Title: Optimising Fusion Detection Through Sequential DNA and RNA Molecular Profiling of Non-Small Cell Lung Cancer

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Highlights:

There are an increasing number of rare but actionable fusions in NSCLC

Targeting fusion panel testing in those negative by DNA-NGS led to an enriched positivity rate of 24%

Failure rate and tissue inadequacy was higher in sequential RNA-NGS than initial DNA-NGS

Collaborating with industry to develop pathways can expand access to identify actionable events

Abstract

Objectives:

There is an increasing number of driver fusions in NSCLC which are amenable to targeted therapy. Panel testing for fusions is increasingly appropriate but can be costly and requires adequate good quality biopsy material. In light of the typical mutual exclusivity of driver events in NSCLC, the objective of this study was to trial a novel testing pathway, supported by industrial collaboration, in which only patients negative for driver mutations on DNA-NGS were submitted for fusion panel analysis.

Materials and Methods:

Over 18 months, all patients from a single centre with non-squamous NSCLC were submitted for DNA-NGS, plus ALK and ROS1 immunohistochemistry +/- FISH. Those which were negative for a driver mutation were then recalled for RNA panel testing.

Results:

307 samples were referred for DNA-NGS mutation analysis, of which, 10% of cases were unsuitable for or failed DNA-NGS analysis. Driver mutations were detected in 61% (167/275) of all those successfully tested. Of those without a driver mutation and with some remaining tissue available, 28% had insufficient tissue/extracted RNA or failed RNA-NGS. Of those successfully tested, 24% (17/72) had a fusion gene detected involving either *ALK, ROS, MET, RET, FGFR* or *EGFR*. Overall, 66% (184/277) of patients had a driver event detected through the combination of DNA and RNA panels.

Conclusion:

Sequential DNA and RNA based molecular profiling increased the efficacy of detecting fusion driven NSCLCs. Continued optimisation of tissue procurement, handling and the diagnostic pathways for gene fusion analysis is necessary to reduce analysis failure rates and improve detection rate for treatment with the next generation of small molecule inhibitors.

Keywords:

Non-small cell lung cancer

Targeted therapy

Molecular profiling

Fusion analysis

1. Introduction

Lung cancer is the world's leading cause of cancer-related death with the majority being metastatic at presentation. Lung adenocarcinoma, a histological subtype of non-small cell lung cancer (NSCLC) can be characterised by the presence or absence of driver aberrations, such as *EGFR* mutations and *ALK* or *ROS1* translocations. Such cancers occur more frequently in never-smokers and were the first to be targeted with oral tyrosine kinase inhibitors, which dramatically improved outcomes and prolonged survival (1,2).

With advances in DNA and RNA sequencing technologies and studies into the molecular landscape of NSCLC, further aberrations have been recognised including mutations in *BRAF, KRAS* and *MET*, as well as gene translocations involving *RET, NTRK* and *FGFR,* amongst others (3,4). These alterations are increasingly targetable with novel therapeutics and it is therefore paramount to establish efficient diagnostic pathways to identify targets for licensed agents or clinical trials of novel agents that can be offered to patients.

One challenge in delivering any predictive biomarker analysis on lung cancer specimens is the availability of diagnostic material; often only single diagnostic biopsies are available for testing. Tissue must therefore be handled sensitively and used judiciously for both diagnostic and predictive markers, whether slide-based or requiring DNA or RNA extraction. Furthermore, the impact of formalin fixation can hamper sufficient quantity and quality of extracted DNA and RNA for clinical biomarker testing (5).

Testing for actionable fusion drivers in NSCLC has commonly been performed using a combination of immunohistochemistry (IHC) and FISH, particularly where *ALK* and *ROS1* translocations are the only fusions routinely tested for. However, as increasing numbers of driver fusions become actionable in NSCLC, performing single fusion gene tests becomes decreasingly tissue-efficient and cost-effective in comparison to multiplex approaches, such as RNA-sequencing.

Within the UK National Health Service (NHS), the current standard of care (SOC) for NSCLC pathological diagnostics is based on the targeted therapies approved for reimbursement by the National Institute for Health and Care Excellence (NICE). Therefore, SOC testing has included *EGFR* analysis, as well as assessment of *ALK* and *ROS1* translocation status.

Driver events in NSCLC are typically mutually exclusive. However, current testing pathways generally perform fusion gene testing for *ALK* and *ROS1* in parallel to *EGFR* mutation analysis, in order to reduce turnaround time. Where mutation analysis for *EGFR* is performed by targeted DNA next generation sequencing (NGS), routine panels typically include other driver genes, such as *KRAS, BRAF* and *ERBB2*. As previously reported, a targeted DNA-NGS panel approach in our laboratory detected a driver event in 64% of patients (6).

There is potential justification for limiting comprehensive fusion analysis to those patients who do not have a driver mutation detected on a DNA-NGS panel, with this subset likely to be enriched for fusion driver events.

Although some centres continue to rely on single gene testing with PCR-based assays, with the expansion of novel agents targeting aberrations beyond *EGFR*, it is increasingly relevant to identify mutations and gene fusions that may qualify patients for clinical trials or 'free-of-charge' early access

schemes. The recent development of the NHS Genomic Medicine Service aims to ensure availability of broader diagnostic NGS analysis universally across England.

Our laboratory has been utilising NGS analysis as SOC for several years before the establishment of The NHS Genomic laboratory Hubs (GLHs) (6). In parallel, our NHS Trust has developed agreements with a number of companies to support extended molecular profiling, set up as a reflex SOC fusion analysis within our service for patients with non-squamous NSCLC without *EGFR* or *KRAS* activating mutations, to facilitate access to novel agents for patients in our network and support our clinical trials portfolio. Here, we describe the UCLH NSCLC molecular diagnostic pathway (Figure. 1A) prior to the roll out of the centralised service via the NHS GLHs.

2. Material and methods

2.1 UCLH Fusion Testing Pathway

All cases of non-squamous NSCLC diagnosed at UCH over an 18-month period were submitted for DNA-NGS analysis as routine SOC. These analyses utilised ISO 15189 accredited diagnostic assays which were designed to identify single nucleotide variants (SNVs) and small insertions/deletions in 'hotspot' regions genes relevant in common solid tumours. The first 12 months of testing was performed using a 22-gene panel, which was upgraded to a 33-gene panel for the remainder. Details of these panels can be found in supplementary tables 1 and 2. These specifically included *EGFR, KRAS, BRAF, NRAS, ERBB2* as well as other less commonly mutated genes. Due to either poor DNA yield/quality or high clinical urgency, a minority of cases were unable to follow this pathway or were submitted for rapid *EGFR* mutation analysis. All cases were submitted for ALK and ROS1 IHC +/- FISH as standard, in parallel to DNA-NGS, in order to capture *ALK* and *ROS1* status at the time of initial diagnosis.

At roughly 6-weekly intervals all DNA-NGS 'driver negative' cases were collated. Those showing minimal tumour tissue remaining in the formalin fixed, paraffin embedded (FFPE) block were excluded from further investigation. All other samples were recalled for RNA-NGS. For internal quality assurance purposes, cases positive for ALK or ROS using IHC/FISH were also included in gene fusion analysis.

Upon tissue block recall to the molecular pathology laboratory, tissue was re-examined and samples which were insufficient for tumour, or extracted RNA, were excluded from further testing. For all remaining cases, gene fusion analysis by RNA-NGS was attempted.

2.2 Fusion Analysis Method

RNA based gene fusion analysis utilised an ISO 15189 accredited diagnostic assay, based upon the fusion component of the Oncomine Focus Assay (OFA) from ThermoFisher Scientific. This assay is not designed to be fusion partner agnostic, though it had been validated (prior to subsequent ISO accreditation) using a custom in-house bioinformatic pipeline designed to enable the detection of all possible 'fusion pairs', rather than the more limited assessment of specific combinations. The ISO scope of the assay was restricted to inter- or intra-genic rearrangement involving *ALK, BRAF, EGFR, MET, ROS1, RET, NTRK1* & *NTRK3* only. Any other findings (detected or not detected) were reported for clinical research use only. NGS library preparation was performed manually, using standard OFA reagents. Sequencing was then undertaken using either OneTouch™ 2/Ion PGM™ or Ion Chef™/GeneStudio™ S5 Prime hardware combinations.

3. Results

3.1 DNA-NGS Results

Over an 18-month period, 307 samples were referred for DNA-NGS mutation analysis, of which 30 samples (9.8%) were rejected following pre-assessment, due to the limited remaining tumour tissue in the FFPE block or very low quantity of extracted DNA. Of the 277 from which DNA was processed for NGS, 2 cases failed analysis (0.7%). Driver mutations or amplifications of driver genes were identified in 167 (61%) of cases, with a breakdown by gene similar to previously published datasets (7). Of KRAS-driven tumours 28% (27/97) showed the KRAS G12C mutation.

The 307 referred non-squamous NSCLC cases came from 171 male (56%) and 136 female (44%) patients. The age range was 23-89 years (mean 65 years).

3.2 Fusion Testing Results

Of 108 samples with no driver mutation or amplification detected on DNA-NGS, one case was excluded from further analysis as subsequent clinical information and IHC revealed this to be metastatic prostate cancer. A further ten cases were not recalled for further testing due to reported exhaustion of tissue at the time of DNA-NGS.

A total of 97 samples were therefore recalled for RNA-NGS, plus an additional 3 cases of lung adenocarcinoma from patients referred from other centres with negative *EGFR* and *KRAS* testing prior to referral. Of this 100-patient cohort, 11 were unsuitable for testing due to the tissue block subsequently 'cutting out' of tumour, or extraction of insufficient RNA. A total of 17 fusion drivers were identified in the 89 samples tested, while a further 17 samples failed RNA-NGS analysis (table 1). Of the 17 fusions, 16 were recognised fusion drivers, while one fusion (*EGFR-ETV1*) is of unknown clinical significance (table 1). A full breakdown of patients is detailed in Figure 1B. Of the fusion positive cases where smoking status was known, half (7/14) were never smokers.

Overall, following both DNA and RNA NGS, 66% of patients had a driver event identified (detailed in Figure 2).

A total of 34% (38/111) of samples which were classed as 'no driver mutation detected' on DNA analysis were either not tested due to tissue exhaustion, extracted RNA quality, or failed RNA-NGS analysis.

Standard ALK and ROS1 IHC and FISH results were studied in this cohort. All 3 cases of ALK and 3 cases of ROS1 rearrangement confirmed on FISH analysis were successfully identified using the fusion panel. One additional case was fusion panel positive for *ALK*, but had been reported as *ALK* FISH negative, despite strong ALK IHC positivity.

3.3 Clinical Impact of Fusion Testing

Treatment information for 11 of the 18 patients identified to have an alteration on RNA NGS was available. Alterations identified that do not yet have NICE approved treatments available included *MET* exon 14 skipping in 3 patients, and a *RET* fusion and *FGFR1* fusion in one patient each. Of these, 1 *MET* exon 14 skipping patient accessed a MET inhibitor via private healthcare and the *FGFR1* fusion patient entered a phase 2 trial based on their result. Treatment details, where available, for the RNA NGS positive cases are included in Table 1.

4. Discussion

There are considerable challenges in predictive biomarker testing in NSCLC patients owing to limited diagnostic material, the effect of formalin fixation upon nucleic acids and the increasing number of actionable alterations.

These data, derived from a single tertiary referral centre over 18 months, show that considering the typical mutual exclusivity of driver events successfully focused testing and enriched for fusion gene detection. In those DNA-driver negative tumours successfully tested, almost one quarter showed a driver fusion event in *ALK, ROS, MET, RET, FGFR* or *EGFR*, a far higher rate than typically described when testing is not restricted to DNA-NGS negative patients.

Having sufficient material of suitable quality for RNA extraction was a major barrier to testing in this cohort. In those cases which were not successfully tested, this was due to either insufficient material or assay failure, with the latter potentially related to formalin fixation. This highlights the importance of adequate biopsy sampling, plus careful handling and judicious use of tissue throughout the diagnostic process. There is also a potential role for circulating tumour DNA (ctDNA) testing, although current evidence indicates a lower sensitivity than tissue-based methods (8).

As we carried out the RNA-NGS at 6 weekly intervals, results from this panel test were not generally available for clinical decision-making for several weeks after biopsy. This is not within a timeframe adequate for first-line treatment for most patients and is a potential disadvantage for sequential DNA and fusion assays. Reflex rather than batch testing would reduce this turnaround time to within 4 weeks for DNA plus reflex RNA analysis, which would be more feasible for clinical management. The high never-smoker rate in the fusion detected samples suggests that these patients could be considered for parallel cDNA and RNA testing upfront.

Additional fusions identified on RNA-NGS may be treated within a clinical trial or expanded access scheme. These are mostly applicable to the second- or third-line setting and would therefore not be affected by a slower turnaround time of testing 'in series' but may move to first-line treatment as data emerge. Some of these targeted agents have already been approved for use outside the UK. In May 2020, the RET inhibitor, selpercatinib, was approved for use by the US FDA for *RET* altered NSCLC based on an overall response rate (ORR) of 64% in platinum treated patients (LIBRETTO-001) (9). In Europe, the EMA approved its use in February 2021. Tepotinib was granted accelerated approval by the FDA in February 2021 for NSCLC associated with *MET* exon-14 skipping based on the results of the VISION trial which reported an ORR of 43% in both treatment naïve and pre-treated NSCLC patients (10). EMA approval is awaited.

5. Conclusion

With the continued advances in precision thoracic oncology that have seen an increase in the number of actionable NSCLC molecular sub-types, developing efficient and cost-effective diagnostic pathways is critical. We have demonstrated that sequential DNA and RNA based molecular profiling allows efficient targeting of fusion driven NSCLC and that collaborating with companies developing new targeted therapies can facilitate access to extended profiling and therefore potential access to novel agents for patients. The move to a centralised genomics service in England via the regional GLHs offers an opportunity to optimise testing pathways and improve access to established and novel treatments for patients, if testing can be delivered successfully and efficiently for the vast majority of patients.

Figure Legends:

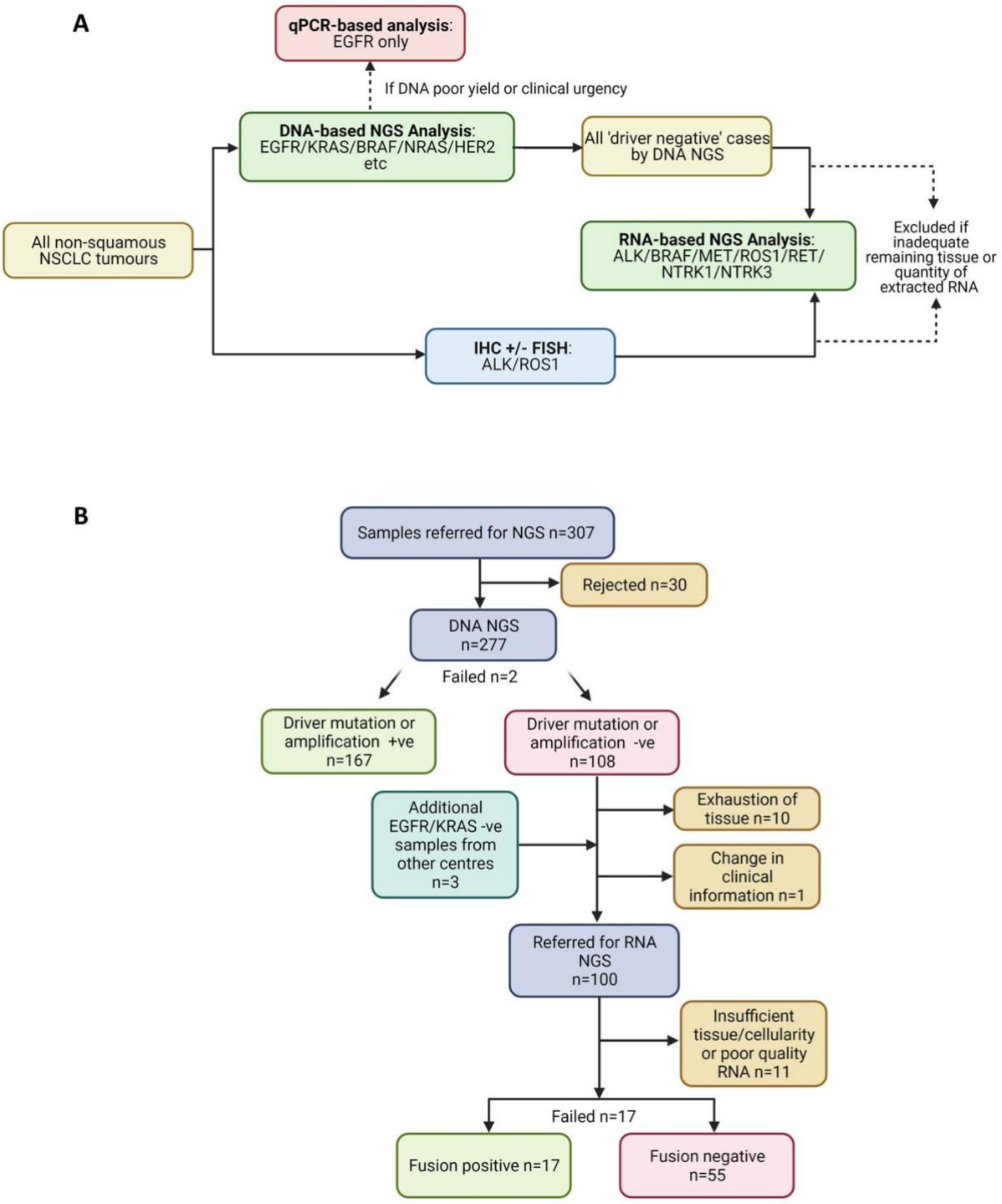

Figure 1A. UCH NSCLC molecular diagnostic pathway. Figure 1B Flowchart of NSCLC tumour samples referred for DNA-NGS +/- RNA-NGS

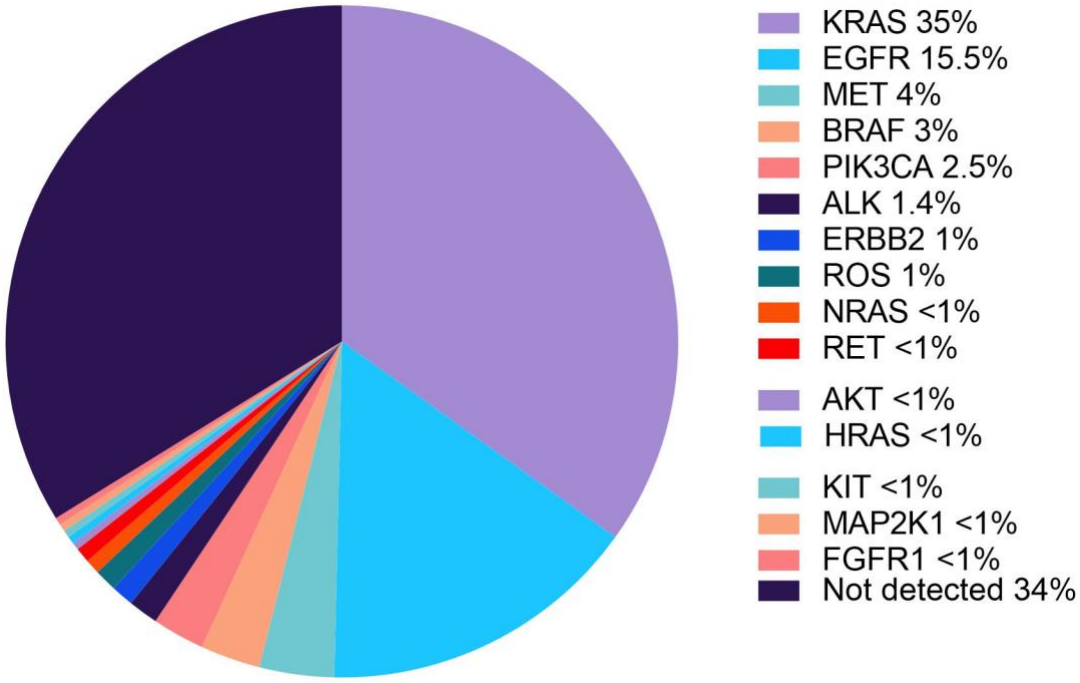

Figure 2. Breakdown of all driver events detected in the cohort including detected driver mutations, fusions and amplifications from both DNA-NGS and RNA-NGS panels.

Tables:

Fusion gene detected	**IHC result**	**FISH result**	**Smoking status**	**Treatment based on result**
ALK-EML4	Positive	Negative	Ex-smoker	Crizotinib received in the second line after chemo-immunotherapy
ALK-EML4	Positive	Positive	Never	Crizotinib first line
ALK-EML4	Positive	Positive	Ex-smoker	Alectinib first line
ALK-EML4	Positive	Positive	Never	No treatment data available
ROS1-CD74	Positive	Positive	Never	No- Early stage NSCLC treated with surgery only
ROS1-EZR	Positive	Positive	Never	No treatment data available
ROS1-SDC4	Positive	Positive	Ex-smoker (<5 PYR)	Crizotinib first line
MET ex14 skipping	N/A	N/A	Never	Capmatinib second line after immunotherapy (accessed in private healthcare setting)
MET ex14 skipping	N/A	N/A	Ex-smoker	No- ongoing first line treatment with immunotherapy

MET ex14 skipping	N/A	N/A	Ex-smoker	No- clinical deterioration before access to MET inhibitor (in 3rd line)
MET ex14 skipping	N/A	N/A	Unknown	No treatment data available
MET ex14 skipping	N/A	N/A	Unknown	No treatment data available
MET ex14 skipping	N/A	N/A	Unknown	No treatment data available
RET-CCDC6	N/A	N/A	Current smoker	No- ongoing first line treatment with chemo-immunotherapy
RET-KIF5B	N/A	N/A	Never	No treatment data available
FGFR1-BAG4	N/A	N/A	Never	Entered phase 2 trial of FGFR inhibitor as 3rd line treatment
EGFR-ETV1	N/A	N/A	Current	Not applicable as no trials for EGFR fusion

Table 1. Details of the 17 fusions detected across the 72 DNA panel driver-negative samples successfully tested by RNA-NGS, including smoking status where available, plus corresponding immunohistochemistry (IHC) and FISH testing results for ALK and ROS1 fusion positive cases.

Competing Interests: DM reports speaker fees from AstraZeneca and consultancy fees from Thermo Fisher. SML is supported by the UCL Hospitals Biomedical Research Centre, London. TA reports Educational bursaries from MSD, Takeda, Pfizer, AstraZeneca, Boehringer Ingelheim and Pierre Fabre and consultancy / speaker fees from AstraZeneca, Pfizer and Takeda. DPP reports consultancy fees and honoraria from Boehringer-Ingelheim, AstraZeneca, Pfizer, Roche, Takeda, Amgen. MJH is a Cancer Research UK Clinician Scientist and has received funding from CRUK (RCCFEL\100099), National Institute for Health Research, Rosetrees Trust, UKI NETs and NIHR University College London Hospitals Biomedical Research Centre. MJH is a member of the Scientific Advisory Board and Steering Committee for Achilles Therapeutics. MDF has research grants from AstraZeneca, Boehringer Ingelheim, MSD and Merck and has received honoraria for advisory, education and consultancy work from Achilles, AstraZeneca, Bayer, Bristol-Myers Squibb, Celgene, Guardant Health, Merck, MSD, Nanobiotix, Novartis, Oxford VacMedix, Pfizer, Roche, Takeda.

References:

1. Sequist LV, Yang JC, Yamamoto N, O'Byrne K, Hirsh V, Mok T, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol*. 2013;31:3327-34.

2. Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, Ahn MJ, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med*. 2013;368:2385-94.

3. Remon J, Ahn MJ, Girard N, Johnson M, Kim DW, Lopes G, et al. Advanced-Stage Non-Small Cell Lung Cancer: Advances in Thoracic Oncology 2018. *J Thorac Oncol*. 2019;14:1134-1155.

4. Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, et al. Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *J Thorac Oncol*. 2018;13:323-358.

5. Kim L, Tsao MS. Tumour tissue sampling for lung cancer management in the era of personalised therapy: what is good enough for molecular testing? *Eur Respir J*. 2014;44:1011-1022.

6. Moore DA, Balbi K, Ingham A, Arkenau H, Bennett P. Analysis of a large cohort of non-small cell lung cancers submitted for somatic variant analysis demonstrates that targeted next-generation sequencing is fit for purpose as a molecular diagnostic assay in routine practice. J Clin Pathol. 2018;71:1001-1006.

7. Fois SS, Paliogiannis P, Zinellu A, Fois AG, Cossu A, Palmieri G. Molecular Epidemiology of the Main Druggable Genetic Alterations in Non-Small Cell Lung Cancer. *Int J Mol Sci.* 2021;22:612.

8. Supplee JG, Milan MSD, Lim LP, Potts KT, Sholl LM, Oxnard GR, et al. Sensitivity of next-generation sequencing assays detecting oncogenic fusions in plasma cell-free DNA. *Lung cancer*. 2019;134:96-99.

9. Drilon A, Oxnard GR, Tan DSW, Loong HHF, Johnson M, Gainor J, et al. Efficacy of Selpercatinib in RET Fusion-Positive Non-Small-Cell Lung Cancer. *N Engl J Med*. 2020;383(9):813-24.

10. Paik PK, Felip E, Veillon R, Sakai H, Cortot AB, Garassino MC, et al. Tepotinib in Non-Small-Cell Lung Cancer with MET Exon 14 Skipping Mutations. *N Engl J Med.* 2020; 383:931-943.

Supplementary Tables

Gene	Exons	Codons
AKT1	4	17-52
ALK	22, 23, 25	1151-1171, 1173-1215, 1251-1278
BRAF	11, 15	439-472, 582-609
CTNNB1	3	9-48
DDR2	5, 8, 12, 13, 14, 15, 17	92-135, 226-265, 440-483, 503-537, 577-607, 621-668, 762-790
EGFR	12, 18, 19, 20, 21	472-499, 693-726, 729-761, 762-800, 854-875
ERBB2	19, 20, 21	753-769, 770-797, 839-882
ERBB4	3, 4, 6, 7, 8, 9, 15, 23	135-140, 167-185, 226-247, 254-290, 296-323, 334-368, 578-622, 917-947
FBXW7	5, 8, 9, 10, 11	261-287, 377-402, 434-472, 478-508, 567-597
FGFR1	4, 7	121-149, 250-275
FGFR2	7, 9, 12	251-278, 363-399, 542-557
FGFR3	7, 9, 14, 16, 18	248-277, 367-402, 631-653, 689-719, 772-807
KRAS	2, 3, 4	5-37, 38-66, 114-150
MAP2K1	2	43-83
MET	2, 14, 16, 19	339-378, 982-1014, 1106-1131, 1244-1274
NOTCH1	26, 27	1566-1602, 1674-1680
NRAS	2, 3, 4	3-31, 41-69, 112-150
PIK3CA	10, 14, 21	522-550, 676-720, 1017-1051, 1063-1069
PTEN	1, 3, 6, 7, 8	1-25, 56-69, 165-184, 213-218, 230-267, 280-302, 312-342
SMAD4	3, 5, 6, 8, 9, 10, 11, 12	98-136, 165-202, 241-262, 307-318, 326-365, 384-426, 444-473, 494-533
STK11	1, 6, 8, 4-5	22-64, 254-286, 317-361, 192-207
TP53	2, 4, 5, 6, 7, 8, 10	1-20, 67-114, 150-186, 126-138, 188-221, 225-257, 262-306, 332-366

Supplementary Table 1: 22-gene DNA NGS Panel used for first 12 months of the cohort.

Gene	Exons	Codons
AKT1	4	17-50
ALK	23, 25	1173-1204, 1249-1275
BRAF	11, 12, 14, 15, 16	450-477, 479-494, 566-589, 582-611, 623-659
CDKN2A	1, 2, 3	13-39, 51-88, 98-139, 154-157
CTNNB1	3	13-50
CXCR4	1	315-354
EGFR	3, 7, 12, 15, 18, 19, 20, 21	85-125, 282-296, 474-499, 583-625, 696-725, 729-761, 762-799, 814-823, 827-865
ERBB2	8, 12, 14, 17 ,18 ,19, 20, 21, 22, 24, 25	302-326, 472-501, 557-579, 650-679, 718-736, 737-760, 770-797, 832-870, 884-903, 969-990, 1007-1048
ESR1	5, 6, 7, 10	233-253, 300-339, 367-384, 533-574
FBXW7	5, 8, 9, 10, 11	256-287, 379-402, 435-472, 479-508, 567-593
FGFR1	3, 4, 8, 10, 12, 14, 17	44-70, 121-147, 315-356, 439-476, 529-554, 640-659, 730-758
FGFR2	5, 7, 9, 12, 14, 16	179-204, 251-269, 284-313, 364-396, 528-558, 648-663, 716-732
FGFR3	7, 9, 13, 15, 16	248-260, 371-414, 561-592, 656-664, 692-723
GNA11	4, 5	166-196, 206-240
GNAQ	2, 4, 5	60-99, 164-201, 203-223
GNAS	8	839-861
HRAS	2, 3, 4	6-33, 45-86, 107-139
IDH1	4	101-138
IDH2	4	162-178
KIT	9, 10, 11, 13, 14, 15, 17	488-513, 517-549, 550-588, 629-663, 665-687, 715-727, 788-826
KRAS	2, 3, 4, 5	6-37, 38-65, 113-150, 155-190
MAP2K1	2, 3, 6	44-81, 98-135, 191-226
MET	2, 9, 11, 13, 14, 15, 16, 19, 20, 21 +5′ & 3′ Flanking regions of exon 14	152-191, 345-383, 751-754, 844-879, 969-980, 982-994, 1009-1027, 1029-1052, 1106-1131, 1234-1274, 1289-1328, 1331-1369
MYD88	3, 4, 5	185-211, 224-242, 255-263
NRAS	2, 3, 4	2-21, 43-68, 122-150
PDGFRA	12, 14, 15, 18	552-584, 644-667, 669-701, 835-854
PIK3CA	2, 3, 5, 8, 10, 14, 21	23-57, 78-108, 119-137, 312-351, 418-443, 533-554, 694-729, 1020-1059
POLE	9, 13, 14, 19, 34	268-303, 410-432, 454-475, 689-724, 1434-1460
PTEN	1, 2, 3, 4, 5, 6, 7, 8, 9	2-26, 28-46, 56-69, 79-84, 86-106, 123-158, 165-181, 213-234, 283-299, 313-342, 343-353
RET	3, 10, 11, 13, 15, 16	114-120, 610-626, 628-636, 665-706, 763-785, 872-904, 911-925
SMAD4	2, 3, 6, 9, 10, 11, 12	18-58, 110-139, 224-254, 336-374, 385-424, 443-478, 484-511, 526-553
STK11	1, 4, 5, 6, 7, 8	27-57, 162-189, 206-235, 269-287, 291-306, 318-359
TP53	2, 3, 4, 5, 6, 7, 8, 9, 10	2-18, 27-32, 53-94, 108-125, 138-180, 188-222, 225-251, 262-302, 308-331, 332-343, 356-366

Supplementary Table 2: 22-gene DNA NGS Panel used for the last 6 months of the cohort.