defines six levels of clinical evidence for targets in relation to their implications for patient management, ranging from tier I (ready for implementation in routine clinical decisions) to tier X (lack of evidence for actionability). The AMP/ASCO/CAP guidelines provide evidence-based categorisation of somatic variants into four tiers based on their clinical significance in cancer diagnosis, prognosis and/or therapeutics [97]. The Precision Oncology Knowledge Base (OncoKB) defines four levels of evidence that support the use of a drug in an indication harbouring that mutation [96]. Finally, the MURIEL database from the German consortium das nationale Netzwerk Genomische Medizin is continuously updated to give harmonised clinical recommendations on actionability of genomic alterations.

Although frameworks for classification of biomarkers are theoretically desirable, limitations exist. Substantial emerging data, especially from NGS assays, show that scores can change quickly, and it is often difficult to assess the relevance of novel findings. Additionally, the evidence base for emerging biomarkers may vary across indications. For example, *KRAS* (and *NRAS*) mutations are potentially prognostic in NSCLC, with *KRAS*<sup>G12C</sup> inhibitors showing promise and currently undergoing investigation in clinical trials in patients with *KRAS*<sup>G12C</sup>-mutated tumours [99,100]. On the other hand, in metastatic colorectal cancer, *KRAS* mutations are already established negative predictors for anti-EGFR antibodies [101]. Therefore, each biomarker cannot be assigned one individual score and must be evaluated in the context of cancer type. Finally, with the extensive adoption of sequencing, clinicians will have to interpret oncogenic driver mutations found in the primary clone and subclonal mutations that may arise as a resistance mechanism. The interpretation of the clinical relevance of subclonal mutations may be challenging, but could be important in guiding choice of second-line therapies. In practice, ESCAT may be more relevant to a molecular tumour board (MTB) setting, or to support policymakers in reimbursement decisions. At a more prosaic level, many molecular laboratories will sometimes confirm NGS findings by alternate, orthogonal testing, as recommended in CAP/IASLC/AMP guidelines [28], and there is an emerging practice of biologically validating fusion gene findings using immunohistochemistry [6,20].

## 5. Best practice for biomarker testing in NSCLC

### 5.1. Multidisciplinary management

Tumour boards are important for optimal diagnosis and treatment of patients [6]. A multidisciplinary approach can provide more complete staging and better adherence to guidelines, resulting in improved patient survival [102]. For example, access to a MTB (distinct from the multidisciplinary team) comprising clinicians, molecular pathologists, clinical molecular biologists, geneticists and bioinformaticians can improve the application of genetics-guided cancer care [103,104]. MTBs were shown to influence providers' initial management plans in 40 % of lung cancer cases [105]. Multidisciplinary management facilitates reflex testing [75]. However, this approach may be limited to centres with in-house laboratories; implementation may be more challenging if testing is outsourced.

### 5.2. Tissue and liquid biopsy considerations

A multidisciplinary approach is vital to obtain an appropriate diagnostic sample, as tissue can be acquired through a multitude of procedures involving different healthcare professionals [106]. Tumour samples are required at baseline (diagnosis/evaluation of predictive markers) and often at the time of disease progression (to identify

mechanisms of resistance to targeted therapies). Samples from the primary tumour or an accessible metastatic site are usually sufficient for diagnosis. When choosing the appropriate site, the safest and most accessible site for the patient should be balanced against getting the largest tumour yield. European Expert Group recommendations state that at least five endobronchial/transbronchial forceps biopsies should be obtained and an additional five forceps biopsies or two cryo-biopsies could be considered; at least four endobronchial ultrasound/endoscopic needle aspiration passes per target needle are recommended; and at least two percutaneous core needle biopsies (18- to 20-gauge needle) or three to six core needle biopsies [75]. However, to ensure sufficient cancer cells for testing, such numbers are best regarded as a minimum. Rapid on-site evaluation (ROSE) is a useful approach allowing rapid assessment of the suitability of material obtained by tissue biopsy [107]. The use of ROSE has several advantages (see Fig. 3) and it should therefore be considered as part of the NSCLC biomarker workflow.

Transthoracic fine-needle aspiration (FNA) under imaging guidance may be a reliable alternative to core needle biopsy [108] in the case of mid-to-peripheral lesions [6]. Cytological specimens may be easier to collect, cause less patient discomfort, and are routinely used in clinical practice when tissue is unavailable. Cytological diagnosis of NSCLC is usually based on endobronchial ultrasound-guided FNA, bronchial cytology, pleural effusions and FNA from distant metastases [75]. Sample formats for molecular testing include previously stained air-dried or alcohol-fixed smears, cell blocks and liquid-based samples, provided the quality and percentage of tumour cells is adequate [109]. Cell blocks provide the greatest flexibility; existing laboratory tissue SOPs will usually apply. Finally, the German S3 guidelines recommend re-biopsies and to have liquid diagnostics available for testing.

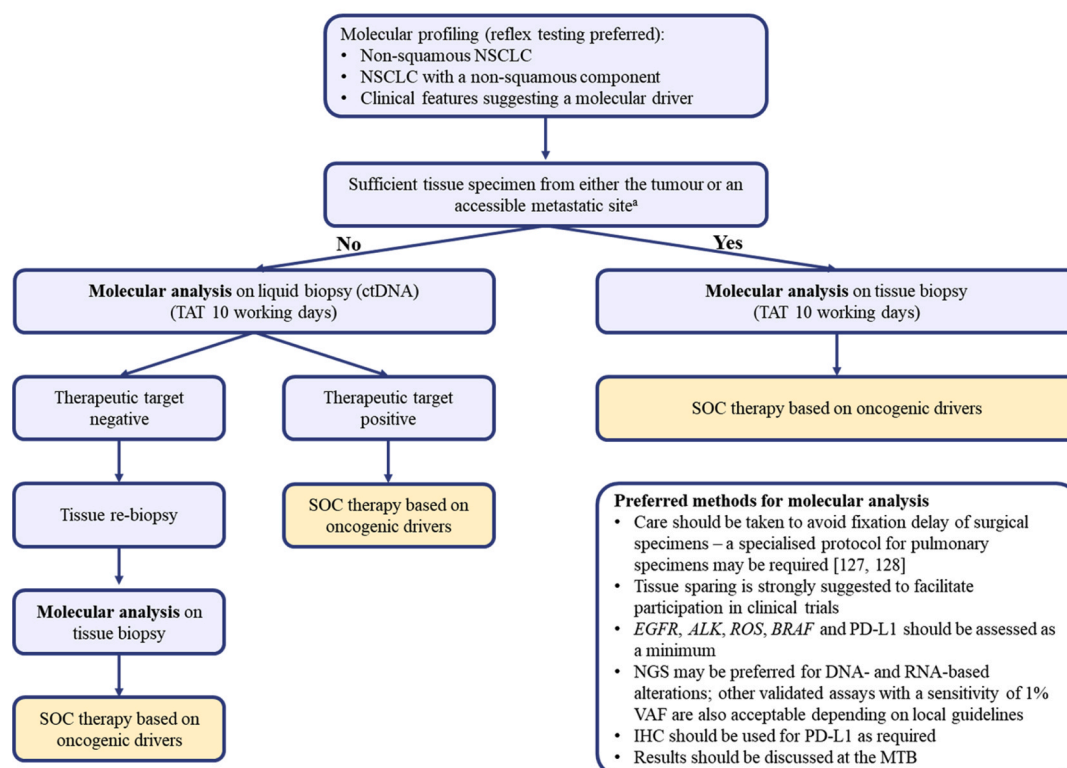
Where the amount of tissue available might prohibit molecular testing, pathologists can maximise its use [106]. Morphological analysis can be sufficient to define histology, but if immunohistochemistry is needed for subtyping, most tumours can be classified using a single adenocarcinoma marker (e.g. TTF-1) and a single squamous marker (e.g. p40) [110,111]. Cell-block preparation of cytology samples is recommended, to retain tissue architecture and provide multiple sections of varying thickness for various analyses including morphology and DNA/RNA testing [106]. Pathologists should mark the most suitable area on the slide to optimise extraction of tumour content, ideally on the blank sections taken for extraction. Microdissection, ideally within a molecularly sterile environment, can be used to achieve a tumour/non-tumour cell ratio above the required threshold for the test. This 'tumour enrichment' is necessary for direct sequencing and NGS [106]. With reflex testing, storing sections at the moment of diagnostic cutting avoids the need to cut new sections from the tissue block for additional testing. Only reflex testing conducted in a molecularly sterile environment, however, should be used for later DNA/RNA extraction and analysis, due to contamination risks. Cytological material (e.g. smears) for isolating DNA can also be used as another approach to maximise tissue [80].

Tissue biopsy remains the 'gold standard' for biomarker testing in NSCLC [75]. Though liquid biopsy can be useful if there is insufficient tumour tissue at diagnosis, services should not accept poor biopsy sampling/handling procedures as a reason to rely on blood testing.

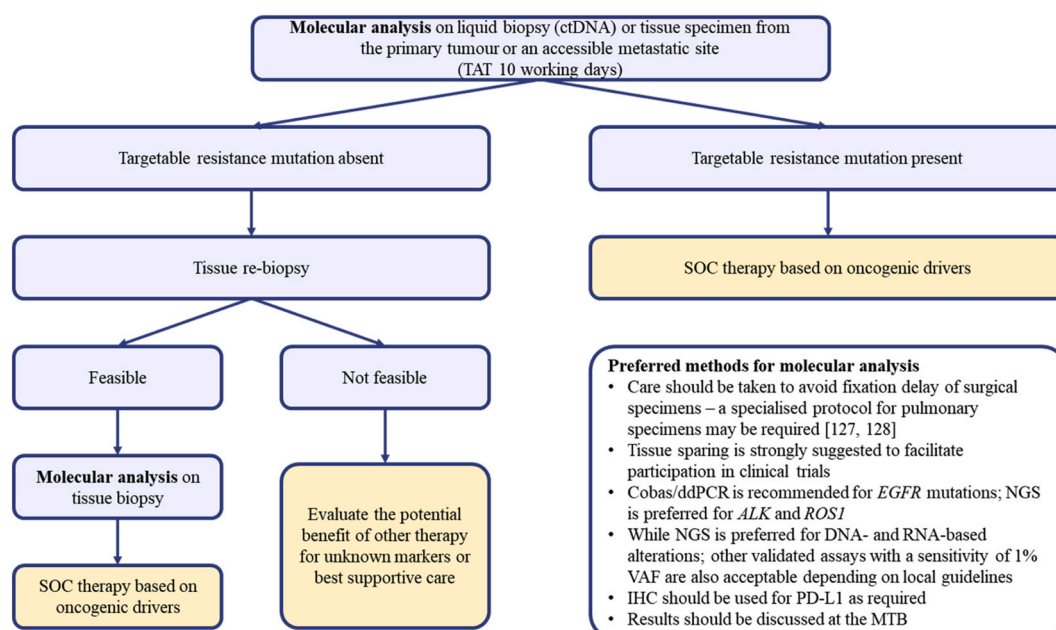
Liquid biopsy may be advantageous if there is a contraindication for biopsy (e.g. bleeding risk), or if re-biopsy is not possible during first-line treatment and there is a need to test for a biomarker relevant to second-line treatment (e.g. *EGFR*<sup>T790M</sup> testing upon progression with *EGFR* tyrosine kinase inhibitors) [112]. Additionally, with the disruption to routine clinical practice resulting from the severe acute respiratory syndrome coronavirus 2 pandemic, liquid biopsies could minimise the requirement for invasive tissue-biopsy procedures. The IASLC recommend the use of plasma over serum for DNA (ctDNA) extraction, a maximum time from blood withdrawal to plasma extraction of 2 h (EDTA tubes) or 3 days (preservative tubes), and that blood should never be frozen before plasma extraction [112]. The volume of blood required



A



B



**Fig. 3.** Best practice recommendations for the treatment of patients with (A) advanced treatment-naïve NSCLC and (B) progressive or recurrent NSCLC during treatment with a tyrosine kinase inhibitor.

<sup>a</sup>Consider the use of ROSE to rapidly assess the suitability of material obtained by tissue biopsy. ROSE may help to improve diagnostic yield, reduce the need for additional procedures, obtain additional passes for molecular testing (if needed) and allow optimal use of laboratory resources [107]. ALK, anaplastic lymphoma kinase; BRAF, B-Raf proto-oncogene; ctDNA, circulating tumour cell DNA; ddPCR, digital droplet PCR; EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; KRAS, Kirsten rat sarcoma viral oncogene homolog; MTB, molecular tumour board; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PD-L1, programmed cell death ligand 1; ROS1, ROS proto-oncogene 1; ROSE, rapid on-site evaluation; SOC, standard of care; TAT, turnaround time; VAF, variant allele frequency.

depends on institutional SOPs and the size of panel to be tested, although many European laboratories request two standard 10-mL tubes [112]. Targeted assays currently approved in Europe for liquid biopsies are limited to *EGFR* mutation testing with the cobas *EGFR* Mutation Test v2 and Therascreen [113]. New NGS-based companion diagnostics are on the horizon in Europe (see Section 6.3), with Guardant360<sup>®</sup> CDx and FoundationOne<sup>®</sup> Liquid CDx already approved by the US Food and Drug Administration (FDA) in 2020 [41,114].

### 5.3. Next-generation sequencing

The adoption of NGS into routine practice should facilitate comprehensive characterisation of current and emerging targetable genomic alterations from available volumes of tumour tissue [106,115]. NGS can sequence a whole genome or exome, transcriptomic RNA, or panels of a few to several hundred regions of exons or, to a lesser extent, introns [112], from tumour tissue and from ctDNA (liquid biopsy). NGS does not address biomarkers that require measurement of protein expression (e.g. PD-L1). Single molecular tests or low multiplex assays are typically faster to perform than NGS (thus shortening overall TAT) and are individually less expensive; however, technological advances have reduced NGS run time [116], and it may offer overall cost savings [72]. Given the high number of currently actionable driver mutations with approved treatments in Europe (*EGFR*, *ALK*, *ROS1*, *NTRK*, *BRAF*), and with others on the horizon (*KRAS*, *MET*, *RET*, *ERBB2/HER2*, *NRG1*, *FGFR1*), it seems that an expanded NGS testing panel at diagnosis (rather than 8–12 different tests) could be the most efficient way of identifying optimal therapeutic approaches and thus improving outcomes for patients while avoiding unnecessary re-biopsies. As noted previously, the use of NGS in conjunction with existing testing methodology is still evolving. Overall, limitations to the use of NGS across Europe may include run-time (which is improving), lack of reimbursement of treatments and testing in some countries (which may reflect the economic situation in each individual country), validation of results, and the interpretation and management of large datasets.

### 5.4. External quality assessment programmes

Acceptable quality control and internal validation procedures must be established and laboratories should participate in external quality assessment (EQA) programmes [6]. EQA is important for achieving accuracy and standardisation across laboratories [75]. Furthermore, adequate performance in EQA schemes is important for comparing global predictive studies of biomarkers [75]. Guidelines for EQA schemes are available [117].

Implementation of EQA has been shown to be clinically beneficial and improve reporting [118,119]. In Europe, several EQA programmes relevant to NSCLC are established, most notably The European Molecular Genetics Quality Network (EMQN) [119–125], and UK NEQAS immunocytochemistry (ICC) and *in situ* hybridisation (ISH) [126].

### 5.5. Summary of best practice recommendations

Based on expert opinion consensus, current best practice recommendations for the diagnosis and management of treatment-naïve advanced NSCLC and progressive/recurrent treated NSCLC are summarised in Fig. 3 [112,127,128]. Best practices require the application of current scientific knowledge to clinical practice in the context of available resources. Therefore, the optimal selection of biomarkers is likely to vary according to country-specific availabilities of the tests and corresponding targeted therapies.

## 6. Future developments in biomarker testing for NSCLC in Europe

### 6.1. New and emerging targeted therapies for NSCLC

Selpercatinib and pralsetinib were FDA approved for *RET* fusion-positive NSCLC in May and September 2020, respectively [40,41]; both drugs are under review in Europe. Tepotinib and capmatinib were approved in 2020 in Japan for NSCLC with *MET*14 skipping alterations [38,129]. In May 2020, the FDA granted accelerated approval to capmatinib for NSCLC with *MET*14 skipping alterations [130].

Historically, *KRAS*—the most frequently mutated oncogene in human cancer [131], and a key regulator of cellular proliferation and differentiation [132]—has proven difficult to target. The majority of *KRAS* mutations in lung adenocarcinoma are thought to be clonal oncogenic drivers arising early in tumour evolution, and while sub-clonal *KRAS* mutations can occur, these events may be fairly rare [133–135]. *KRAS* mutations rarely overlap with other actionable oncogenic driver mutations (e.g. *EGFR*, *ALK*, *ROS-1*), so patients with *KRAS*-mutant NSCLC are unlikely to benefit from therapies targeted to these mutations [11,136]. The majority of patients with *KRAS*-mutant NSCLC are current or former smokers; however, as approximately 5–10 % of patients are never or light smokers, all patients should be tested for *KRAS* mutations regardless of smoking history [137]. In the absence of *KRAS*-specific agents, patients with *KRAS*-mutant solid tumours have limited treatment options. Up to one-third of NSCLC adenocarcinoma cases in Europe have *KRAS* mutations [138,139]. *KRAS*<sup>G12C</sup> is the most common, comprising ~40–46 % of *KRAS* mutations and ~13 % of all NSCLC adenocarcinoma cases [27,140–144]. However, the independent prognostic impact of *KRAS* mutation status has been difficult to assess as it is confounded by association with smoking and concurrent smoking-related co-mutations (e.g. *TP53/STK11*) [145].

Advances in understanding the structure of *KRAS* have led to the development of sotorasib (AMG 510), a small molecule that covalently and irreversibly binds to the cysteine amino acid of *KRAS*<sup>G12C</sup>-mutant protein, locking it in its inactive state and preventing *KRAS*-dependent oncogenic signalling without affecting wild-type *KRAS* signalling (thus inhibiting cancer cell growth and survival) [146–148]. Sotorasib demonstrated anti-tumour activity in patients with NSCLC in the Phase 1 dose-escalation part [100] and registrational Phase 2 part [149] of the CodeBreaK 100 study; Phase 2 and 3 clinical studies are ongoing (NCT03600883, NCT04303780) in patients with locally advanced and unresectable/metastatic NSCLC. Another agent targeting *KRAS*<sup>G12C</sup> (adagrasib; MRTX 849) has demonstrated objective responses in patients with NSCLC in an ongoing Phase 1/2 trial [36,99]. Other direct inhibitors of *KRAS*<sup>G12C</sup> are in earlier-stage clinical trials (e.g. GDC-6036 [150]) or preclinical testing. Several other targeted therapies are under clinical evaluation for NSCLC, and pan-RAS/SOS mutation inhibitors are also in the very early stages of clinical development [151] (Table 1). Overall, the published Phase 1 data for sotorasib and adagrasib are encouraging and suggest that long-awaited targeted therapies for patients with *KRAS*-mutant NSCLC may be on the horizon. The data support the testing of *KRAS*<sup>G12C</sup> in a broad molecular panel before first-line therapy to identify patients that may benefit from emerging *KRAS*-targeted therapies.

### 6.2. Technological developments

The plethora of targeted NSCLC therapies currently under evaluation emphasises the need to continually validate and revise molecular testing strategies. An increase in the number of actionable targets confers a cost-

effectiveness benefit favouring NGS [72]. The anticipated increase in NGS in NSCLC will permit concomitant testing of multiple actionable targets while minimising additional tissue requirements. Ultimately, uptake of NGS is likely to depend on the availability of appropriate technologies (e.g. combined RNA/DNA testing capabilities and fully automated platforms), as well as reimbursement and organisational considerations [152]. Equally, an increased use of liquid biopsies is anticipated. The IASLC recently concluded that ‘liquid biopsy approaches have significant potential to improve patient care, and immediate implementation in the clinic is justified in a number of therapeutic settings relevant to NSCLC’ [112]. However, given its significantly lower overall test sensitivity [112], liquid biopsy is unlikely to replace molecular testing of tissue in the near future. It is more likely that liquid biopsy will complement tissue assessment in a combined approach, mitigating the limitations associated with either type of testing material. It remains to be seen how such an approach will be best implemented.

### 6.3. Legislative changes in Europe

Preference in Europe will likely remain for accredited laboratory-developed tests over commercial kits, although this depends on how the new European Regulation for IVD Medical Devices (2017/746) [86, 153] is implemented over the coming years. If implemented in its present form, this would favour the use of commercial tests as manufacturers will need to perform clinical performance analyses and demonstrate safety and performance according to the risk class of the test. Although a step towards standardisation, this is not without problems: currently, laboratories providing predictive testing must be accredited, or at least have an implemented quality management system (including internal and external quality assurance). On the other hand, higher-priced CE-IVD kits, as well as a need to upgrade reagents and existing platforms, could increase costs and limit access to testing in countries with limited resources. Finally, for diagnostic testing companies, the effort required to get CE-IVD accreditation for each iteration of a test may discourage development of new or improved tests.

## 7. Conclusions

All patients with unresectable NSCLC require fast-track screening of biomarkers (results within 5–10 days) for selection of first-line targeted therapy, immunotherapy, or immunotherapy/chemotherapy combinations. In Europe, national clinical guidelines for molecular testing and targeted therapies reflect ESMO/NCCN guidelines and are tailored for compatibility with national healthcare models and resources. Molecular testing for *EGFR*, *ALK* and PD-L1 is widespread across Europe, with some countries additionally testing for *ROS1*, *BRAF* and *NTRK*. Currently, testing of *KRAS*, *MEK*, *MET*, *ERBB2/HER2*, *RET*, *FGFR1/2/3* and *NRG1* is generally limited to academic institutions or clinical trial settings. Country-specific differences concerning the availability of molecular tests and corresponding targeted therapies partly reflect reimbursement status and/or insurance coverage at the national or regional level.

To improve the application of genetics-guided lung cancer care, ESMO guidelines recommend the use of MTBs involving clinicians, molecular pathologists, molecular biologists, geneticists and bioinformaticians. Where institutional structures permit, reflex testing in patients with advanced disease is desirable to minimise the interval between histological diagnosis and initiation of first-line targeted therapies. Improvements in NGS technology have enhanced the analysis of driver mutations in a group of genes together in one assay; however, the run time for NGS remains relatively long (1–2 weeks), even in optimal settings [154]. Currently, NGS alongside rapid screening technologies for single-driver mutations are being implemented to circumvent this. Thus, further improvements in NGS are needed to reduce run time and to avoid double testing. NGS testing with liquid and tissue biopsies can be considered complementary, with liquid biopsy giving quicker results but

tissue biopsy having a lower false-negative rate. Furthermore, the combined use of tissue and liquid biopsies may permit extensive testing of re-biopsies to provide insights into tumour evolution and heterogeneity during the course of NSCLC. This may identify targetable biomarkers arising and guide subsequent lines of therapy, and also support the discovery of new biomarkers and therapeutic agents; however, as the complexity of testing increases, it is important to ensure that reported results are fully understood by clinicians.

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## Data sharing statement

This article does not include any patient data; all reported data are publicly available in the scientific literature.

## Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

## CRediT authorship contribution statement

**Keith M. Kerr:** Conceptualization, Writing - review & editing. **Frédéric Bibeau:** Conceptualization, Writing - review & editing. **Erik Thunnissen:** Conceptualization, Writing - review & editing. **Johan Botling:** Conceptualization, Writing - review & editing. **Aleš Ryška:** Conceptualization, Writing - review & editing. **Jürgen Wolf:** Conceptualization, Writing - review & editing. **Katarina Öhrling:** Conceptualization, Writing - review & editing. **Peter Burdon:** Conceptualization, Writing - review & editing. **Umberto Malapelle:** Conceptualization, Writing - review & editing. **Reinhard Büttner:** Conceptualization, Writing - review & editing.

## Declaration of Competing Interest

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Katarina Öhrling is an employee of Amgen (Europe) GmbH and is a stockholder in Amgen.

Peter Burdon was an employee of Amgen (Europe) GmbH at the time the research was conducted and is a stockholder in Amgen; he also owns shares in Guardant Health Inc, Mirati Therapeutics Inc, Novartis and Verastem Inc. He is currently an employee of MSD International GmbH.

Umberto Malapelle reports personal fees (for service in a speaker bureau and as an advisor) from Amgen, AstraZeneca, Boehringer



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