Sequential Biomarkers testing versus upfront NGS in mNSCLC

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What are we currently doing with Single Markers?

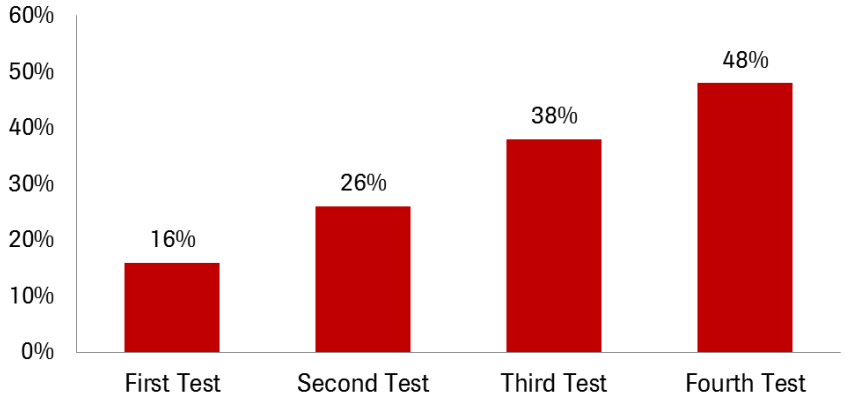
Are we even exploring all the NCCN recommended options for our patients?

Pathology Confirmation and Subtyping | **EGFR** | **ALK** | **ROS1** | **RET** | **MET** | **BRAF** | **PD-1/PD-L1**

Sequential Testing Leads to High Tissue Depletion Rates

~50% patients do not have any tissue specimen remaining after the first 4 biomarkers have been tested[1]

Time wasted in serially testing is another key consideration



% tissue no longer available to perform biomarker testing on NSCLC lung tissue samples

Source: 1.Roche/Genentech Lung Tracker Survey Q2 2015

Guidelines highlight the requirement for broad molecular testing techniques to support therapy selection

CAP / IASLC / AMP[1] / ESMO[2]

Molecular testing guideline

"In general, capture-based [NGS] methods may be preferable for initial testing of lung cancer samples in order to detect rearrangements… as well as a broader range of potential genetic markers"[1]

"If available, multiplex platforms (NGS) for molecular testing are preferable"[2]

ASCO Educational Book[3]

Biomarker testing for advanced NSCLC

"For very limited samples… for which multiple tests cannot be performed, [hybrid capture-based] assays are preferable for upfront comprehensive assessment"

Molecular testing method	Point mutations and small indels					Copy number alterations		Rearrangements			
	EGFR	BRAF	KRAS	HER2	MET[4]	HER2	MET[4]	ALK	ROS1	NTRK	RET[4]
PCR and conventional sequencing			✓								
FISH									✓		
IHC							✓*			✓*	
NGS (amplicon-based)			✓								
NGS (hybrid capture-based)			✓				✓			✓	

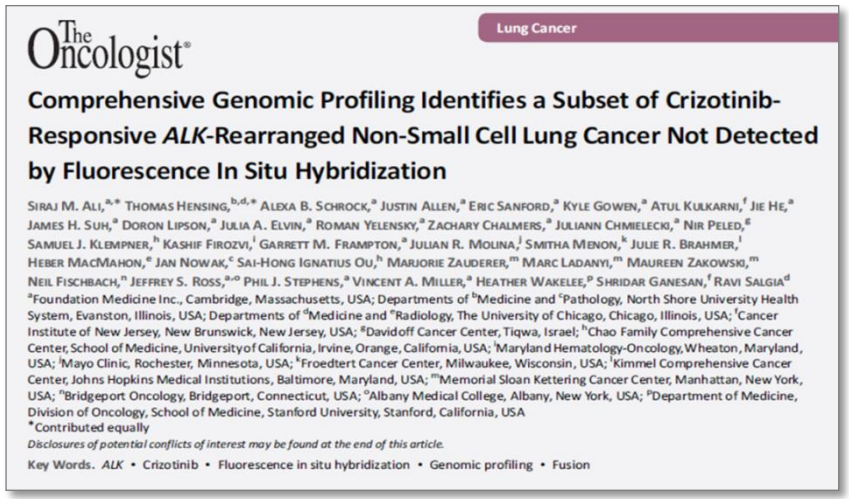
*IHC is used to detect *MET* overexpression and *ALK* translocations respectively.

AMP: Association for Molecular Pathology; ASCO: American Society of Clinical Oncology; CAP: College of American Pathologists; FISH: fluorescence in situ hybridisation; IASLC: International Association for the Study of Lung Cancer; NGS: next-generation sequencing; NSCLC: non-small cell lung cancer; PCR: polymerase chain reaction. Table adapted from Pennell, N.A., et al. (2019).

1. Lindeman, L. I., et al. (2018) *J Mol Diagn* 20:129-59; 3. Pennell, N.A., et al. (2019) *ASCO Educational Book* 39:531-42;
4. Domagala-Kulawik, J., et al. (2019) *Front Med (Lausanne)* 6:284.

Limitations of single sequential biomarker approach

35% ALK cases missed by FISH



The Oncologist

Lung Cancer

Comprehensive Genomic Profiling Identifies a Subset of Crizotinib-Responsive *ALK*-Rearranged Non-Small Cell Lung Cancer Not Detected by Fluorescence In Situ Hybridization

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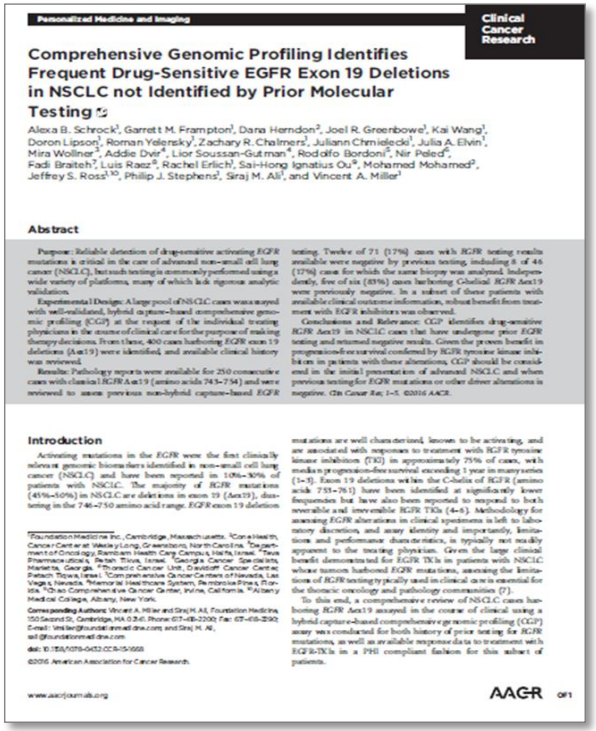
Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. *ALK* • Crizotinib • Fluorescence in situ hybridization • Genomic profiling • Fusion

17% EGFR cases missed by Hot Spots



Personalized Medicine and Imaging

Clinical Cancer Research

Comprehensive Genomic Profiling Identifies Frequent Drug-Sensitive EGFR Exon 19 Deletions in NSCLC not Identified by Prior Molecular Testing

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Abstract

Purpose: Reliable detection of drug-sensitive activating *EGFR* mutations is critical in the care of advanced non–small cell lung cancer (NSCLC), but such testing is commonly performed using a wide variety of platforms, many of which lack rigorous analytic validation.

Experimental Design: A large pool of NSCLC cases was assayed with well-validated, hybrid capture–based comprehensive genomic profiling (CGP) at the request of the individual treating physicians in the course of clinical care for the purpose of making therapy decisions. From these, 400 cases harboring *EGFR* exon 19 deletions (Ex19) were identified, and available clinical history was reviewed.

Results: Pathology reports were available for 250 consecutive cases with classical *EGFR* Ex19 (amino acids 743–754) and were reviewed to assess previous non-hybrid capture–based *EGFR* testing. Twelve of 71 (17%) cases with *EGFR* testing results available were negative by previous testing, including 8 of 46 (17%) cases for which the same biopsy was analyzed. Independently, five of six (83%) cases harboring G-helical *EGFR* Ex19 were previously negative. In a subset of these patients with available clinical outcome information, robust benefit from treatment with EGFR inhibitors was observed.

Conclusions and Relevance: CGP identifies drug-sensitive *EGFR* Ex19 in NSCLC cases that have undergone prior *EGFR* testing and returned negative results. Given the proven benefit in progression-free survival confirmed by EGFR tyrosine kinase inhibitors in patients with these alterations, CGP should be considered in the initial presentation of advanced NSCLC and when previous testing for *EGFR* mutations or other driver alterations is negative. (*Clin Cancer Res*; 1–5. ©2016 AACR.)

Introduction

Activating mutations in the *EGFR* were the first clinically relevant genomic biomarkers identified in non–small cell lung cancer (NSCLC) and have been reported in 10%–30% of patients with NSCLC. The majority of *EGFR* mutations (45%–50%) in NSCLC are deletions in exon 19 (Ex19), clustering in the 746–750 amino acid range. *EGFR* exon 19 deletion mutations are well characterized, known to be activating, and are associated with responses to treatment with EGFR tyrosine kinase inhibitors (TKI) in approximately 75% of cases, with median progression-free survival exceeding 1 year in many series [1–5]. Exon 19 deletions within the C-helix of EGFR (amino acids 753–761) have been identified at significantly lower frequencies but have also been reported to respond to both reversible and irreversible EGFR TKIs [4–6]. Methodology for assessing *EGFR* alterations in clinical specimens is left to laboratory discretion, and assay identity and importantly, limitations and performance characteristics, is typically not readily apparent to the treating physician. Given the large clinical benefit demonstrated for EGFR TKIs in patients with NSCLC whose tumors harbored *EGFR* mutations, assessing the limitations of *EGFR* testing typically used in clinical care is essential for the thoracic oncology and pathology communities [7].

To this end, a comprehensive review of NSCLC cases harboring *EGFR* Ex19 assayed in the course of clinical using a hybrid capture-based comprehensive genomic profiling (CGP) assay was conducted for both history of prior testing for *EGFR* mutations, as well as available response data to treatment with EGFR-TKIs in a PHI compliant fashion for this subset of patients.

[1]Foundation Medicine Inc., Cambridge, Massachusetts. [2]Core Health, Cancer Center at Wesley Long, Greensboro, North Carolina. [3]Department of Oncology, Rambam Health Care Campus, Haifa, Israel. [4]Teva Pharmaceuticals, Petah Tikva, Israel. [5]Georgia Cancer Specialists, Marietta, Georgia. [6]Thoracic Cancer Unit, Davidoff Cancer Center, Petach Tikva, Israel. [7]Comprehensive Cancer Centers of Nevada, Las Vegas, Nevada. [8]Memorial Healthcare System, Pembroke Pines, Florida. [9]Chao Comprehensive Cancer Center, Irvine, California. [10]Albany Medical College, Albany, New York.

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doi: 10.1158/1078-0432.CCR-15-1668

Source: Ali, S., et al. (2016). *Oncologist*. Jun;21(6):762-70 | Schrock, AB et al., Clin Cancer Res 2016; 22(13) 3281-5

NGS identifies *EGFR* mutations in lung cancer patients that were missed by standard of care testing

400 NSCLC cases **with *EGFR* exon 19 deletions** identified by CGP[1]

77 cases **with previous testing results** available

22% of cases were tested **(false) negative** for *EGFR* mutations in previous **non-hybrid capture-based testing**

*In a subset of patients with available clinical outcome information, a **robust benefit from treatment with EGFR inhibitors** was observed*

482 NSCLC cases **with *EGFR* point mutations*** identified by CGP[2]

103 cases **with previous testing results** available

21% of cases were tested **(false) negative** for *EGFR* mutations in previous **SoC testing**

*In cases with available clinical data, **benefit from EGFR inhibitor therapy** was observed*

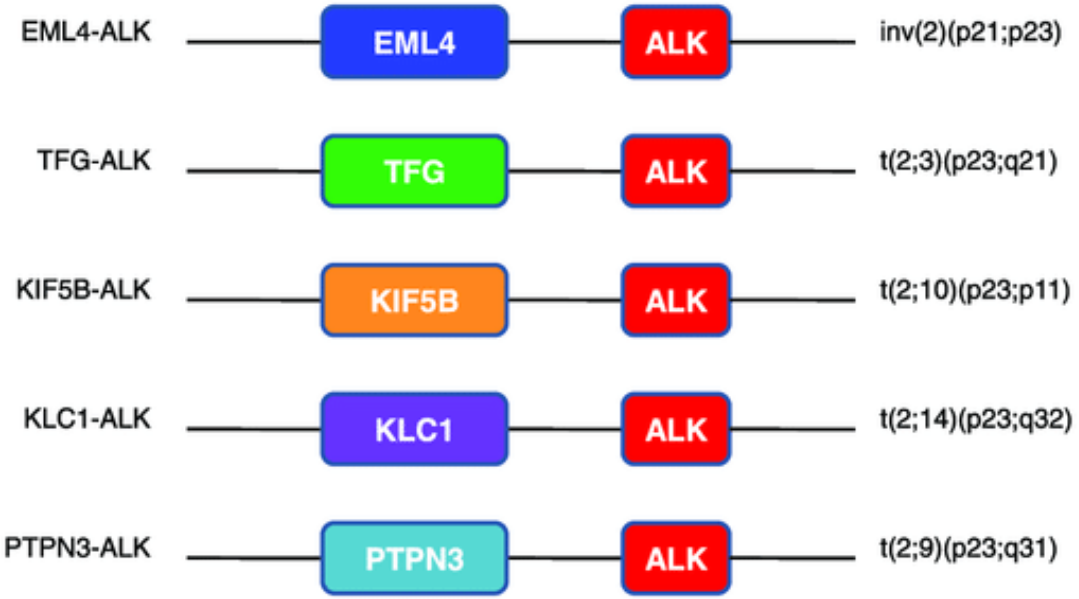
*CGP identified EGFR-activating mutations in over **20%** of patients who previously tested negative by SoC EGFR mutation testing[1,2]*
Many patients could experience improved clinical outcomes when CGP is used to inform therapeutic decisions[1,2]

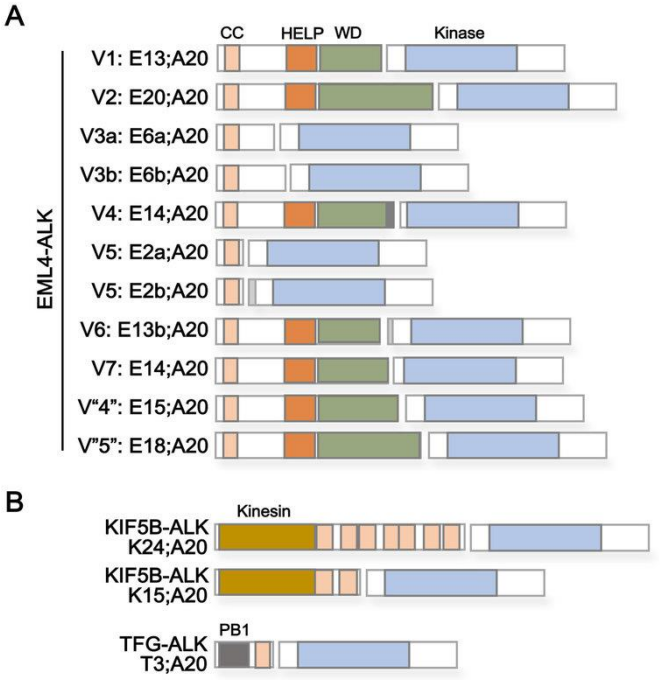
*Some patients had multiple *EGFR* point mutations.

CGP: comprehensive genomic profiling; NSCLC: non-small cell lung cancer; SoC: standard of care.

1. Schrock, A.B., et al. (2016) *Clin Can Res* 22:3281-5; 2. Suh, J.H., et al. (2018) *Oncologist* 23:776-81.

Limitations of single sequential biomarker approach





The added clinical value in using CGP for the treatment of lung cancer

NGS can uncover clinically valuable genetic drivers even after other tests are negative or inconclusive

A retrospective cohort study assessed the clinical impact of CGP in lung cancer by conducting hybrid capture-based broad-panel NGS in 101 advanced lung cancer patients[1]

86 patients were previously tested for *EGFR* and / or *ALK* alterations using standard molecular testing*†

EGFR — ***81 tested negative and 5 were inconclusive***

ALK — ***71 tested negative and 1 was inconclusive***

NGS

15 *patients were found to* ***harbour genomic alterations in EGFR or ALK*** *despite previous negative standard molecular testing results*

80% *of patients (12 / 15) went on to* ***receive targeted therapy*** *based on NGS results*

67% *of patients (8 / 12)* ***experienced complete or partial response to the treatment***

Broad use of CGP in lung cancer may provide a key for therapeutic decision making with high probability to identify actionable driver alterations despite negative standard molecular tests

**EGFR* mutations were assessed with real-time PCR or narrow-spectrum NGS assays (amplicon-based hotspot NGS); *ALK* rearrangements were assessed with immunohistochemistry and / or FISH; †15 patients were not previously tested with standard methods ; ‡22.8% of patients carried 2 actionable genomic alterations and 5.9% 3 or more. CGP: comprehensive genomic profiling; NCCN: National Comprehensive Cancer Network; NGS: next-generation sequencing.

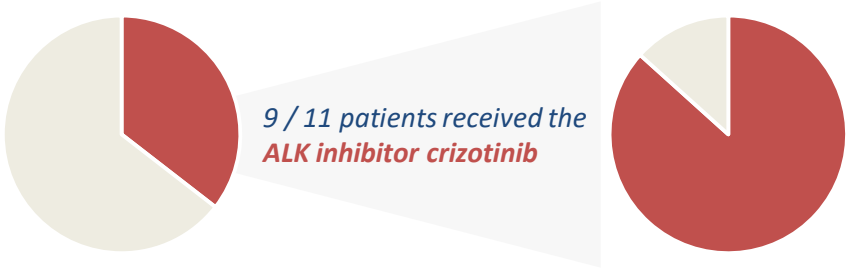
Rozenblum, A.B., et al. (2017) *J Thorac Oncol* 12:258-68.

NGS identifies *ALK* rearrangements in NSCLC undetected by other testing approaches

1,070 patient samples were profiled using **CGP**

47 cases were found to **harbour *ALK* rearrangements**

31 of cases had **prior FISH** results available



35%

*of cases (11 / 31) were tested **(false) negative** for ALK rearrangements using FISH, but were identified subsequently with CGP*

78%

*of treated patients (7 /9) had **confirmed responses***

CGP: comprehensive genomic profiling; FISH: fluorescence *in situ* hybridisation; NSCLC: non-small cell lung cancer.

Ali, SM., et al. (2016) *Oncologist* 21:762-70.

NGS shows clinical utility in real-world practice

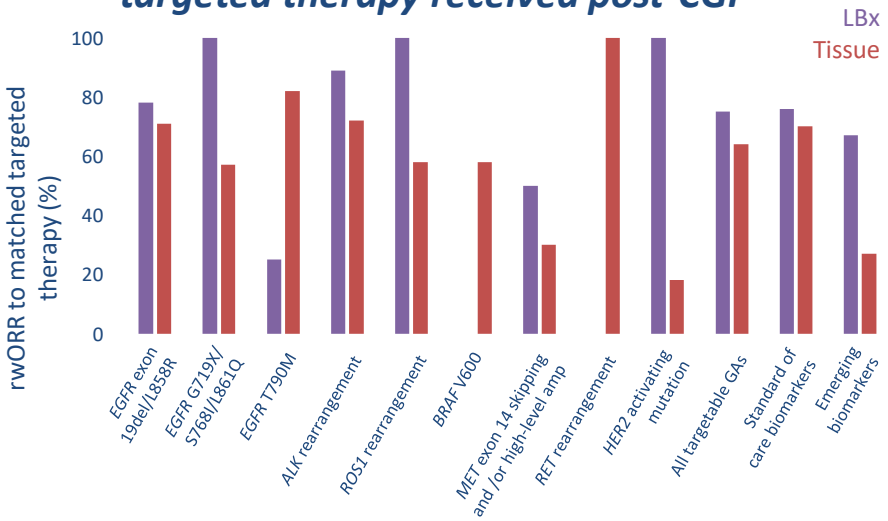
5,188 **advanced NSCLC** patients with **tissue-based CGP (n = 4486)** or **liquid-based CGP (n = 702)** results were identified in the Flatiron Health-Foundation Medicine, Inc. clinico-genomic database and evaluated for real-world tumour (rwTR) response to **matched targeted therapy**

22% of both **liquid- and tissue-based** CGP specimens **contained targetable GAs**

62% of **liquid-based** CGP specimens **received** subsequent **matched targeted therapy**

54% of **tissue-based** CGP specimens **received** subsequent **matched targeted therapy**

Real-world tumour response to matched targeted therapy received post-CGP



*Frequency of detected targetable GAs and rwTR to treatment with matched therapy was similar for LBx and TBx CGP. CGP identified **all types of GAs** in a large proportion of patients who may benefit from matched targeted therapy*

biopsy.

Madison, R., et al. presented at WCLC 2019; abstract P1.01-23.

Guidelines are recognising the usefulness of liquid biopsy for lung cancer management

Liquid biopsy can be considered…

At time of initial diagnosis, in all patients who need tumour molecular profiling[1]

If a patient is medically unfit for invasive tissue sampling[2]

If there is insufficient tissue for molecular analysis[1-3]

At disease progression[1]

~ 30% of patients have inadequate tumour tissue for molecular analysis at diagnosis & repeat biopsies are not feasible in ~20% of patients with aNSCLC[4,5]

1. Rolfo, C., et al. (2018) *J Thorac Oncol* 13:1248-68; 2. NSCLC NCCN Guidelines Version 2.2020;
3. Lindeman, N.I., et al. (2018*) J Mol Diagn* 20:129-59; 4. Zugazagoitia, J., et al. (2019) *Lung Cancer* 134:72-78;
5. Chouaid, C., et al. (2014) *Lung Cancer* 86:170-3.

Liquid biopsy can complement tissue based profiling

Liquid biopsy is not currently recommended as a replacement for solid biopsy but is a convenient option when tissue is insufficient or upon disease progression[1]

ctDNA NGS* (62 gene panel) was used to characterise samples from **1,552 aNSCLC patients**[2]

86% of ctDNA samples had genomic alterations in **≥ 1 pathway**†

64% **concordance**‡ was observed for 33 temporally matched ctDNA and tissue samples

Most alterations detected in matched tissue were also detected in ctDNA, suggesting ctDNA testing should be used as a complementary approach to tissue testing in aNSCLC[2]

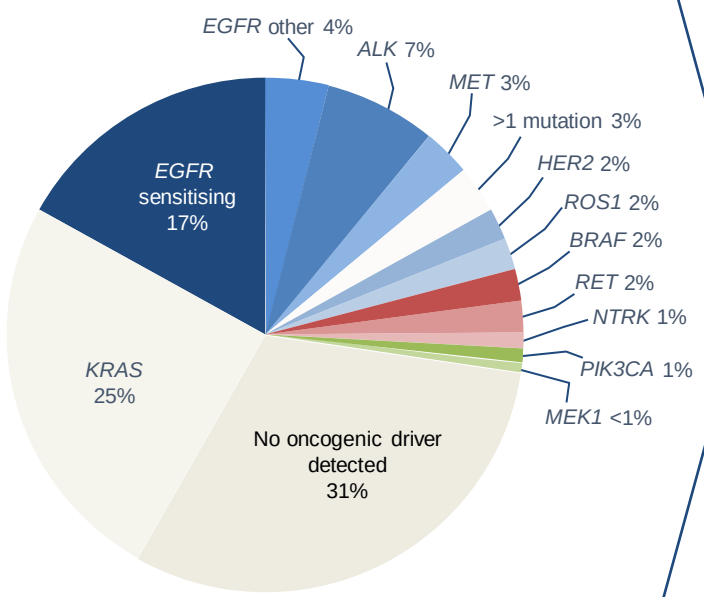
*FoundationACT was used: current version of the assay is known as FoundationOne Liquid. †Percentage of 1,243 samples with a maximum somatic allele frequency greater than 0. ‡Percentage of alterations detected in tissue that were also detected in ctDNA. aNSCLC: advanced non-small cell lung cancer; ctDNA: circulating tumour DNA; NGS: next-generation sequencing.

1. Rolfo, C., et al. (2018) *J Thorac Oncol* 13:1248-68; 2. Schrock, A.B., et al (2018) *J Thorac Oncol* 14:255–64.

New treatment options in NSCLC driven by biomarkers and genomic signatures

Advanced diagnostics inform therapy selection

Targetable mutations in lung cancer[1]



Identifying actionable mutations with broad genomic profiling[2]

Approved drugs

Investigational drugs

EGFR

- Afatinib
- Dacomitinib ▼
- Erlotinib (± anti-VEGF / VEGFR)
- Gefitinib
- JNJ-372[3]
- Necitumumab ▼[1]
- Osimertinib ▼
- Poziotinib[6]
- TAK-788[5]
- U3-1402[4]

BRAF

- Dabrafenib (± trametinib)
- Vemurafenib

NTRK

- Cabozantinib ▼[1]
- Entrectinib
- Larotrectinib ▼
- Repotrectinib[7]
- Selitrectinib[17]

ALK

- Alectinib ▼
- Brigatinib ▼
- Ceritinib ▼
- Crizotinib
- Ensartinib[8]
- Lorlatinib ▼
- Repotrectinib[7]

RET

- Apatinib[1]
- Cabozantinib ▼
- Lenvatinib ▼[1]
- Selpercatinib[15]
- Ponatinib ▼[1]
- Pralsetinib[16]
- Vandetanib ▼

ROS1

- Ceritinib ▼
- Crizotinib
- DS-6051b[1]
- Entrectinib
- Lorlatinib ▼
- Repotrectinib[7]

MET

- Cabozantinib ▼[1]
- Crizotinib
- Capmatinib[9]
- Savolitinib[10]
- Tepotinib[11]

HER2

- Afatinib[1]
- Dacomitinib ▼[1]
- Pertuzumab + trastuzumab ▼[13]
- Poziotinib[6]
- TAK-788[5]
- Trastuzumab emtansine[1] / deruxtecan[14]

PIK3CA

- Copanlisib[12]

MEK1

- Cobimetinib ▼[1]
- Selumetinib[1]
- Trametinib[1]

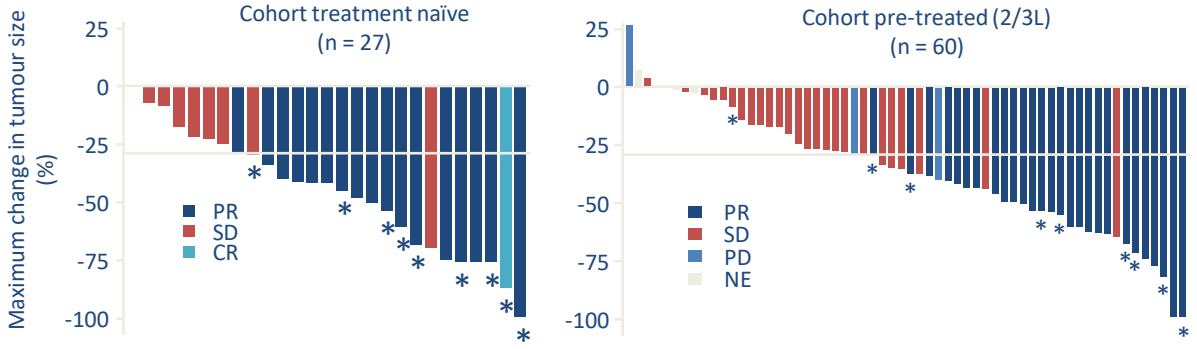
All drugs listed are included in NSCLC NCCN Guidelines unless otherwise indicated.

Some drugs are investigational and not approved in any indication. Some non-investigational drugs are only approved for use in specific indications in Europe and / or USA and / or Japan. Therapies marked with ▼ are subject to additional monitoring. Reporting suspected adverse reactions after authorisation of the medicinal product is important. Adverse events should be reported to your respective local office. Amgen Europe B.V.: Trastuzumab (Kanjinti); AstraZeneca AB: Osimertinib; Bayer AG: Larotrectinib; Celltrion Healthcare Hungary Kft.: Trastuzumab (Herzuma); Eli Lilly Nederland B.V.: Necitumumab; Eisai Europe Limited: Lenvatinib; Genzyme Europe B.V.: Vandetanib; Incyte Biosciences Distribution B.V.: Ponatinib; Ipsen Pharma: Cabozantinib; Mylan S.A.S.: Trastuzumab (Oqivri); Novartis Europharm Limited: Ceritinib; Pfizer Europa MA EEG: Trastuzumab (Trazimera); Pfizer Europe MA EEIG: Dacomitinib, Lorlatinib; Roche Registration GmbH: Alectinib, Cobimetinib; Samsung Bioepis UK Limited: Trastuzumab (Ontruzant); Takeda Pharma A/S: Brigatinib. 1. Adapted from Tsao, A.S., et al. (2016) *J Thorac Oncol* 11:613-38; 2. NSCLC NCCN Guidelines Version 2.2020; 3. NCT02609776; 4. NCT03260491; 5. NCT02716116; 6. NCT03318939; 7. NCT03093116; 8. NCT02767804; 9. NCT03693339; 10. NCT03778229; 11. NCT02864992; 12. NCT02465060; 13. NCT03845270; 14. NCT03505710; 15. NCT04268550; 16. NCT04204928; 17. NCT03206931.

GEOMETRY mono-1 shows high response rate in mNSCLC patients with *MET*ex14 mutation treated with capmatinib

2 cohorts (pre-treated and treatment naïve), both with *MET*ex14 mut regardless of *MET* GCN were treated with capmatinib (400 mg BID)

Primary endpoint: objective response rate† by central review (BIRC)



	Cohort treatment naïve	Cohort pre-treated (2/3 line)
ORR % (95% CI)	67.9 (47.6, 84.1)‡	40.6 (28.9, 53.1)‡
mDoR	11.14 months	9.72 months

Capmatinib has also demonstrated preliminary efficacy in patients with brain metastases

54% *(7 / 13) showed intracranial response*

92% *(12 / 13) achieved intracranial disease control*

Based on GEOMETRY mono-1 the FDA approved FoundationOne®CDx as a companion diagnostic to capmatinib in mNSCLC

*Patients still on treatment; †per RECIST v1.1. ; ‡Evaluated by BIRC. aNSCLC: advanced non-small cell lung cancer; BID: twice daily; BIRC: blinded independent review committee; CR: complete response; GNC: gene copy number; mDoR: median duration of response; NE: not evaluable; ORR: overall response rate; PD; progressive disease; PR: partial response; SD: stable disease.

Garon, E.B., et al. presented at AACR 2020, abstract CT082.

Primary efficacy and biomarker analyses from the VISION study of Tepotinib in NSCLC patients with *MET*ex14 skipping

Phase II VISION trial

Led to regulatory approval of tepotinib in Japan in March 2020

Previously treated aNSCLC pts with *MET*ex14 skipping mutations identified using LBx or TBx

Pts received oral tepotinib (500 mg QD), efficacy, safety and biomarker analyses performed

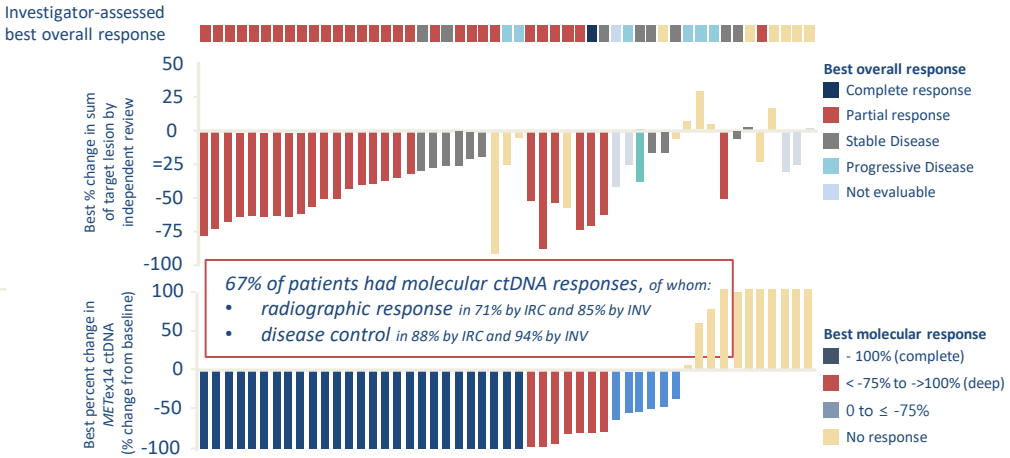
Tumour shrinkage was observed in 89% of pts

ORR was 46.5-50.0% by IRC and 55.6-61.7% by INV

In the combined group median PFS was 8.5 and 8.6 months by IRC and INV

Tepotinib had a manageable tolerability profile with few adverse events leading to discontinuation

Patients with molecular ctDNA responses (reduction in METex14 mutant allele frequency) had high ORRs



Association with molecular ctDNA and clinical responses support that MET inhibition in METex14 skipping tumour cells can lead to clinical benefit

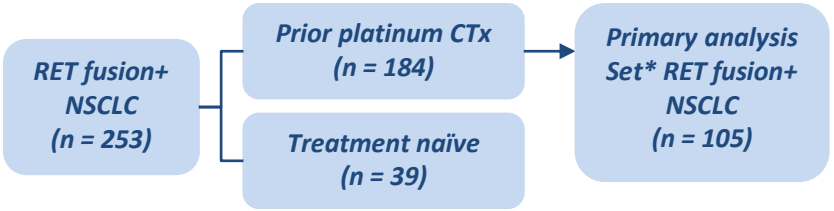
CR: complete response; ctDNA: circulating tumour DNA; INV: investigator assessment; IRC: independent review committee; LBx: liquid biopsy; NE: not evaluable; NSCLC: no small cell lung cancer; ORR: objective response rate; PD: progressive disease; PFS: progression free survival; PR: partial response; pts: patients; QD: once daily; SD: stable disease; TBx: tissue biopsy.

Le, X., et al. presented at ASCO 2020, abstract 9556.

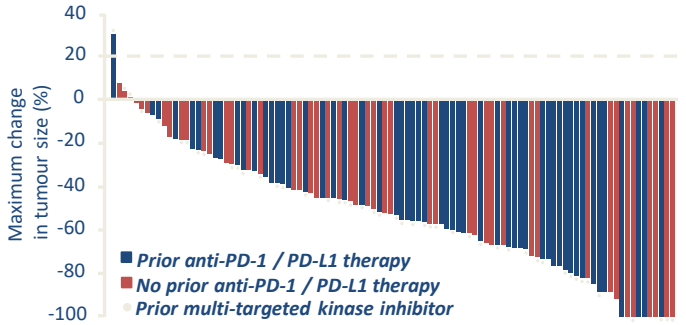
Selpercatinib (LOXO-292) in patients with *RET* fusion+ NSCLC

***RET* fusions** drive lung cancer. **Selpercatinib (LOXO-292)** is a highly selective and potent **RET kinase inhibitor, FDA-approved** for use **in *RET* fusion+ NSCLC,** based on phase I/II trials showing **antitumour activity**

Reported here is an **update on the efficacy**, including tumour assessment by BIRC and safety.
***RET* alteration determined** by local CLIA or similarly accredited laboratories using **NGS, FISH, or PCR**



Marked antitumour activity with selpercatinib in pts with *RET* fusion+ NSCLC pretreated with platinum-based CTx by BIRC



ORR BICR: *64% (95% CI: 54-73)*
ORR IR: *70% (60-78)*
Intracranial ORR: *91% (59-100)*
Median DoR: *18 mo (12-NE)*
Median PFS: *17 mo (14-NE)*

Marked **antitumour activity with selpercatinib** in patients with *RET* fusion+ NSCLC **naïve to proir treatment** by BIRC

ORR BICR: *85% (95% CI: 70-94)*
ORR IR: *90% (76-97)*
Median DoR: *NE (12-NE)*
Median PFS: *NE (14-NE)*

Selpercatinib demonstrated robust and durable anti-tumour activity in RET fusion+ NSCLC and had a favourable safety profile. A randomised, global phase 3 trial is underway

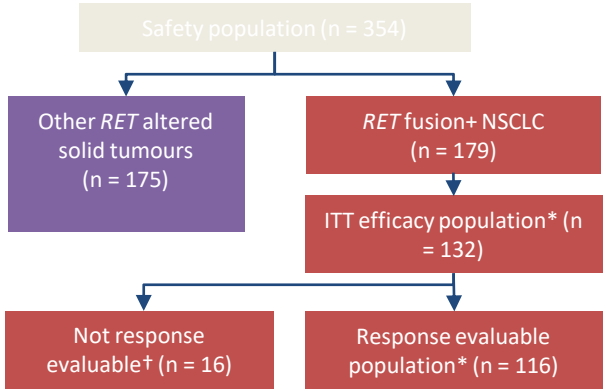
*The primary analysis set (PAS) was defined through health authority agreement as the first 105 consecutively enrolled patients with *RET* fusion+ NSCLC previously treated with platinum chemotherapy. Patients with non-measurable disease enrolled in phase 1 dose escalation were included in the PAS. BIRC: blinded independent review committee; CLIA: Clinical Laboratory Improvement Amendments CTx: chemotherapy; DoR: duration of response; FISH: fluorescence in situ hybridisation; IR: investigators review; mo: months; NE: not evaluable; NGS: next-generation sequencing; NR: not reached; NSCLC: non-small cell lung cancer; ORR: overall response rate; PCR: polymerase chain reaction; PFS: progression-free survival. Goto, K., et al. presented at ASCO 2020, abstract 3584.

Registrational dataset from the phase I/II ARROW trial of pralsetinib in pts with advanced *RET* fusion+ NSCLC

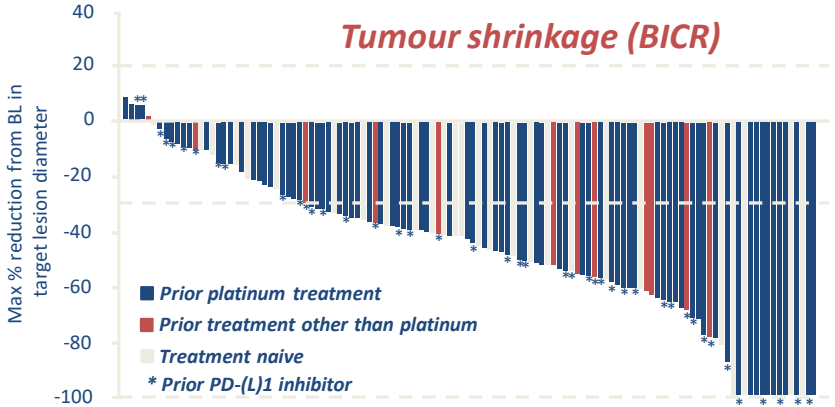
Phase I/II ARROW trial

Pralsetinib is a **RET kinase inhibitor** targeting oncogenic *RET* alterations including fusions

ARROW is an ongoing phase I/II trial investigating **pralsetinib in pts with advanced solid tumours with *RET* alterations**



- ORR was 65% and was similar despite *RET* fusion genotype or prior therapies



96% of evaluable pts had tumour reductions *(100% of treatment naïve pts)*

6% CR rate in evaluable pts *12% CR in treatment naïve pts*

- Pralsetinib has robust intracranial activity with an ORR of 56% and 3 pts (33%) with CR
- Well tolerated across tumour types with predominantly grade 1-2 treatment related adverse events

Pralsetinib has the potential to change SoC for the treatment of RET fusion+ NSCLC pts

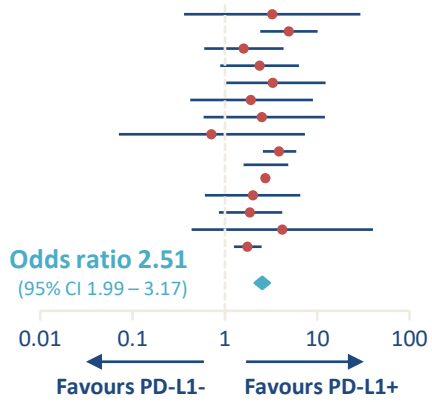
*Initiated pralsetinib by July 11, 2019; †Due to alternative driver mutation, insufficient evidence of RET fusion, incomplete baseline imaging, no measurable disease per BICR, no post-treatment disease assessment. BICR: blinded independent centralised review; BL: baseline; CR: complete response; ITT: intent to treat; NSCLC: non-small cell lung cancer; ORR: objective response rate; pts: patients; QD: once daily; SoC: standard of care. Gainor, J.F., et al. presented at ASCO 2020, abstract 9515

Predictive biomarkers for response to immunotherapy are highly sought after

Tumour PD-L1 expression is associated with greater likelihood of objective response to PD-(L)1 inhibition[1]

Meta-analysis of 14 studies in NSCLC published between 2014 – 2017 found that objective response to PD-(L)1 inhibitor therapy is more likely in PD-L1+ patients

PD-L1+ patients; n = 1,295
PD-L1- patients; n = 1,984



While PD-L1 expression is associated with a greater likelihood of response to PD-(L)1 inhibition, ***the association is not absolute***[2,3]

~8% NSCLC patients with **negative PD-L1 staining** (< 1%) on tumour cells **will respond** to pembrolizumab[2]

Assay performance, interpretation and PD-L1 expression heterogeneity may **limit the sensitivity and specificity of PD-L1 IHC**[3]

Additional predictive tools may be able to better enrich the population of potential responders to anti-PD-(L)1 monotherapy[2,4] *or anti-PD-(L)1 + anti-CTLA-4 combination immunotherapy*[4]

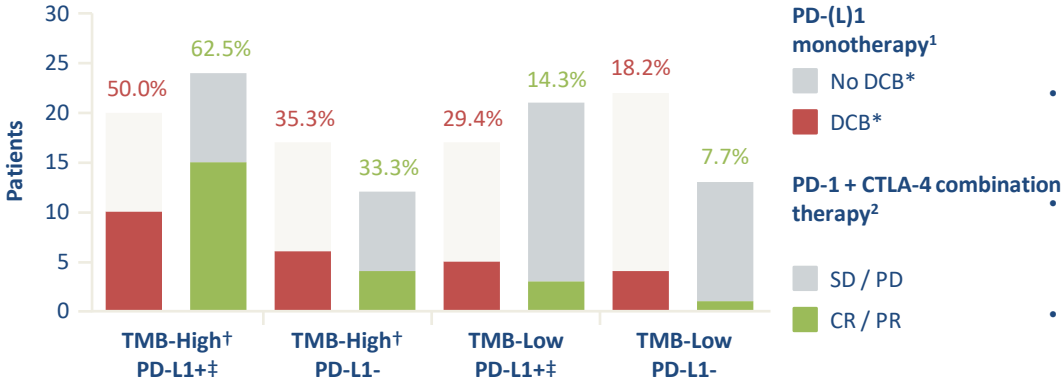
IHC: immunohistochemistry; NSCLC: non-small cell lung cancer; PD-L1: programmed death-ligand 1.

1. Khunger, M., et al. (2017) *JCO Precis Oncol* doi:10.1200/PO.16.00030 [Epub];
2. Garon, E.B., et al. (2015) *N Engl J Med* 372:2018-28; 3. Cottrell, T. and Taube, J.M. (2018) *Cancer J* 24:41-6;
4. Hellmann, M.D., et al. (2018) *Cancer Cell* 33:843-52.

NGS accurately estimates TMB[1], which may be associated with response to immunotherapy

TMB does not correlate with PD-L1 expression and is independently associated with ORR and PFS in NSCLC treated with single-agent or combination immunotherapy[1,2]

Composite measurement of PD-L1 expression and TMB may improve prediction of response to immunotherapies in advanced NSCLC



The relationship between TMB and immuno-therapy efficacy in NSCLC remains uncertain based on recent exploratory analyses

- KEYNOTE-021/-189/-407 showed no significant association between TMB and efficacy ofpembrolizumab + chemotherapy[3]
- KEYNOTE-010/-042 show that high TMB is associated with improved outcomes in PD-L1+ NSCLC treated with pembrolizumab[4]
- Other biomarkers such as *EGFR* / *HER* family, *STK11* and *KRAS* mutational status may provide additional prognostic information[2,5]
- Based on KEYNOTE-158, pembrolizumab was approved as monotherapy for TMB-High (≥ 10 mut/Mb) advanced solid tumours with no satisfactory alternative in June 2020[6]

*Durable clinical benefit defined as SD or PR lasting > 6 months. †TMB-High defined as > median TMB in both studies. Medians may differ between studies. ‡PD-L1+ defined as ≥ 1% tumour membranous staining by immunohistochemistry in both studies.
CR: complete response; DCB: durable clinical benefit; NDB: no durable benefit; NSCLC: non-small cell lung cancer; ORR: objective response rate; PD: progressive disease; PD-L1: programmed-death-ligand 1; PFS: progression-free survival; PR: partial response; SD: stable disease; TMB: tumour mutational burden.
1. Rizvi, H., et al. (2018) *J Clin Oncol* 36:633-41; 2. Hellmann, M.D., et al. (2018) *Cancer Cell* 33:843-52; 3. Paz-Ares, L., et al. (2019) presented at ESMO 2019, abstract LBA80; 4. Herbst, R.S., et al. (2019) presented at ESMO 2019, abstract LBA79; 5. Cinausero, M., et al. (2019) *Ther Adv Med Oncol* 11:1-13; 6. FDA Drug Approvals and Databases (2020) Available at: https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-pembrolizumab-adults-and-children-tmb-h-solid-tumors (Accessed July 2020).

bTMB as a predictor of clinical utility in NSCLC patients receiving atezolizumab

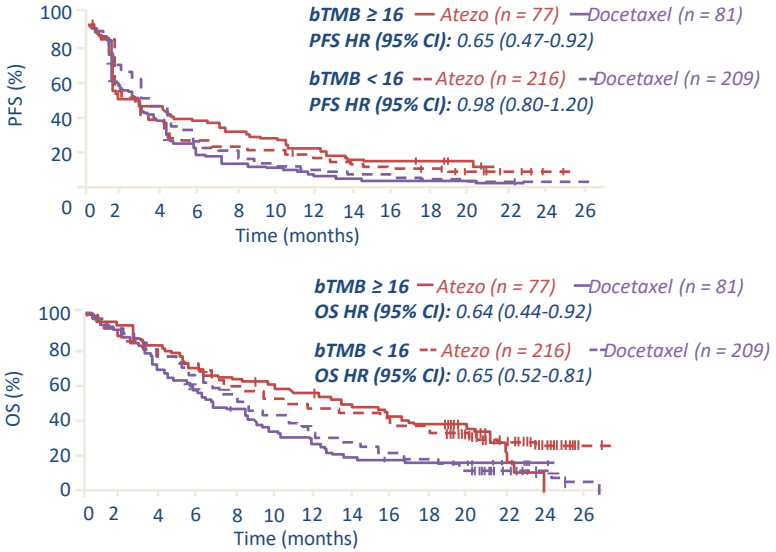
Clinical utility of the **bTMB assay** was tested using **> 1,000 plasma samples** from 2L or higher **aNSCLC pts** prospectively collected from 2 RCTs: **POPLAR and OAK**

POPLAR: bTMB predicts clinical outcome

Improved PFS and OS benefit was observed for all three bTMB cut-points (≥ 10, ≥ 16 and ≥ 20) relative to evaluable pt populations BEP (n = 211) and ITT (n = 287)

	atezo		docetaxel
mPFS:	4.2	vs	2.9 months
mOS:	13.0	vs	7.4 months

OAK: Pts with bTMB ≥ 16 obtained significant survival benefit from atezo



bTMB reproducibly identified aNSCLC pts who derive clinically significant improvements in PFS from atezo

High bTMB is a clinically actionable biomarker for atezo in NSCLC. Use of plasma instead of tissue makes bTMB particularly useful in pts who are not amenable to biopsy or whose tumour tissue is unavailable

1L: first line; atezo: atezolizumab; aNSCLC: advanced NSCLC; BEP: biomarker evaluable population; bTMB: blood based tumour mutational burden; ITT: intent to treat; mPFS: median PFS; mOS: median OS; NSCLC: non small cell lung cancer; OS: overall survival; PFS: progression free survival; pts: patients.

Gandara, D.R., et al. (2018) *Nat Med* 24:1441-48.

Blood TMB and clinical benefit from durvalumab

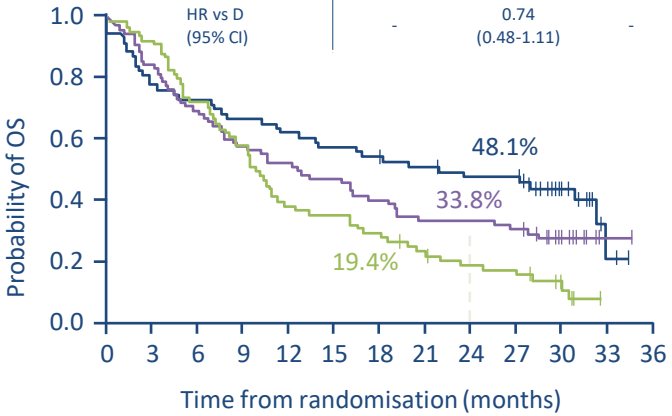
MYSTIC
Phase 3 trial

First line durvalumab with or without tremelimumab (D or D+T) versus platinum-based standard of care chemotherapy (CT) in metastatic NSCLC (n = 1118)

Patients were *EGFR*- and *ALK*-negative, unselected for PD-L1 status, and immunotherapy and chemotherapy naïve

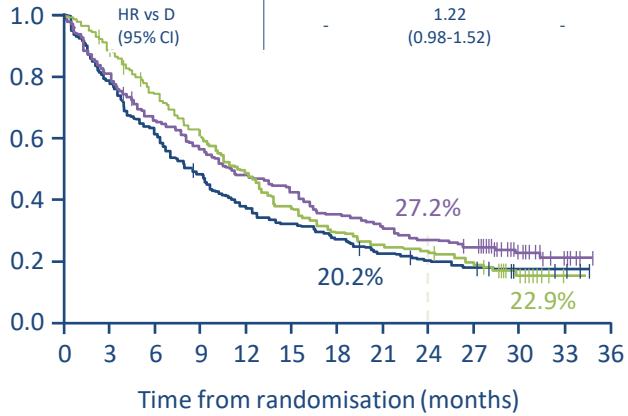
bTMB ≥ 20 mut/Mb

	D (n = 77)	D+T (n = 64)	CT (n = 70)
mOS (months) (95% CI)	12.6 (7.8-18.6)	21.9 (11.4-32.8)	10.0 (8.1-11.7)
HR vs CT (95% CI)	0.72 (0.50-1.05)	0.49 (0.32-0.74)	-
HR vs D (95% CI)	-	0.74 (0.48-1.11)	-



bTMB < 20 mut/Mb

	D (n = 209)	D+T (n = 204)	CT (n = 185)
mOS (months) (95% CI)	11.0 (8.9-14.9)	8.5 (6.7-9.8)	11.6 (9.6-13.1)
HR vs CT (95% CI)	0.93 (0.74-1.16)	1.16 (0.93-1.45)	-
HR vs D (95% CI)	-	1.22 (0.98-1.52)	-



Exploratory analysis
bTMB ≥ 20 associated with:

1. Improved OS (51% reduced risk of death)
2. Improved PFS (47% reduced risk of disease progression)
3. No benefit in bTMB < 20 or tTMB < 10 mut/Mb

bTMB: blood tumour mutational burden; CI: confidence interval; CT: chemotherapy; D: durvalumab;
D+T: durvalumab + tremelimumab; HR: hazard ratio; mOS: median overall survival; mut/Mb: mutations per megabase; NSCLC: non-small cell lung cancer.

Rizvi, N.A., et al. (2020) *JAMA Oncol* 6(5):661-74.

Plasma TMB and outcomes in first line NSCLC treated with pembrolizumab ± chemotherapy

Stage IV NSCLC, treatment naïve, starting first line pembrolizumab based therapy with or without chemotherapy (n = 66)

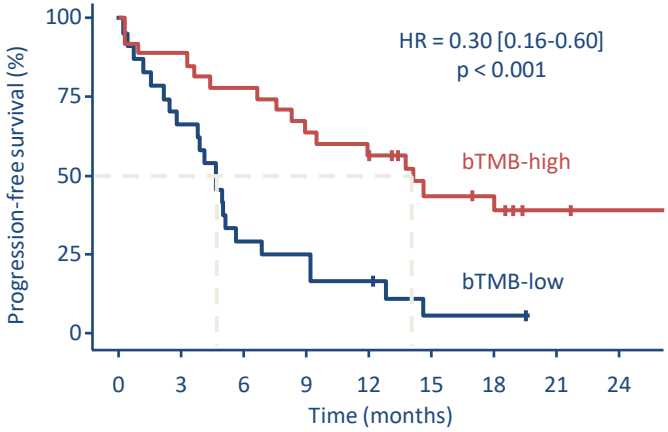
EGFR, *ALK*, *ROS1*, *BRAF* mutated excluded

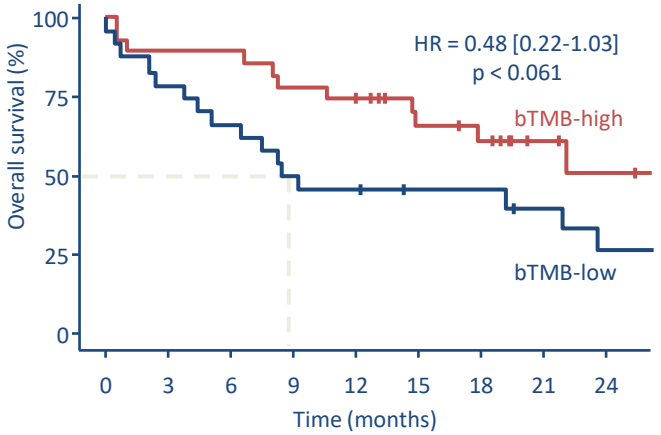
Plasma collected before SoC treatment

bTMB derived from 500 gene panel (~2.1 Mb coverage)

52 patients (78.8%) were TMB evaluable

Survival outcomes – PFS and OS by bTMB using a cutoff of 16 mut/Mb



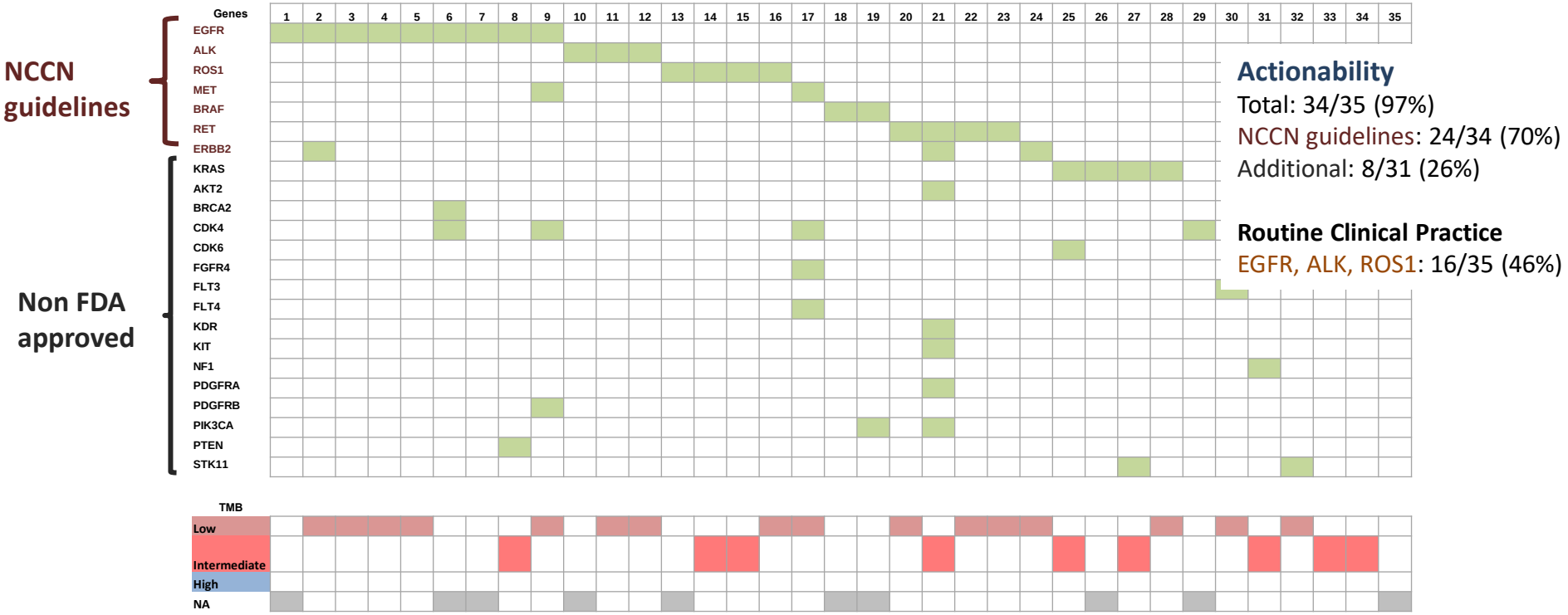


	bTMB ≥ 16 mut/Mb (n = 28)	bTMB < 16 mut/Mb (n = 24)
Median PFS	14.1 months	4.7 months
	HR 0.30 (95% CI = 0.16-0.60)	
Median OS	Not reached	8.8 months
	HR 0.48 (95% CI = 0.22-1.03)	

HR: hazard ratio; mut/Mb: mutations per megabase; OS: overall survival; PFS: progression-free survival; bTMB: blood tumour mutational burden; SoC: standard of care.

Aggarwal, C., et al. (2020) *Clin Cancer Res* 26(10):2354-2361.

Comprehensive Genomic Profiling



Actionability

Total: 34/35 (97%)

NCCN guidelines: 24/34 (70%)

Additional: 8/31 (26%)

Routine Clinical Practice

EGFR, ALK, ROS1: 16/35 (46%)

Verma et al (Unpublished work)

Summary

	Single Platform (PCR, IHC, FISH)	NGS Platform
EGFR	23%	26%
ALK	10%	9%
ROS1		11%
MET		6%
BRAF		6%
RET		11%
ERBB2		9%
KRAS		11%

EGFR, ALK and ROS1 pick-up rate was higher in NGS (approx. 45%) as compared to single platform (33%)

Case Study

56 y/o M Dx with Metastatic NSCLC. EGFR mutation (Del19). Rapid Progression on Erlotinib and Afatinib. T790M present – Progression on Osimertinib.

ABOUT THE TEST:

FoundationOne™ is a next-generation sequencing (NGS) based assay that identifies genomic alterations within hundreds of cancer-related genes.

PATIENT RESULTS

- 12 genomic findings
- 12 therapies associated with potential clinical benefit
- 0 therapies associated with lack of response
- 28 clinical trials

TUMOR TYPE: LUNG ADENOCARCINOMA

Genomic Alterations Identified†

EGFR amplification, exon 19 deletion (L747_S752del)
ERBB2 amplification
RICTOR amplification – equivocal‡
BCL2L1 amplification – equivocal‡
NFKBIA amplification
NKX2-1 amplification
SRC amplification
TOP1 amplification
TP53 L194R

Additional Findings†

Microsatellite status MS-Stable
Tumor Mutation Burden TMB-Low; 4 Muts/Mb

Summary

Standard molecular tests, such as IHC-FISH and CGP complement each other effectively

Immunohistochemistry is important for accurate diagnosis and determination of subtypes in lung cancer, as well
as for assessing expression of specific predictive protein markers such as PD-L1[1-3]

However, utilisation of CGP can:

detect several markers and genomic signatures at once[4]

avoid tissue exhaustion[1,2]

save time[2,5]

IHC and CGP are both important tools in the management of lung cancer and may be used complementary[1,2,6]

CGP: comprehensive genomic profiling; FISH: fluorescence *in-situ* hybridisation; IHC: immunohistochemistry.
1. Domagala-Kulawik, J., et al. (2019) *Front Med (Lausanne)* 6:284; 2. Osmani, L., et al. (2018) *Semin Cancer Biol* 52:103-9;
3. Lindeman, L. I., et al. (2018) *J Mol Diagn* 20:129-59; 4. Frampton, G.M., et al (2013) *Nat Biotech* 31:1023-31;
5. Singh, A.P., et al. (2020) *Cancers* 12:E1156 6. NSCLC NCCN Guidelines Version 2.2020

THANKS