Pathology - Research and Practice 248 (2023) 154660

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Improving the turnaround time of molecular profiling for advanced non-small cell lung cancer: Outcome of a new algorithm integrating multiple approaches

G. Treichler [a,d,f], S. Hoeller [a,b,c,d], J.H. Rueschoff [b], M. Rechsteiner [b], C. Britschgi [a,c,d], F. Arnold [b], M. Zoche [b], S. Hiltbrunner [a,d,g], H. Moch [b], D. Akhoundova [a,d,h], I. Opitz [c,e], A. Curioni-Fontecedro [a,c,d,g,i,*]

[a] Department of Medical Oncology and Hematology University Hospital Zurich, Switzerland
[b] Department of Pathology and Molecular Pathology, University Hospital Zurich, 8091 Zurich, Switzerland
[c] University of Zurich, Switzerland
[d] Comprehensive Cancer Center Zurich, Switzerland
[e] Department of Thoracic Surgery, University Hospital Zurich, Switzerland
[f] Department of Medical Oncology and Hematology, Cantonal Hospital Winterthur, Switzerland
[g] Faculty of Science and Medicine, University of Fribourg, Switzerland
[h] Department of Oncology, University Hospital Bern, Switzerland
[i] Department of Oncology, Cantonal Hospital Fribourg, Switzerland

ARTICLE INFO

Keywords:
Non-small cell lung cancer
Diagnostic
Genomic profiling
Personalized treatment

ABSTRACT

Background: Molecular tumor profiling to identify oncogenic drivers and actionable mutations has a profound impact on how lung cancer is treated. Especially in the subgroup of non-small cell lung cancer (NSCLC), molecular testing for certain mutations is crucial in daily clinical practice and is recommended by international guidelines. To date, a standardized approach to identify druggable genetic alterations are lacking. We have developed and implemented a new diagnostic algorithm to harmonize the molecular testing of NSCLC.

Patients and methods: In this retrospective analysis, we reviewed 119 patients diagnosed with NSCLC at the University Hospital Zurich. Tumor samples were analyzed using our standardized diagnostic algorithm: After the histological diagnosis was made, tissue samples were further analyzed by immunohistochemical stainings as well as the real-time PCR test Idylla™. Extracted DNA was further utilized for comprehensive genomic profiling (FoundationOne®CDx, F1CDx).

Results: Out of the 119 patients were included in this study, 100 patients were diagnosed with non-squamous NSCLC (nsqNSCLC) and 19 with squamous NSCLC (sqNSCLC). The samples from the nsqNSCLC patients underwent testing by Idylla™ and were evaluated by immunohistochemistry (IHC). F1CDx analysis was run on 67 samples and 46 potentially actionable genomic alterations were detected. Ten patients received the indicated targeted treatment. The median time to test results was 4 days for the Idylla test, 5 days for IHC and 13 days for the F1CDx.

Abbreviations: ALK, anaplastic lymphoma kinase; ATM, ataxia telangiectasia mutated; BRAF, v-raf murine sarcoma viral oncogene homolog B1; cDNA, complementary DNA; CGP, comprehensive genomic profiling; CNV, copy number variants; ddPCR, digital droplet polymerase chain reaction; EGFR, epidermal growth factor receptor; ERBB2, Erb-B2 Receptor Tyrosine Kinase 2; ERBB3, Erb-B2 Receptor Tyrosine Kinase 3; F1CDx, FoundationOne®CDx; FANCA, Fanconi anaemia complementation group A; FFPE, formalin-fixed, paraffin embedded; FISH, fluoresence in-situ hybridization; ICI, immune checkpoint inhibitors; IHC, immunohistochemistry; KRAS, Kristen rat sarcoma; LC, lung cancer; MTB, molecular tumor board; NDA, new drug application; NGS, next-generation sequencing; NRAS, neuroblastoma RAS viral oncogene homolog; NSCLC, non-small cell lung cancer; NTRK1, neurotrophic tyrosine kinase receptor 1; nsqNSCLC, non-squamous non-small cell lung cancer; OFA, Oncomine™ Focus Assay; PCR, polymerase chain reaction; PD-L1, programmed cell death 1 ligand 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN, Phosphatase and tensin homolog; RET, rearranged during transfection; RT-PCR, real-time polymerase chain reaction; ROS1, ROS Proto-Oncogene 1; SCLC, small cell lung cancer; SNV, single nucleotide variants; sqNSCLC, squamous non-small cell lung cancer; TC, tumor cell; TMB, tumor mutational burden; USZ, University Hospital Zurich.

* Correspondence to: Cantonal Hospital Fribourg Department of Oncology, Chem. des Pensionnats 2/6 1752 Villar-sur-Glane, Fribourg, Switzerland.
E-mail address: alessandra.curioni-fontecedro@h-fr.ch (A. Curioni-Fontecedro).

https://doi.org/10.1016/j.prp.2023.154660
Received 6 April 2023; Received in revised form 29 June 2023; Accepted 30 June 2023
Available online 1 July 2023

Conclusion: In patients with NSCLC, the implementation of a standardized molecular testing algorithm provided information on predictive markers for NSCLC within a few working days. The implementation of broader genomic profiling led to the identification of actionable targets, which would otherwise not have been discovered.

1. Introduction

The use of molecular tumor profiling and rational therapeutic decision-making based on oncogenic "driver" alterations in the tumor genome has revolutionized the way lung cancer (LC) is treated. Molecular testing for single genes or comprehensive genomic profiling (CGP) has become an essential part of the diagnostic work-up and guides daily decision making for therapeutic management. International guidelines integrate up-front molecular profiling to sub-classify non-small cell lung cancer (NSCLC), and therapeutic algorithms are based on the identification of actionable genomic alterations. Due to the broad number of therapeutically targetable gene alterations, genomic analysis of tumor tissue using next-generation sequencing (NGS) is recommended [1,2]. However, algorithms guiding optimal selection and sequence of testing assays in the clinic are still lacking, and molecular testing is performed in a heterogeneous way across centers [3]. Therefore, for optimal patient management, it is crucial to establish clear diagnostic paths with short turnaround time in routine diagnostics [4]. Multiple genomic testing panels, with a more limited to broader sequencing spectrum, can be implemented for tumor profiling. Distinct assays based on real-time PCR (RT-PCR), NGS or digital droplet PCR (ddPCR) are used in the clinical routine. RT-PCR methods amplify DNA or complementary DNA (cDNA) sequences using thermal cycling. This technique can detect the presence of single nucleotide variants (SNVs), fusions, deletions or changes in DNA methylation, in a time-efficient way [5]. However, broad simultaneous genomic testing with this method is not possible, since only limited nucleotide sequences can be screened at the same time. On the contrary, a simultaneous multiplexed testing can be provided with the use of NGS panels. The relevance of NGS analysis has been demonstrated in a study aiming to assess the rate of detection of actionable genomic alterations using a comprehensive NGS assay. In 26% of NSCLC patients previously classified as negative by a non-NGS-based test, actionable genomic alterations could be identified. In an additional 39% of patients, an alteration suitable for inclusion in a clinical trial was uncovered [6]. In NSCLC specifically, the recent emergence of novel targeted treatments for *EGFR* exon 20 insertions makes the identification of these mutations paramount. Whereas direct sequencing techniques such as RT-PCR lead to a high rate of false negative results due to the limited coverage of the assay, NGS-based genetic testing is able to detect low frequency alterations as well as unknown mutations [7–9]. Additionally, less tumor tissue is required for NGS as compared to separate single-gene assays and is cost-effective [10]. On the other hand, despite the recent technical advances, turnaround time of broad sequencing panels is still considerably longer. In certain clinical instances, rapid diagnosis within days to allow early start of therapy is crucial for optimal patient management [11]. In particular, genomic alterations with approved targeted therapeutics in the first line setting need to be identified in rapid time. To address these issues, we have established and evaluated the use of a new algorithm for molecular analysis of tumor specimens from patients with NSCLC. This algorithm was developed with the aim to optimize the use of patients' material, and to improve the turnaround time as well as to permit a homogenous evaluation of all NSCLC patients. Through this algorithm, we also evaluated the impact of comprehensive molecular testing on treatment decisions.

2. Methods

2.1. Patients

We retrospectively collected and systematically reviewed the medical records of 100 consecutive patients with mostly advanced or recurrent non-squamous NSCLC (nsqNSCLC) as well as 19 patients with squamous NSCLC (sqNSCLC), who underwent oncological evaluation with the newly developed diagnostic algorithm at the University Hospital Zurich (USZ) between April 2019 and January 2021. Our study was approved by the local ethics committee (KEZ-ZH-2021–00381) and was conducted in accordance with local laws and regulations. All included patients had signed the approved general informed consent form.

2.2. Diagnostic lung algorithm

As the first step, conventional immunohistochemical (IHC) stainings were performed in order to confirm the diagnosis of NSCLC and to categorize the sample into a histological subtype. After the histological diagnosis was made, the tissue samples were further analyzed through IHC staining, as well as molecular profiling (Fig. 1): For nsqNSCLC, the real-time PCR (RT-PCR) test Idylla™ was used to interrogate for specific hotspots alterations in *EGFR*, *KRAS* and *BRAF*. Simultaneously, IHC for PD-L1, ALK and ROS1 expression was performed. DNA was extracted for further comprehensive genomic profiling using the FoundationOne®CDx (F1CDx) platform. If there was not enough DNA for this test (i.e. <50 ng), the Oncomine™ Focus Assay (OFA) was performed instead. For sqNSCLC, only IHC for determining the PD-L1 status was conducted (Fig. 1).

2.3. Evaluation of ALK, ROS1 and PD-L1 expression by immunohistochemistry

IHC for ALK was performed with the clone 5A4 (Leica, 1:5 dilution) and for ROS1 with the clone D4D6 (Cell signaling, 1:100 dilution), as a screening tool for potential molecular alterations [12,13]. PD-L1 status was assessed with the clone E1L3N (Cell signaling, 1:100 dilution). In cases of dubious interpretation (including cytological cell block specimens), repetition with PD-L1 clone SP263 (Ventana, prediluted, pretreated 20 min) was performed. For ALK and ROS1 any homogeneous cytoplasmic positivity (additionally membranous for ROS1) in the tumor cells is considered positive. In doubtful cases (very low positivity or patchy positivity), a fluorescence *in-situ* hybridization (FISH) analysis with break apart probes (both Abbott Molecular) was added to confirm or exclude *ALK* and *ROS1* translocations. PD-L1 was classified according to the recommended scoring system into tumor cell status (TC) 0–3 and immune cell status (IC) 0–3 subgroups [14,15].

2.4. Idylla™ testing

The automated RT-PCR based Idylla™ test was used to investigate the mutational status of *EGFR*, *KRAS* and *BRAF*. To this end, 10 μm sections of formalin-fixed, paraffin embedded (FFPE) tumor tissue with a tumor cell content of at least 10% were analyzed. Within the Idylla console cell lysis, DNA extraction, allele-specific RT-PCR, data analysis and reporting was automatically performed within 2–3 h per gene [16]. Specifically, the Idylla™ EGFR Mutation Test covers 51 mutations in common hotspots of exons 18, 19, 20 and 21, including T790M. The Idylla™ KRAS Mutation Test covers 21 mutations in exons 2, 3 and 4,

and the Idylla™ NRAS-BRAF Mutation Test covers 5 mutations in the hotspot V600 of *BRAF*.

2.5. Comprehensive Genomic Profiling

Tumor DNA was extracted from FFPE diagnostic tissues and analyzed by the F1CDx assay. The F1CDx assay detects genomic alterations in a panel of 324 genes (all exons). In addition, the tumor mutational burden (TMB) and microsatellite status (stable or instable) are reported. For analysis, the Illumina® HiSeq 2500 platform is used, hybrid capture-selected libraries were sequenced (targeting >500x median coverage with >99% of exons). Sequence data were analyzed by a customized analysis pipeline designed to detect all classes of genomic alterations, including base substitutions, insertions/deletions (indels), genomic rearrangements (e.g. gene fusions), and copy number alterations (CNVs; i.e. gene amplifications and homozygous gene deletions). The threshold used in F1CDx for identifying a copy number amplification is 4 for *ERBB2* and 6 for all other genes (FMI technical information sheet).

The Oncomine™ Focus Assay (OFA) panel interrogates 52 genes for the presence of mutations, small indels, CNVs and gene fusions. Since the latter is performed on RNA and the molecular profiling algorithm presented here is based on DNA only as input material, the fusion part of the OFA panel was not performed. Shortly, DNA was isolated using Maxwell 16 FFPE Plus LEV DNA Purification Kit (Promega, WI, US). The DNA concentration was measured with Qubit and 10 ng were used for NGS library preparation. Emulsion-PCR, enrichment and chip loading was carried out on the Ion Chef with the Ion 510&520&530 or 540 Kit. The S5 platform was used for sequencing with the Ion S5 Sequencing Kit. The protocols from Life Technologies / Thermo Fisher Scientific (MA, US) were followed in all steps. Alignment, variant calling and annotation is performed with the Ion Reporter software 5.14 workflow from Thermo Fisher Scientific (Oncomine Focus w2.6 – DNA - Single Sample; Filter chain: Oncomine 5% CI, CNV ploidy >= gain of 2 over normal).

2.6. Molecular tumor board

After obtaining the results of the F1CDx or the OFA panel testing, the results were presented and discussed at the multidisciplinary molecular tumor board, which takes place at our institution weekly (Step 3 Fig. 1). If actionable genomic alterations were detected, a suitable recommendation based on available data concerning treatment was made.

3. Results

3.1. Patient characteristics

A total of 100 patients with nsqNSCLC underwent molecular profiling following the diagnostic algorithm between April 1, 2019, and January 31, 2021 (Table 1). Thirty-six (36%) patients were female and sixty-four (64%) male, the median age at tumor tissue analysis was 66 years (range 45–81 years). The majority of patients were diagnosed with the histological subtype of adenocarcinoma (83%). The other subtypes were adenosquamous (3%), adenoid cystic (3%), or other (13%) histological types. Eighty-seven patients (87%) were diagnosed with advanced stage nsqNSCLC at the time of analysis. The most frequently biopsied tumor location was the lung (47%), followed by lymph nodes (27%). Forty-three (43%) out of the 87 patients with advanced stage disease received a combination of chemotherapy and immunotherapy with immune checkpoint inhibitors (ICI) as a first-line treatment, reflecting current treatment guidelines [2]. Eight (8%) patients were treated with ICI alone (due to PD-L1 TC3) and eight (8%) patients with a molecularly targeted therapy. Twenty-eight (28%) patients received either chemotherapy alone (4%), chemotherapy with concomitant radiotherapy (2%), surgery alone (3%), radiotherapy alone (5%), best

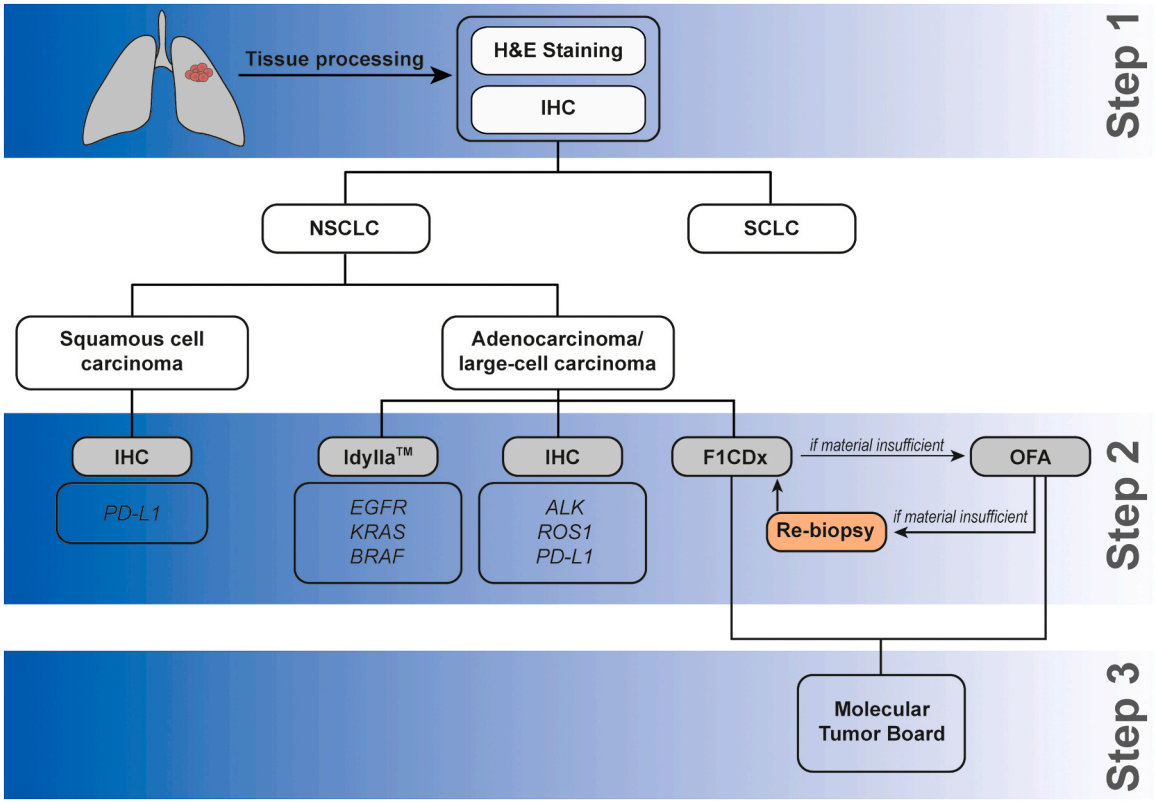

Fig. 1. : Diagnostic algorithm for molecular profiling of NSCLC tumors at the University Hospital Zürich. Abbreviations: ALK: anaplastic lymphoma kinase, EGFR: epidermal growth factor receptor, F1CDx: FoundationOne®CDx, IHC: immunohistochemistry, KRAS: Kirsten rat sarcoma, NSCLC: non-small cell lung cancer, OFA: OncomineTM Focus Assay, PD-L1: programmed cell death 1 ligand 1, ROS1: ROS Proto-Oncogene 1, SCLC: small cell lung cancer.

Table 1
Patient characteristics of the non-squamous NSCLC cohort (n = 100).

Patient characteristics	Number (%)
Gender	
Female/Male	36 (36)/64 (64)
Age	
Median	66
Range	45–81
Histology	
Adenocarcinoma	83 (83)
Adenosquamous	3 (3)
Adenoid cysitic	1 (1)
Other	13 (13)
Stage (at diagnosis)	
I/II/IIIA	9/11/12, total 32 (32)
IIIB/IIIC/IV	5/0/63, total 68 (68)
Stage (at analysis)	
I/II/IIIA	3/5/5, total 13 (13)
IIIB/IIIC/IV	3/0/84, total 87 (87)
Tissue used for analysis	
Lung	47 (47)
Bone	5 (5)
Brain	10 (10)
Lymph node	27 (27)
Pleura	4 (4)
Pleural effusion	3 (3)
Liver	1 (1)
Oesophagus	1 (1)
Soft Tissue	2 (2)
First line treatment for advanced stage	87 (87)
Immunotherapy	8 (8)
Chemo-Immunotherapy	43 (43)
Targeted	8 (8)
Other	28 (28)

supportive care (11%) or were included in a clinical trial (3%).

Following our lung algorithm, 19 patients with a squamous histology were evaluated between April 1, 2019 and February 28, 2020 (Table 2). The median age was 65 years (range 52–85 years), the majority were men (74%) with an advanced stage (79%) (stage IIIB-IV). Most of the tissue samples were obtained from the lung (47%), followed by lymph nodes (27%) and pleura (4%).

Table 2
Patient characteristics in the squamous-cell histology cohort (n = 19).

Patient characteristics	Number (%)
Gender	
Female/Male	5 (26)/16 (74)
Age	
Median	65
Range	52–85
Histology	
Squamous Cell Carcinoma	19 (100)
Stage (at diagnosis)	
I/II/IIIA	0/4/5, total 9 (47)
IIIB/ IV	2/8, total 10 (53)
Stage (at analysis)	
I/II/IIIA	0/2/2, total 4 (21)
IIIB/IIIC/ IV	3/0/12, total 15 (79)
Tissue used for analysis	
Lung	14 (73)
Liver	1 (5)
Lymph node	2 (11)
Pleura	2 (11)
First line treatment advanced stage	15 (79)
Immunotherapy	3 (15.7)
Chemo-Immunotherapy	5 (26.3)
Targeted	-
Other	7 (36.8)

3.2. Molecular profiling with the USZ lung algorithm

All 100 tumors from patients with nsqNSCLC underwent RT-PCR with Idylla™ and were evaluated by IHC. Ninety-nine (99%) were stained by IHC for ALK protein expression. In 97 (97%) samples IHC was performed for ROS1, in 99 (99%) samples for PD-L1 expression. The F1CDx test was run on 67 (67%) patient samples.

Out of the 33 (33%) patients who did not receive testing by F1CDx, 15 (15%) had an oncogenic driver mutation identified through the previously performed Idylla™ or IHC tests. Six (6%) cases (out of these 33) did not have enough DNA for the completion of F1CDx and underwent analysis with the OFA panel. In ten (10%) patients (out of these 33), the test was not performed because of lack of potential therapeutic consequences, e.g. patients refusing active treatment. In five (5%) patients, there was not enough material to conduct either F1CDx or OFA panel testing. In one case (1%), patient's written consent for performing molecular testing was not obtained (Table 3). Concerning the group of sqNSCLC, PD-L1 expression was available in 17 out of 19 (89.5%) cases.

3.3. Concordance between assays

We aimed to evaluate the accordance between the results obtained by the different testing modalities. We could not find any mismatch between the findings of the RT-PCR with Idylla™ and the NGS either performed with the OFA panel or the F1CDx.

3.4. Actionable genomic alterations and their detection method

We were able to detect 46 (46%) potentially targetable genomic alterations with all the diagnostics performed. Among the 100 patients with nsqNSCLC, two (2%) *ALK* rearrangements were identified by IHC. Idylla™ test detected eight (8%) *EGFR* mutations, 11 (11%) *KRAS p.G12C* mutations and 1 (1%) *BRAF* mutation. F1CDx detected the other actionable mutations in *MET, ERBB2, ERBB3, FGFR1, FGFR3, AKT2, NTRK1, ATM, FANCA, PTEN, RET, PIK3CA* in 25 (25%) cases. Ten (10%) patients received targeted treatment matching the genomic alterations detected. Precisely, two patients were diagnosed with *ALK*-rearranged tumors by IHC as well as with OFA or F1CDx, respectively. Both patients with ALK-rearrangements received targeted treatments. Three (3%) tumors with *EGFR* mutations detected by Idylla™ were confirmed with F1CDx; only F1CDx allowed to detect *MET* and *NTRK* alterations in four (4%) patients and 3 out of these 4 received targeted treatments

Table 3
Molecular profiling assays performed in the cohort of non-squamous cell histology.

Assay Total number of samples n = 100	Number of samples analyzed	Time to results median days
IHC		5
ALK	99	
ROS1	97	
PD-L1	99	
TC0	28	
TC1	37	
TC2	15	
TC3	19	
Idylla™	100	4
OFA	10	14
F1CDx	67	13

Abbreviations: ALK: anaplastic lymphoma kinase, F1CDx: FoundationOne®CDx, IHC: Immunohistochemistry, OFA: OncomineTM Focus Assay, PD-L1: Programmed cell death 1 ligand 1, ROS1: ROS Proto-Oncogene 1, TC: Tumor cells

Table 4
Most relevant molecular alterations uncovered by the performed assays in the non-squamous cell histology cohort and targeted treatment received. A (-) means the gene was not found with the respective method.

	IHC	Idylla	OFA	F1	Total	Received targeted treatments
ALK	2	-	1	1	2	2 (100%)
EGFR	-	8	5	3	8	5 (62.5%)
MET	-	-	-	3	3	2 (66.7%)
ERBB2/ HER2	-	-	1	2	2	0 (0%)
ERBB3	-	-	-	1	1	0 (0%)
KRASp. G12C	-	11	2	6	11	0 (0%)
ROS1	-	-	-	-	-	0 (0%)
FGFR1	-	-	-	4	4	0 (0%)
FGFR3	-	-	-	1	1	0 (0%)
AKT2	-	-	-	1	1	0 (0%)
NTRK1	-	-	-	1	1	1 (100%)
ATM	-	-	-	2	2	0 (0%)
FANCA	-	-	-	1	1	0 (0%)
PTEN	-	-	-	4	4	0 (0%)
RET	-	-	-	2	2	0 (0%)
ATR	-	-	-	-	-	
NRAS	-	-	-	-	-	
IDH1	-	-	-	-	-	
BRAF	-	1	-	1	1	0 (0%)
PIK3CA	-	-	-	3	3	0 (0%)
Total	2	20	3	36	46	10

(Table 4). Specifically, one *NTRK1* fusion, one *MET* exon 14 skipping mutation, one rare *MET* mutation (p.D1128N) as well as one *MET* amplification were identified.

3.5. Turnaround time for molecular testing

The median time until availability of results was 4 days for the Idylla™ test and 5 days for the IHC (defined as the time from placing the order of the analysis until completion of report). These two tests were performed in parallel. The running time of the F1CDx test has a median of 13 days (defined as the time from when the patient signature and the FFPE material is available until completion of report). The median duration for testing by OFA panel to the report was to 14 days (defined as the time from placing the order of the analysis until completion of report). The turnaround time for each molecular test is described in Table 3.

3.6. Evaluation of genomic profiling results at molecular tumor board

All the patients assessed with the lung algorithm were discussed at the interdisciplinary tumor board for thoracic malignancies at the University Hospital Zurich. All patients who received further molecular profiling, either by OFA or F1CDx assay, were additionally discussed at our multidisciplinary molecular tumor board (MTB) within a median of 8 days from the results of NGS testing to the day of MTB.

4. Discussion

The presence of alterations in *EGFR, ALK, ROS1, BRAF*, *MET, RET*, *NTRK* and very recently also *KRAS* p.G12C have relevant therapeutic implications for patients with NSCLC [17,18] and need fast identification. Despite a certain consensus about which genes to test, different methods are used which might cause delay in molecular diagnosis. Therefore, we designed a diagnostic algorithm for systematic genotyping of patients with advanced or recurrent NSCLC. This algorithm has the advantage to minimize the interval between the diagnosis and the start of treatment and it guaranteed a homogenous evaluation of all cases. In this work, we illustrate the algorithm and analyze the molecular profiling data generated from 100 patients with advanced nsqNSCLC as well as 19 sqNSCLC patients, treated at our institution between April 2019 and January 2021. The time to results for those alterations, which are fundamental for treatment decision in the first-line setting, were available within 5 working days. Some of the clinically most relevant genomic alterations in our cohort, uncovered only through NGS, (using either F1CDx or OFA assays), were *MET* mutations, *FGFR* mutations/amplifications or *ERBB2/3* amplifications/mutations/deletions. Moreover, the F1CDx allowed unraveling an *NTRK* fusion in one patient. Of note, in 67 NGS cases (F1CDx and OFA), 25 potentially actionable genomic alterations were discovered only through this method, thus highlighting the clinical impact and advantages of larger panels over single gene testing. Three out of these patients received molecularly targeted treatment, which would otherwise not have been possible, namely two patients with *MET* and one with *NTRK1* alterations. Considering the recent FDA and EMA approval for capmatinib, the drug is expected to be broader accessible for the treatment of *MET* exon 14 skipping mutations and *MET* amplifications. Following this development, it might be sensible to include testing for these alterations at an earlier step in the diagnostic algorithm. This might also be evaluated for the identification of *NTRK* fusions, although they are rare in NSCLC (<0.1%). At the time of diagnosis, there were no available targeted treatments for patients with *KRAS* mutations. Currently, through the approval of sotorasib and the acceptance of a new drug application (NDA) for adagrasib by the FDA, the use of our algorithm might be crucial to screen patients who might benefit from these drugs as well in the near future. It also bears mentioning that new guidelines recommend NGS testing not only for nsqNSCLC but also for sqNSCLC as targeted therapies are approved in NSCLC irrespective of histological subtype [19]. This might be implemented in our algorithm in the future.

The department of medical oncology and hematology and the department of pathology and molecular pathology cooperated to develop the diagnostic algorithm. The collaborative effort between the two institutions created a hub for molecular testing with established procedures for analysis of tumor samples. The applicability of the algorithm is broad, as it can be performed on different diagnostic specimens.

The fact that a sufficiently large tissue sample is required to perform NGS at a high success rate can pose a concern in NSCLC. In some patients, obtaining ample biopsy material is challenging due to the location of the tumor or its metastases. In our cohort, F1CDx testing failed in six patients due to insufficient amount of DNA extracted. In five of these six samples, NGS could then be performed using the OFA panel instead.

Here, data based on DNA sequencing has been discussed. However, a combinatorial approach of DNA- and RNA-based assays should be taken into consideration in the future, since gene rearrangements and gene fusions can be often detected with more precision using RNA-based methods. In particular, in patients with driver-gene negative DNA NGS results, a more precise analysis method can lead to better outcomes. For example, the detection of amplifications or novel variants in the *EGFR* gene might allow ameliorating prognosis [20,21]. It would be ideal to evaluate the impact of genomic testing on survival within a randomized trial but this would necessitate the access to the recommended drugs and is unfortunately not feasible worldwide [22].

Importantly, all patients undergoing NGS were systematically discussed at our institution's multidisciplinary MTB, providing actualized evidence-based therapeutic recommendations. We envision that implementing such an approach might be of fundamental use for the treatment of patients with advanced NSCLC as well as for earlier stages. On the one side, most clinical trials adopting immunotherapy in the neoadjuvant setting, exclude patients with actionable alterations of *EGFR* and *ALK*. On the other side, neoadjuvant trials are already ongoing for patients whose tumors harbor such genetic alterations. In the adjuvant context, testing for *EGFR* is expected to become standard as the use of Osimertinib leads to a significant prolongation of disease free survival (DFS) in case of an *EGFR*-mutated tumor and PD-L1 is used to address the indication of Atezolizumab in the adjuvant setting [23,24].

Therefore, our algorithm is of potential use for all patients with NSCLC to permit timely access to the best possible treatment option with a reflex testing.

5. Conclusion

With our work, we show that early and systematic broad molecular profiling of advanced or recurrent NSCLC is feasible and has relevant therapeutic impact in the daily management of these patients.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

G. Treichler: Conceptualization, Formal analysis, Investigation, Writing – review & editing. **S. Hoeller:** Formal analysis, Investigation. **J. H. Rueschoff:** Formal analysis, Investigation. **M. Rechsteiner:** Formal analysis; Investigation; Writing – review & editing. **C. Britschgi:** Methodology. **F. Arnold:** Formal analysis. **M. Zoche:** Formal analysis, Writing – review & editing. **S. Hiltbrunner:** Formal analysis; Investigation, Writing – review & editing. **H. Moch:** Conceptualization, Writing – review & editing. **D. Akhoundova**: Supervision. **I. Opitz:** Methodology. **A. Curioni-Fontecedro:** Conceptualization, Formal analysis, Investigation; Supervision.

Declaration of Competing Interest

No conflict of interest.

References

[1] D.S. Ettinger, D.E. Wood, C. Aggarwal, et al., NCCN guidelines insights: non-small cell lung cancer, version 1.2020, J. Natl. Compr. Canc Netw. 17 (2019) 1464–1472.
[2] D. Planchard, S. Popat, K. Kerr, et al., Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, iv192-iv237, Ann. Oncol. 29 (2018).
[3] K.M. Kerr, F. Bibeau, E. Thunnissen, et al., The evolving landscape of biomarker testing for non-small cell lung cancer in Europe, Lung Cancer 154 (2021) 161–175.
[4] J.C. Thompson, C. Aggarwal, J. Wong, et al., Plasma genotyping at the time of diagnostic tissue biopsy decreases time-to-treatment in patients with advanced NSCLC-results from a prospective pilot study, JTO Clin. Res Rep. 3 (2022), 100301.
[5] D. Chen, T. Xu, S. Wang, et al., Liquid biopsy applications in the clinic, Mol. Diagn. Ther. 24 (2020) 125–132.
[6] A. Drilon, L. Wang, M.E. Arcila, et al., Broad, hybrid capture-based next-generation sequencing identifies actionable genomic alterations in lung adenocarcinomas otherwise negative for such alterations by other genomic testing approaches, Clin. Cancer Res. 21 (2015) 3631–3639.
[7] U. Malapelle, S. Pilotto, M.L. Reale, et al., Epidermal growth factor receptor exon 20 insertion variants in non-small cell lung cancer patients, Crit. Rev. Oncol. Hematol. 169 (2022), 103536.
[8] F. Passiglia, U. Malapelle, N. Normanno, et al., Optimizing diagnosis and treatment of EGFR exon 20 insertions mutant NSCLC, Cancer Treat. Rev. 109 (2022), 102438.
[9] S. Ou, J. Hong, H. Lin, et al., Distribution and detectability of EGFR Exon 20 insertion variants in nonesmall cell lung cancer, J. Thorac. Oncol. 16 (2021) S928–S929.
[10] V. Endris, R. Penzel, A. Warth, et al., Molecular diagnostic profiling of lung cancer specimens with a semiconductor-based massive parallel sequencing approach: feasibility, costs, and performance compared with conventional sequencing, J. Mol. Diagn. 15 (2013) 765–775.
[11] R.E. Smith, M.L. Johnson, L.N. Gordan, et al., Evaluation of outcomes in patients (pts) with stage 4 non-small cell lung cancer (NSCLC 4) harboring actionable oncogenic drivers (AOD) when treated prior to report of mutation without tyrosine kinase inhibitors (TKI): an Integra Connect Database (ICD) retrospective observational study, 1530-1530, J. Clin. Oncol. 40 (2022).
[12] G. Hutarew, C. Hauser-Kronberger, F. Strasser, et al., Immunohistochemistry as a screening tool for ALK rearrangement in NSCLC: evaluation of five different ALK antibody clones and ALK FISH, Histopathology 65 (2014) 398–407.
[13] S. Savic, B. Bode, J. Diebold, et al., Detection of ALK-positive non-small-cell lung cancers on cytological specimens: high accuracy of immunocytochemistry with the 5A4 clone, J. Thorac. Oncol. 8 (2013) 1004–1011.
[14] A. Gagne, E. Wang, N. Bastien, et al., Impact of specimen characteristics on PD-L1 testing in non-small cell lung cancer: validation of the IASLC PD-L1 testing recommendations, J. Thorac. Oncol. 14 (2019) 2062–2070.
[15] H.U. Schildhaus, Predictive value of PD-L1 diagnostics, Pathologe 39 (2018) 498–519.
[16] S.M. Evrard, E. Taranchon-Clermont, I. Rouquette, et al., Multicenter evaluation of the fully automated PCR-Based Idylla EGFR mutation assay on formalin-fixed, paraffin-embedded tissue of human lung cancer, J. Mol. Diagn. 21 (2019) 1010–1024.
[17] D.S. Hong, M.G. Fakih, J.H. Strickler, et al., KRAS(G12C) inhibition with sotorasib in advanced solid tumors, NewEngl. J. Med 383 (2020) 1207–1217.
[18] F. Skoulidis, B.T. Li, G.K. Dy, et al., Sotorasib for lung cancers with KRAS p.G12C mutation, New Engl. J. Med. 384 (2021) 2371–2381.
[19] D. Chakravarty, A. Johnson, J. Sklar, et al., Somatic genomic testing in patients with metastatic or advanced cancer: ASCO provisional clinical opinion, J. Clin. Oncol. 40 (2022) 1231–1258.
[20] R. Benayed, M. Offin, K. Mullaney, et al., High yield of RNA sequencing for targetable kinase fusions in lung adenocarcinomas with no mitogenic driver alteration detected by DNA sequencing and low tumor mutation burden, Clin. Cancer Res. 25 (2019) 4712–4722.
[21] A.L. Volckmar, J. Leichsenring, M. Kirchner, et al., Combined targeted DNA and RNA sequencing of advanced NSCLC in routine molecular diagnostics: analysis of the first 3,000 Heidelberg cases, Int .J. Cancer 145 (2019) 649–661.
[22] V. Prasad, Why the US centers for medicare and medicaid services (CMS) should have required a randomized trial of foundation medicine (F1CDx) before paying for it, Ann. Oncol. 29 (2018) 298–300.
[23] Y.L. Wu, M. Tsuboi, J. He, et al., Osimertinib in Resected EGFR-Mutated Non-small-cell Lung Cancer, New Engl. J. Med 383 (2020) 1711–1723.
[24] E. Felip, N. Altorki, C. Zhou, et al., Adjuvant atezolizumab after adjuvant chemotherapy in resected stage IB-IIIA non-small-cell lung cancer (IMpower010): a randomised, multicentre, open-label, phase 3 trial, Lancet 398 (2021) 1344–1357.