Mechanism of Resistance to ALK Inhibitors

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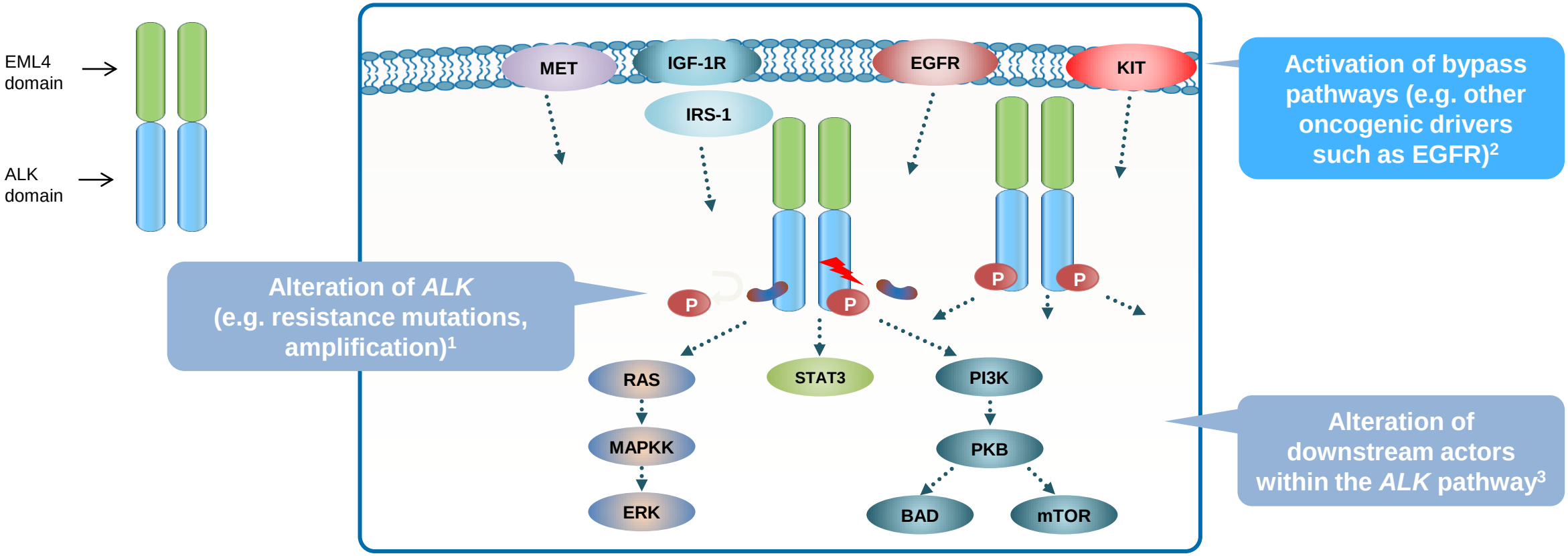
Mechanisms of Resistance

ALK dependent
ALK independent
Activation of bypass pathways
Alterations in downstream actors within the ALK pathway
Epithelial to mesenchymal transition (EMT)
Transformation to SCLC

Acquired resistance is the most common mechanism resulting in disease progression for patients with advanced *ALK*+ NSCLC



Acquired resistance to an ALK TKI can be mediated through *ALK*-dependent or *ALK*-independent mechanisms

ALK = anaplastic lymphoma kinase; BAD = BCL2 associated agonist of cell death; EGFR = epidermal growth factor receptor; EML4 = echinoderm microtubule-associated protein-like 4; ERK = extracellular receptor kinase; IGF-1R = insulin growth factor 1 receptor; IRS-1 = insulin receptor substrate 1; MAPKK = mitogen-activated protein kinase ; mTOR = rapamycin; NSCLC = non-small cell lung cancer; P = phosphate; PI3K = phosphatidylinositol-3; PKB = protein kinase B; STAT3 = signal transducer and activator of transcription 3; TKI = tyrosine kinase inhibitor

1. Golding, et al. Molecular Cancer 2018
2. Malapelle, et al. Transl Genet Genom 2019
3. Pinto, et al. Exp Rev Resp Med 2020

ALK TKI Resistance

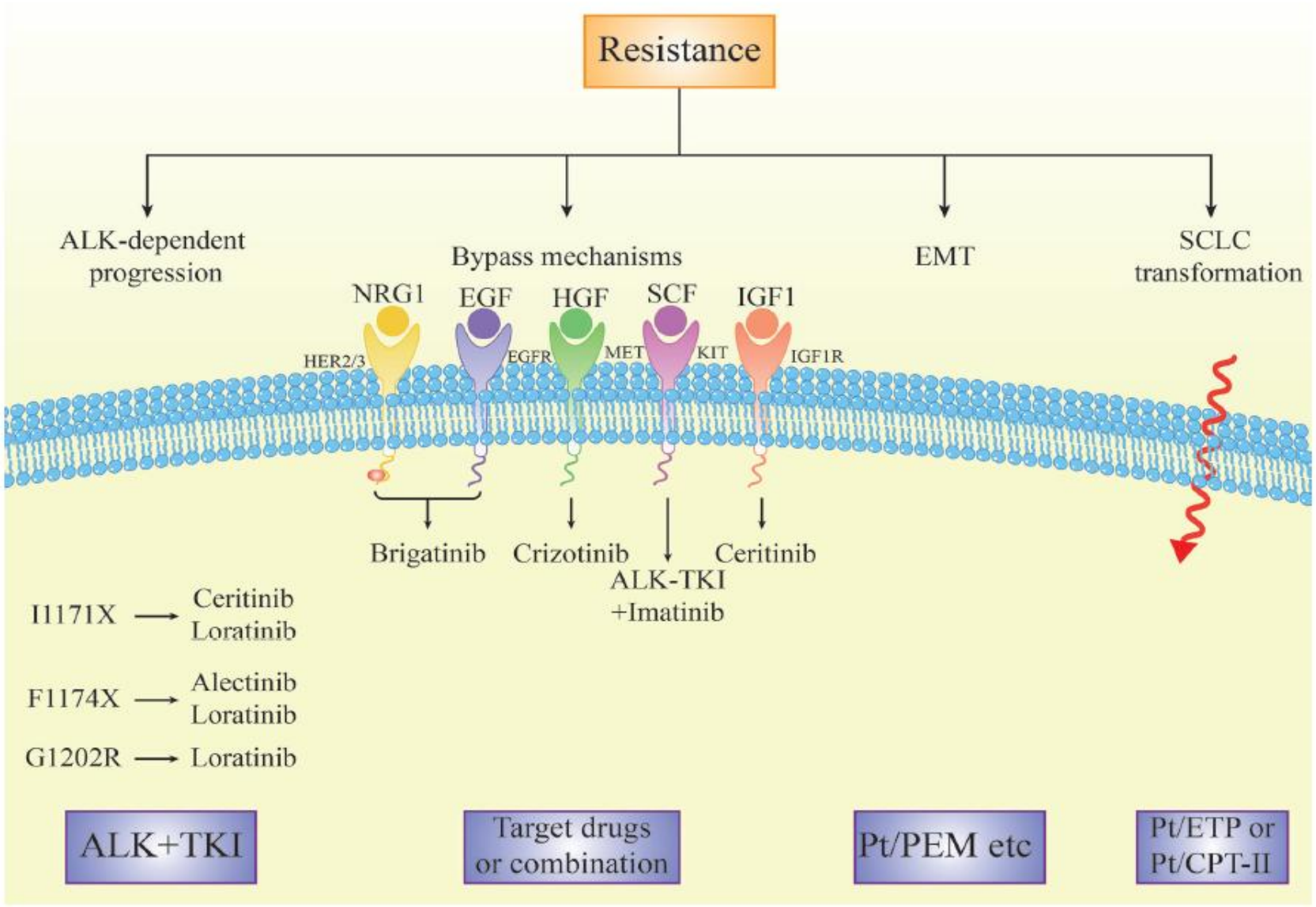


FIGURE 4 | The resistance mechanisms of ALK TKIs in advanced NSCLC and next treatment strategy.

ALK Dependent Resistance

Does prior treatment impact the type of resistance?

Resistance mechanisms after prior Crizotinib

- acquired secondary mutation within the ALK tyrosine kinase domain.
- most common resistance mutation is the **gatekeeper L1196M** mutation, followed closely by the G1269A mutation- deep binding pocket of ATP.
- Other mutations occur at residues 1151, 1152, 1156, 1174, 1202, 1203, and 1206.
- The G1202R confers resistance to crizotinib & 2nd gen ALK inhibitors.
- Amplification of the ALK fusion gene
- Alternative signaling (bypass) pathways

Resistance Mechanisms on 1st and 2nd Generation ALK TKIs-

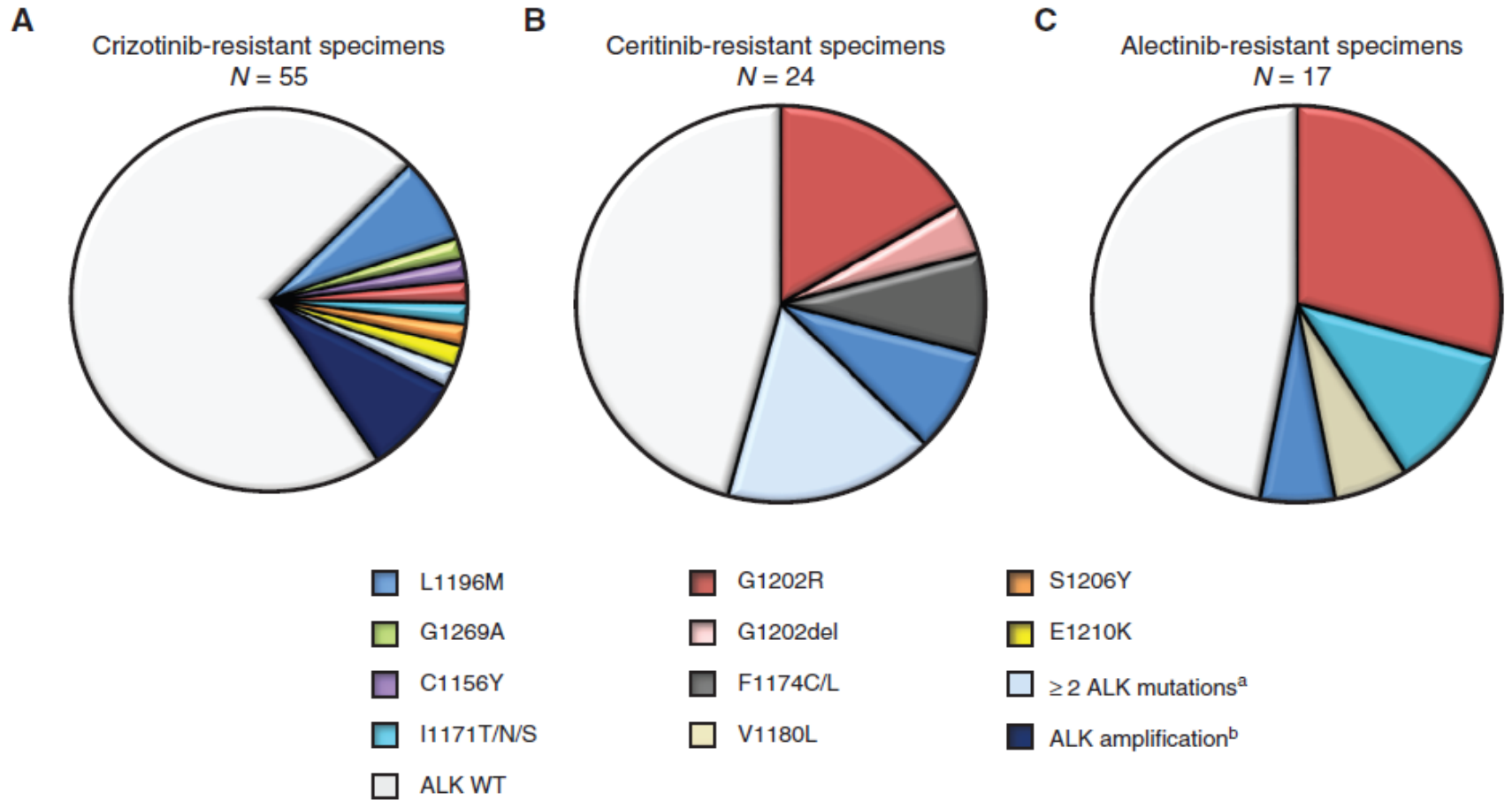


Figure 1. Overview of on-target mechanisms of resistance among ALK-positive specimens obtained from patients progressing on **A,** crizotinib; **B,** ceritinib; and **C,** alectinib. Pie charts depict the frequency and distribution of *ALK* resistance mutations and *ALK* fusion gene amplification in each cohort. Four patients underwent two separate biopsies while on crizotinib; one patient underwent two separate biopsies while on ceritinib. Note that if a specimen is listed as having ≥ 2 *ALK* resistance mutations, the individual mutations are not listed separately. [a]One post-crizotinib specimen harbored *ALK* G1269A and 1151Tins mutations. Four post-ceritinib samples contained ≥ 2 ALK resistance mutations. These included: I1171N+C1156Y, D1203N+F1174C, F1174L+G1202R, and C1156Y+G1202del+V1180L mutations. [b]ALK FISH to assess for fusion gene amplification was performed in only crizotinib-resistant specimens ($N = 36$), of which 8% had amplification. Ceritinib- and alectinib-resistant specimens were not assessed for *ALK* amplification by FISH. WT, wild-type.

Resistance Mechanisms after 2nd gen TKI

RESEARCH ARTICLE

Molecular Mechanisms of Resistance to First- and Second-Generation ALK Inhibitors in *ALK*-Rearranged Lung Cancer

Justin F. Gainor[1], Leila Dardaei[1], Satoshi Yoda[1], Luc Friboulet[1,2], Ignaty Leshchiner[3], Ryohei Katayama[1,4], Ibiayi Dagogo-Jack[1], Shirish Gadgeel[5], Katherine Schultz[1], Manrose Singh[1], Emily Chin[1], Melissa Parks[1], Dana Lee[1], Richard H. DiCecca[1], Elizabeth Lockerman[1], Tiffany Huynh[6], Jennifer Logan[1], Lauren L. Ritterhouse[6], Long P. Le[6], Ashok Muniappan[7], Subba Digumarthy[8], Colleen Channick[1], Colleen Keyes[1], Gad Getz[3], Dora Dias-Santagata[6], Rebecca S. Heist[1], Jochen Lennerz[6], Lecia V. Sequist[1], Cyril H. Benes[1], A. John Iafrate[6], Mari Mino-Kenudson[6], Jeffrey A. Engelman[1], and Alice T. Shaw[1]

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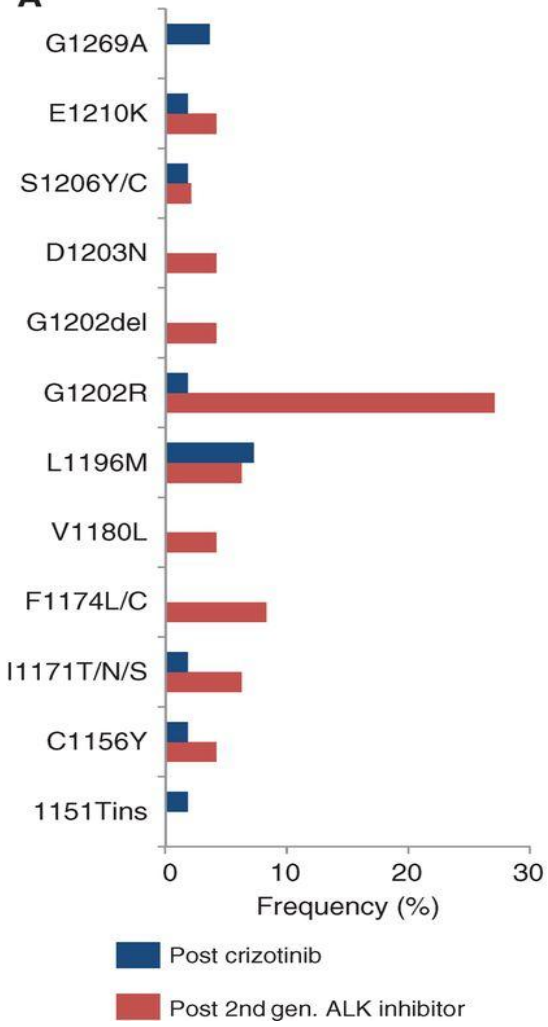
Fig A: comparison of the frequency and distribution of ALK resistance mutations in biopsy specimens obtained after disease progression on crizotinib (blue) or second-generation ALK inhibitors (red).

Fig B: breakdown of specific ALK resistance mutations in ALK-positive patients progressing on crizotinib, ceritinib, alectinib, or brigatinib.

A



B

ALK resistance mutations[a]	Crizotinib (*N* = 55)	Ceritinib (*N* = 24)	Alectinib (*N* = 17)	Brigatinib (*N* = 7)
1151Tins	2%	0%	0%	0%
C1156Y	2%	8%	0%	0%
I1171T/N/S	2%	4%	12%	0%
F1174L/C	0%	17%	0%	0%
V1180L	0%	4%	6%	0%
L1196M	7%	8%	6%	0%
G1202R	2%	21%	29%	43%
G1202del	0%	8%	0%	0%
D1203N	0%	4%	0%	14%
S1206Y/C	2%	0%	0%	14%
E1210K	2%	0%	0%	29%
G1269A	4%	0%	0%	0%
ALK mutations[b]	**20%**	**54%**	**53%**	**71%**

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ALK resistance mutations were present in 56% of ALK-positive patients progressing on second-generation ALK inhibitors (ceritinib, 54%; alectinib, 53%; and brigatinib, 71%).

ALK resistance mutations were observed in only 20% of ALK-positive patients progressing on crizotinib,

Treatment with more potent second-generation ALK inhibitors was associated with a significantly higher frequency of ALK resistance mutations (P = 0.0002) and a **different spectrum of such mutations.**

ALK G1202R emerged as the most common ALK resistance mutation among patients receiving second-generation ALK inhibitors

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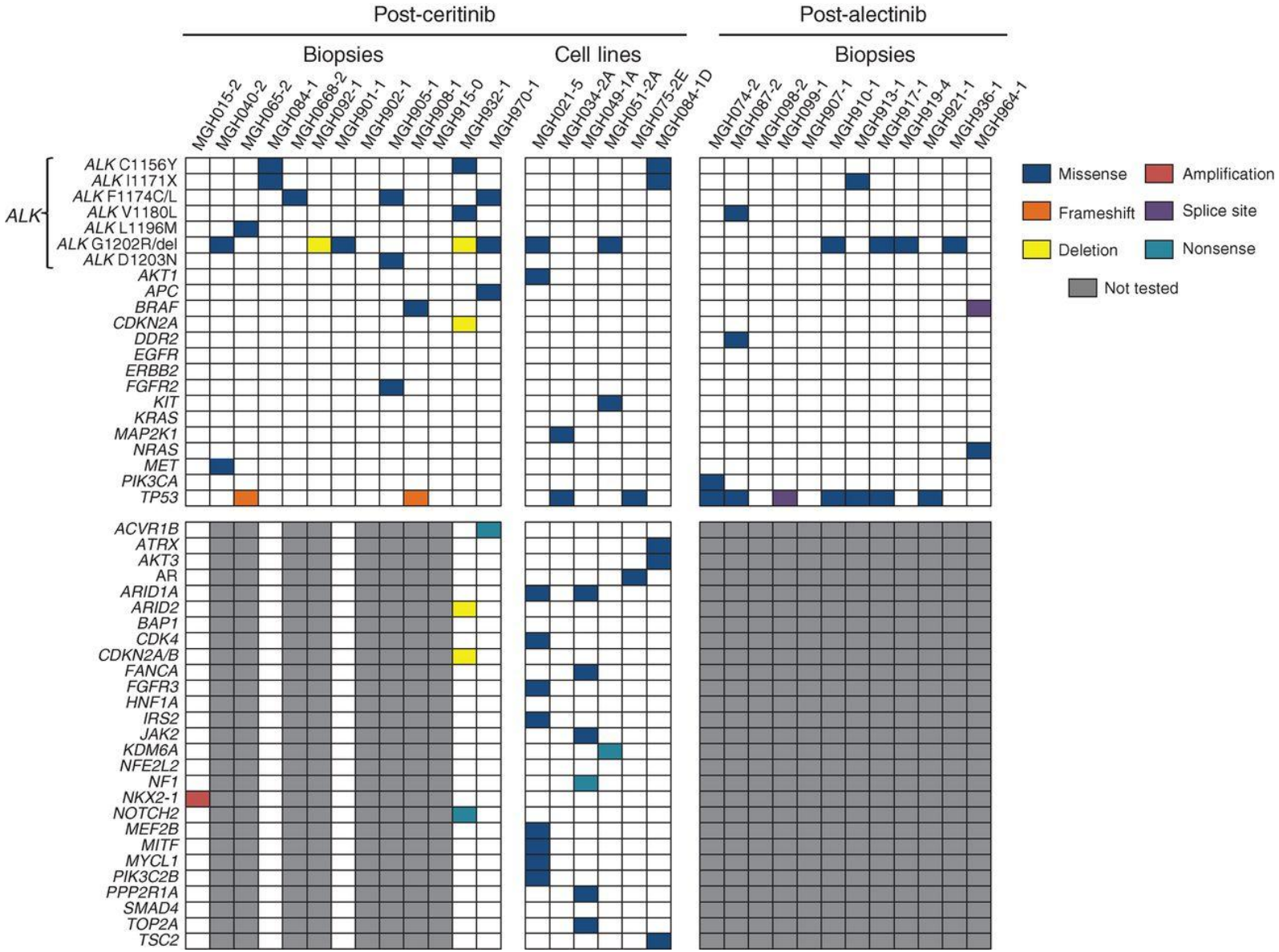
Second generation ALKi have increased activity,

The larger molecular volume of their compounds, which is heavily dependent on the direct binding to the **solvent front region such as G1202** in order to increase its activity

“inducing” resistance mutations within this region.

Summary of genetic alterations in resistant biopsies among patients progressing on ceritinib or alectinib.



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Cellular ALK phosphorylation mean IC_{50} (nmol/L)

Mutation status	Crizotinib	Ceritinib	Alectinib	Brigatinib	Lorlatinib
Parental Ba/F3	763.9	885.7	890.1	2774.0	11293.8
EML4–ALK V1	38.6	4.9	11.4	10.7	2.3
EML4–ALK C1156Y	61.9	5.3	11.6	4.5	4.6
EML4–ALK I1171N	130.1	8.2	397.7	26.1	49.0
EML4–ALK I1171S	94.1	3.8	177.0	17.8	30.4
EML4–ALK I1171T	51.4	1.7	33.6[a]	6.1	11.5
EML4–ALK F1174C	115.0	38.0[a]	27.0	18.0	8.0
EML4–ALK L1196M	339.0	9.3	117.6	26.5	34.0
EML4–ALK L1198F	0.4	196.2	42.3	13.9	14.8
EML4–ALK G1202R	381.6	124.4	706.6	129.5	49.9
EML4–ALK G1202del	58.4	50.1	58.8	95.8	5.2
EML4–ALK D1203N	116.3	35.3	27.9	34.6	11.1
EML4–ALK E1210K	42.8	5.8	31.6	24.0	1.7
EML4–ALK G1269A	117.0	0.4	25.0	ND	10.0
EML4–ALK D1203N+F1174C	338.8	237.8	75.1	123.4	69.8
EML4–ALK D1203N+E1210K	153.0	97.8	82.8	136.0	26.6

$IC_{50} \leq 50$ nmol/L

$IC_{50} > 50 < 200$ nmol/L

$IC_{50} \geq 200$ nmol/L

Lorlatinib potently inhibits ALK resistance mutations, including $ALK^{G1202R.}$

Absolute IC50 values of crizotinib, ceritinib, alectinib, brigatinib, and lorlatinib on cellular ALK phosphorylation in Ba/F3 cells harboring wild-type EML4–ALK variant 1 or various EML4–ALK resistance mutants are depicted.

In Ba/F3 cells, ALK^{F1174C} and ALK^{I1171T} appear sensitive to ceritinib and alectinib, respectively; however, these mutations may not be susceptible to these agents in vivo based upon prior clinical reports. ND, not done.

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Double /Complex Mutations

Compound ALK Mutations as Resistance mechanism

TABLE 3 | Compound mutations and Treatment recommendations.

Team	Previous treatment	Compound mutation	Note
Takashi, Ken et al	Crizotinib,alectinib,lorlatinib	I1171S+G1269A	Recommended drugs: ceritinib, brigatinib
Shaw AT et al (45)	Crizotinib, lorlatinib	C1156Y+L1198F	re-sensitization: Crizotinib
Okada K et al (65)	Alectinib, lorlatinib	I1171N+L1256F	re-sensitization: Alectinib
Okada K et al (65)		I1171N+L1198F	Compound mutations are more sensitive to crizotinib than I1171N single mutants

Complex Mutations

Sequential ALKi treatment, the cancer cells accumulate new mutations in addition to the previously acquired mutations, making treatment more complex. [1]

Not all complex mutations increase the difficulty of treatment

Some compound mutations that lead to lorlatinib resistance led to re-sensitization of the first or second generation ALKi . [2]

1- Pan Y, et al. Front. Oncol. 11:713530.Okada K, et al.
2- EBioMedicine (2019) 41:105–19.

A patient receiving sequential treatment for ALK-positive NSCLC was resistant to crizotinib due to the mutation C1156Y in the ALK kinase region. [1]
Sequencing revealed the mutation ALK L1198F in addition to C1156Y. [1]
The L1198F mutation developed resistance to lorlatinib through spatial interference with drug binding. [1]
The **L1198F mutation** enhanced its binding to crizotinib, making it sensitive to the C1156Y mutation. The patient was treated again with crizotinib. [1]
Multiple studies **- L1198F** mutation leads to conformational changes in the inhibitor site as well as changes in the binding affinity of ALK to crizotinib and lorlatinib [2].

1-Shaw A.T. et al. N Engl J Med (2016) 374(1):54–61.
2- Li J, et al. Int J Mol Sci (2017) 18(3):482.

Molecular Mechanisms of Resistance to First- and Second-Generation ALK Inhibitors in ALK-Rearranged Lung Cancer

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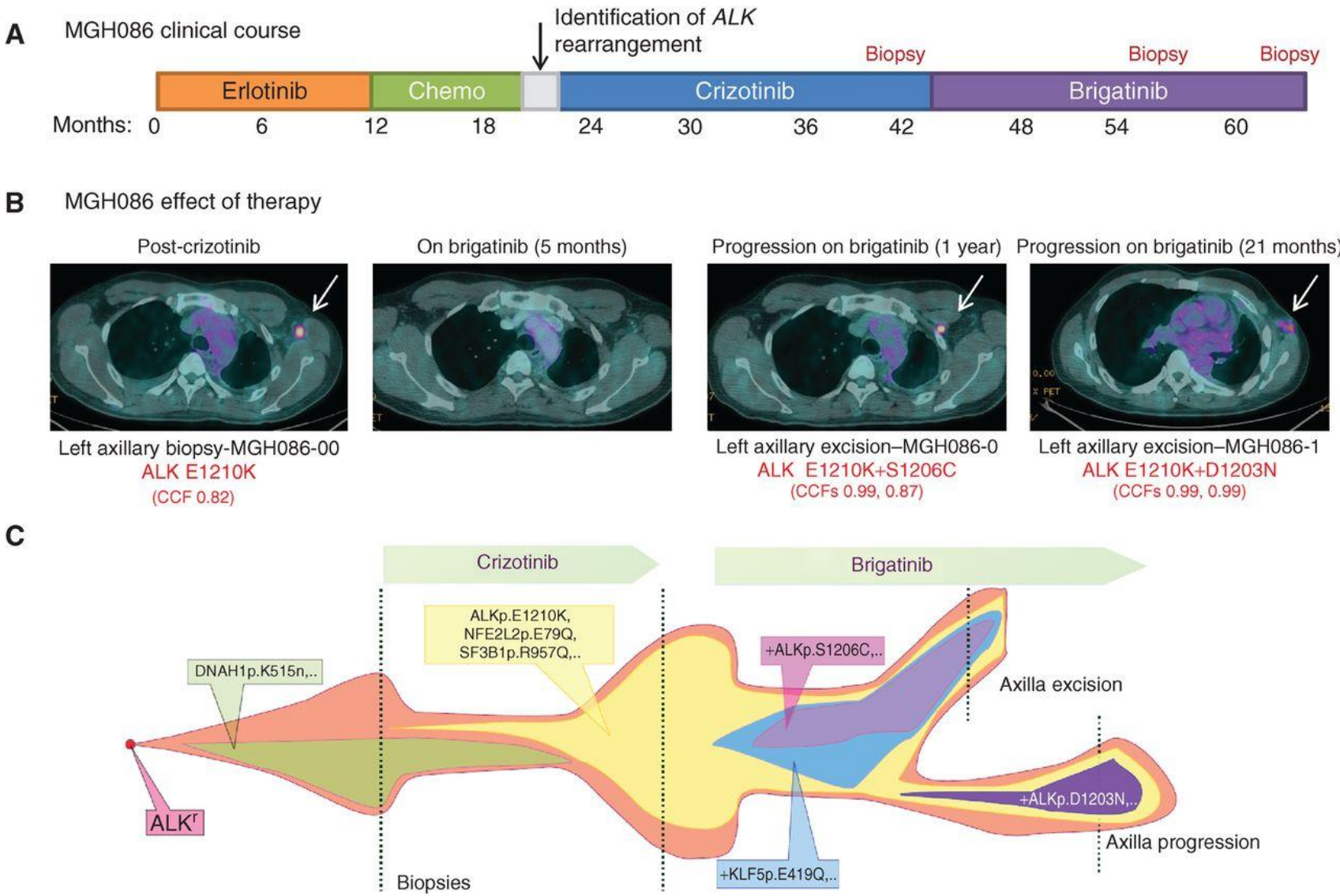
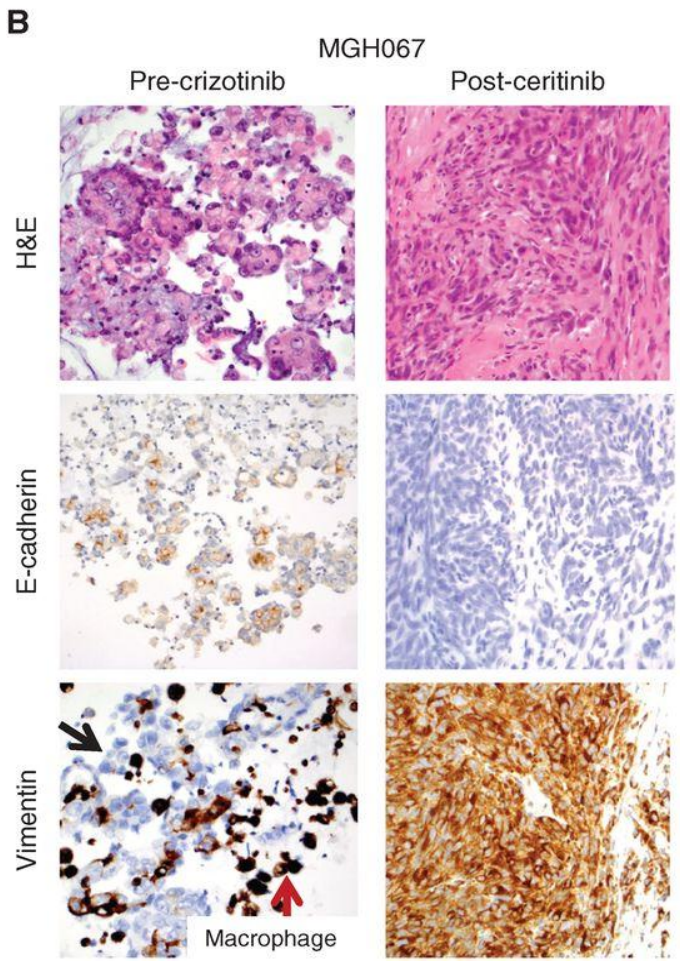


Epithelial to Mesenchymal Transformation

EMT is associated with ceritinib resistance



C

Assessments of EMT in ceritinib-resistant biopsies

Patient ID	ALK resistance mutation	Vimentin	E-cadherin
MGH023-2	ALK F1174C	**Positive**	**Negative**
MGH034-2	WT	**Positive**	**Negative**
MGH049-1	WT	Positive	Positive
MGH051-2	ALK G1202R	Positive	Positive
MGH061-1	WT	Negative	Positive
MGH065-2	ALK L1196M	**Positive**	**Negative**
MGH067-1	ALK L1196M	**Positive**	**Negative**
MGH084-1	ALK I1171N, C1156Y	Negative	Positive
MGH089-1	WT	Negative	Positive
MGH092-1	ALK G1202del	Negative	Positive
MGH902-1	WT	**Positive**	**Negative***
MGH908-1	WT	Negative	Positive

*Partial loss

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- Epithelial-to-mesenchymal transition (EMT) is a morphological change in which epithelial cells lose their polarity and intercellular connections becoming more mobile and invasive.
- Through EMT, tumor cells acquire mesenchymal morphology and the ability to migrate and invade.
- There are four pathways associated with EMT: proteoglycan in cancer, HIF-1 signaling, FoxO signaling, and extracellular matrix receptor interactions, related to the drug resistance mechanisms of crizotinib.
- ALK mutants L1196M and EMT were simultaneously detected

Lorlatinib Resistance

Precision Medicine and Imaging

Clinical Cancer Research

Treatment with Next-Generation ALK Inhibitors Fuels Plasma *ALK* Mutation Diversity

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Abstract

Purpose: Acquired resistance to next-generation ALK tyrosine kinase inhibitors (TKIs) is often driven by secondary *ALK* mutations. Here, we investigated utility of plasma genotyping for identifying *ALK* resistance mutations at relapse on next-generation ALK TKIs.

Experimental Design: We analyzed 106 plasma specimens from 84 patients with advanced *ALK*-positive lung cancer treated with second- and third-generation ALK TKIs using a commercially available next-generation sequencing (NGS) platform (Guardant360). Tumor biopsies from TKI-resistant lesions underwent targeted NGS to identify *ALK* mutations.

Results: By genotyping plasma, we detected an *ALK* mutation in 46 (66%) of 70 patients relapsing on a second-generation ALK TKI. When post-alectinib plasma and tumor specimens were compared, there was no difference in frequency of *ALK* mutations (67% vs. 63%), but plasma specimens were more likely to harbor ≥2 *ALK* mutations (24% vs. 2%, $P = 0.004$). Among 29 patients relapsing on lorlatinib, plasma genotyping detected an *ALK* mutation in 22 (76%), including 14 (48%) with ≥2 *ALK* mutations. The most frequent combinations of *ALK* mutations were G1202R/L1196M and D1203N/1171N. Detection of ≥2 *ALK* mutations was significantly more common in patients relapsing on lorlatinib compared with second-generation ALK TKIs (48% vs. 23%, $P = 0.017$). Among 15 patients who received lorlatinib after a second-generation TKI, serial plasma analysis demonstrated that eight (53%) acquired ≥1 new *ALK* mutations on lorlatinib.

Conclusions: *ALK* resistance mutations increase with each successive generation of ALK TKI and may be underestimated by tumor genotyping. Sequential treatment with increasingly potent ALK TKIs may promote acquisition of *ALK* resistance mutations leading to treatment-refractory compound *ALK* mutations.

Lorlatinib

Lorlatinib is a reversible third-generation ALK and ROS1 inhibitor that can overcome multiple ALK resistance mutations.

Lorlatinib has strong activity for common mutations such as **L1196M and G1269A.**

The **G1202R** mutation is particularly important as it is the primary resistance mechanism to ceritinib, alectinib, and brigatinib,

Only lorlatinib can inhibit the ALK G1202R mutation.

The whole exome sequencing of compound ALK mutations occurring in several lorlatinib-resistant patients confirms the stepwise accumulation of ALK mutations during sequential treatment.

Several of these ALK kinase compound mutations that have been described include the L1196M/D1203N, F1174L/ G1202R, and C1156Y/G1269A mutations

Role of CGP in treatment of ALK rearranged NSCLC after failure of 2nd/3rd Gen ALK TKI

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Genomic Profiling (CGP) on the Decision-Making Process in the Treatment of ALK-Rearranged Advanced Non-Small Cell Lung Cancer (aNSCLC) After Failure of 2nd/3rd-Generation ALK Tyrosine Kinase Inhibitors (TKIs)

Ari Raphael[1,2†], Amir Onn[2,3†], Liran Holtzman[2], Julia Dudnik[4], Damien Urban[3], Waleed Kian[5], Aharon Y. Cohen[4], Mor Moskovitz[6], Alona Zer[6], Jair Bar[2,3], Natalie Maimon Rabinovich[7], Shirly Grynberg[3], Cecilie Oedegaard[3], Abed Agbarya[8], Nir Peled[5,9], Tzippy Shochat[10] and Elizabeth Dudnik[9,11,12*] on behalf of Israel Lung Cancer Group on behalf of Israel Lung Cancer Group

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Background: The use of CGP in guiding treatment decisions in aNSCLC with acquired resistance to ALK TKIs is questionable.

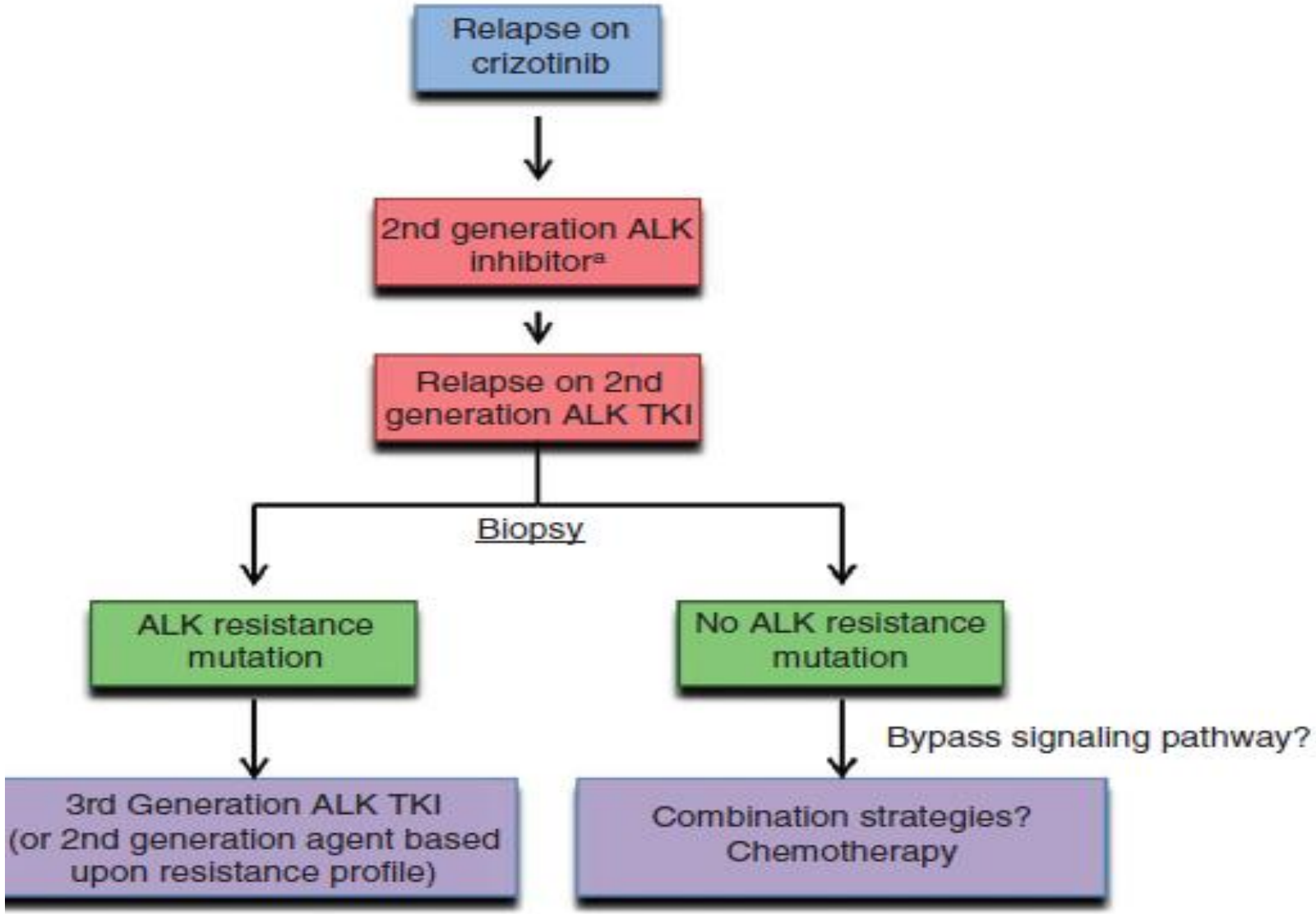
Methods: We prospectively assessed the impact of CGP on the decision-making process in ALK-rearranged aNSCLC patients following progression on 2nd/3rd-generation ALK TKIs. Physician's choice of the most recommended next-line systemic treatment (NLST) was captured before and after receival of CGP results; the percentage of cases in which the NLST recommendation has changed was assessed along with the CGP turnaround time (TAT). Patients were divided into groups: patients in whom the NLST was initiated after (group 1) and before (group 2) receival of the CGP results. Time-to-treatment discontinuation (TTD) and overall survival (OS) with NLST were compared between the groups.

Results: In 20 eligible patients (median [m]age 63 years [range, 40-89], females 75%, adenocarcinoma 100%, failure of alectinib 90%, FoundationOne Liquid CDx 80%), CGP has altered NLST recommendation in 30% of cases. CGP findings were as follows: ALK mutations 30% (I1171X 10%, G1202R, L1196M, G1269A, G1202R+I1171N+E1210K 5% each), CDKN2A/B mutation/loss 10%, c-met amplification 5%. CGP mTAT was 2.9 weeks [IQR, 2.4-4.4]. mTTD was 11.3 months (95% CI, 2.1-not reached [NR]) and 5.4 months (95% CI, 2.0-NR) in groups 1 and 2, respectively (p-0.34). mOS was 13.2 months (95% CI, 2.9-NR) and 13.0 months (95% CI, 6.0-NR) in groups 1 and 2, respectively (p-0.86).

Conclusion: CGP has a significant impact on the decision-making process in ALK-rearranged aNSCLC following progression on 2nd/3rd-generation ALK TKIs.

Keywords: comprehensive genomic profiling, next-generation sequencing, ALK, failure of ALK TKI, acquired resistance, decision impact

Tissue biopsy post relapse on 2nd Generation ALK TKI-



ALK Treatment Algorithm- Sequencing

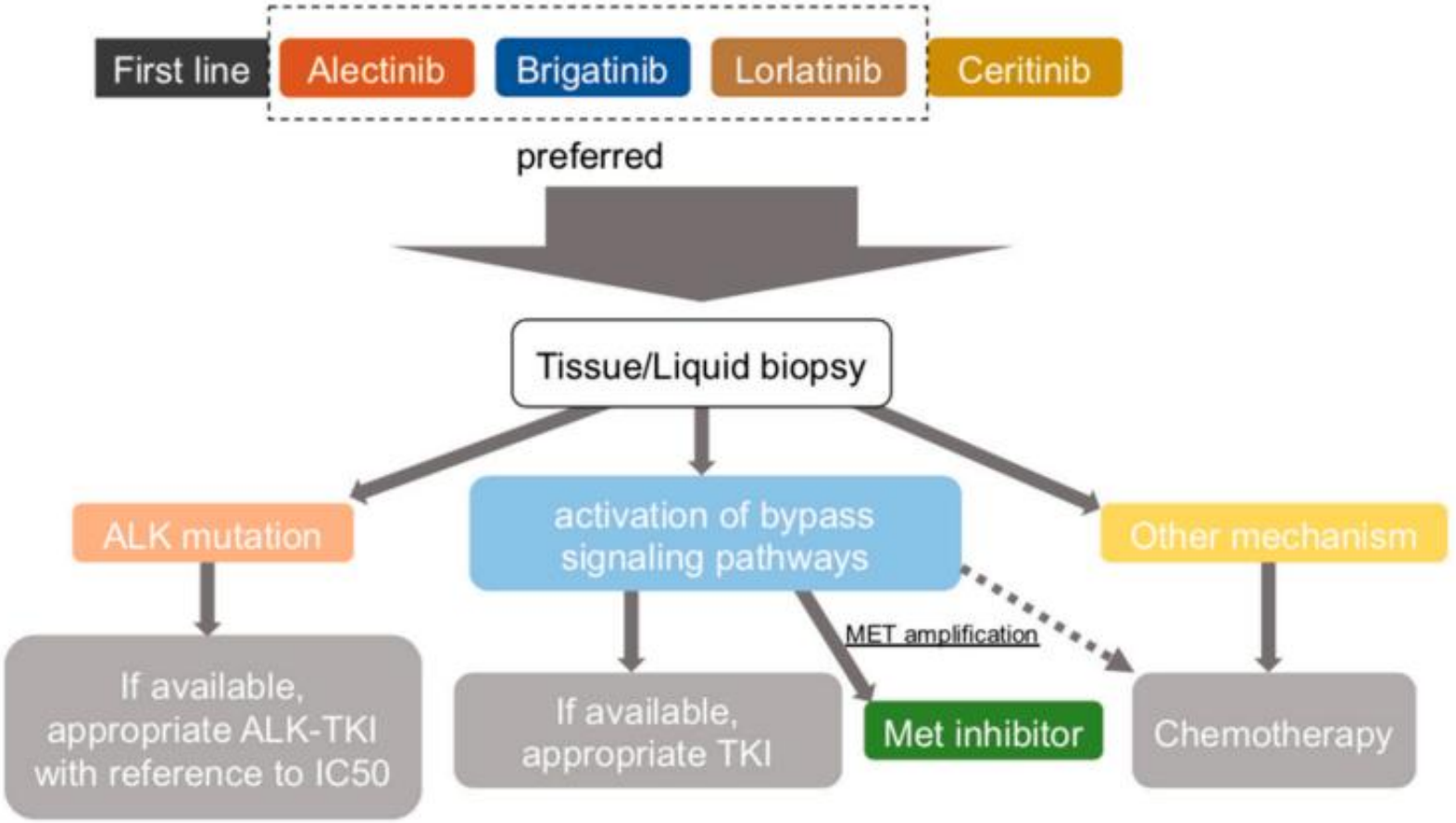


Figure 2. Proposed treatment algorithm for ALK-rearranged advanced NSCLC.

- Need to look at $2^{nd}/3^{rd}$ Line data of Lorlatinib through resistance mechanism lens

Tissue Biopsy on ALK TKI Progression-

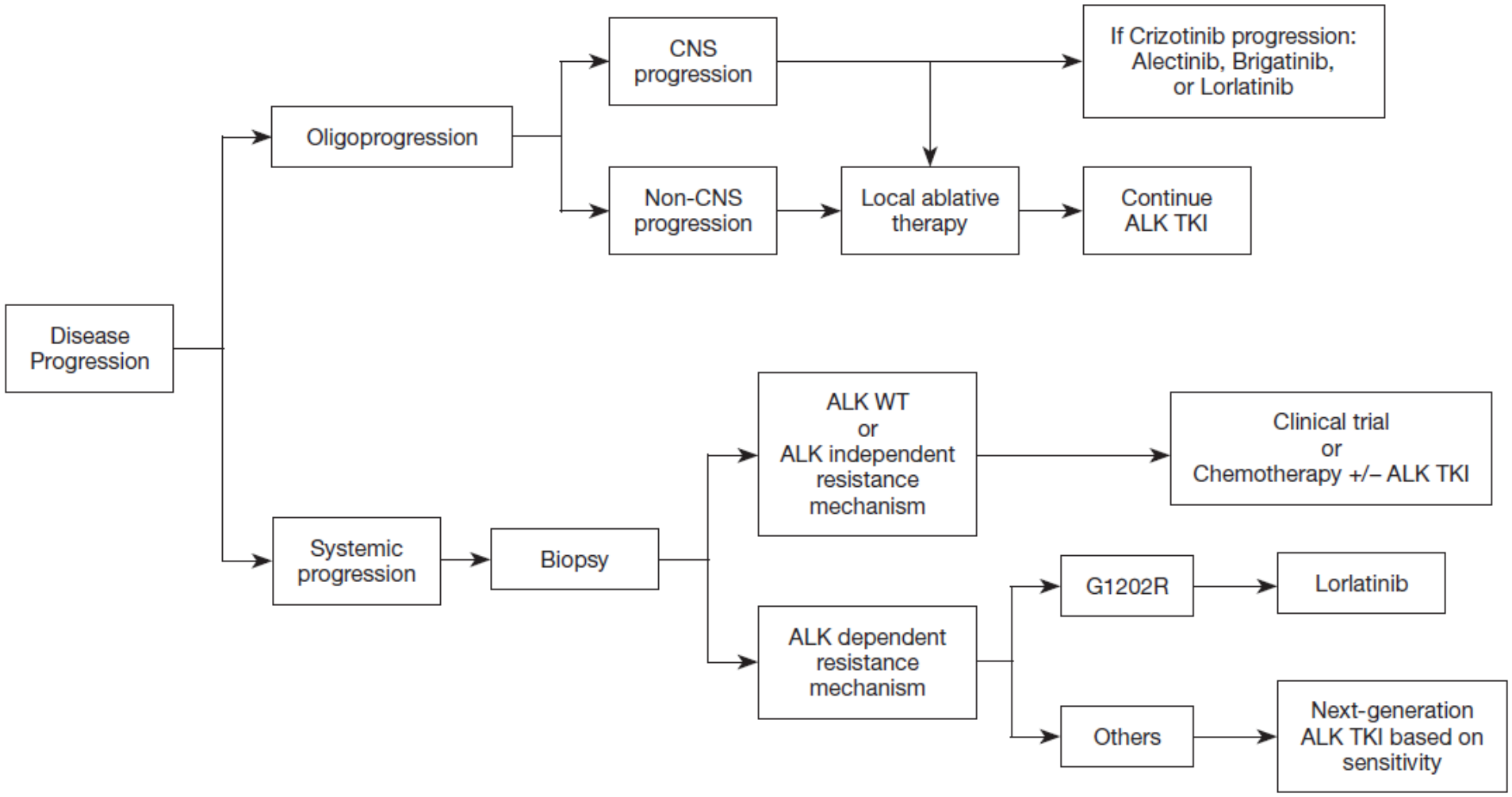
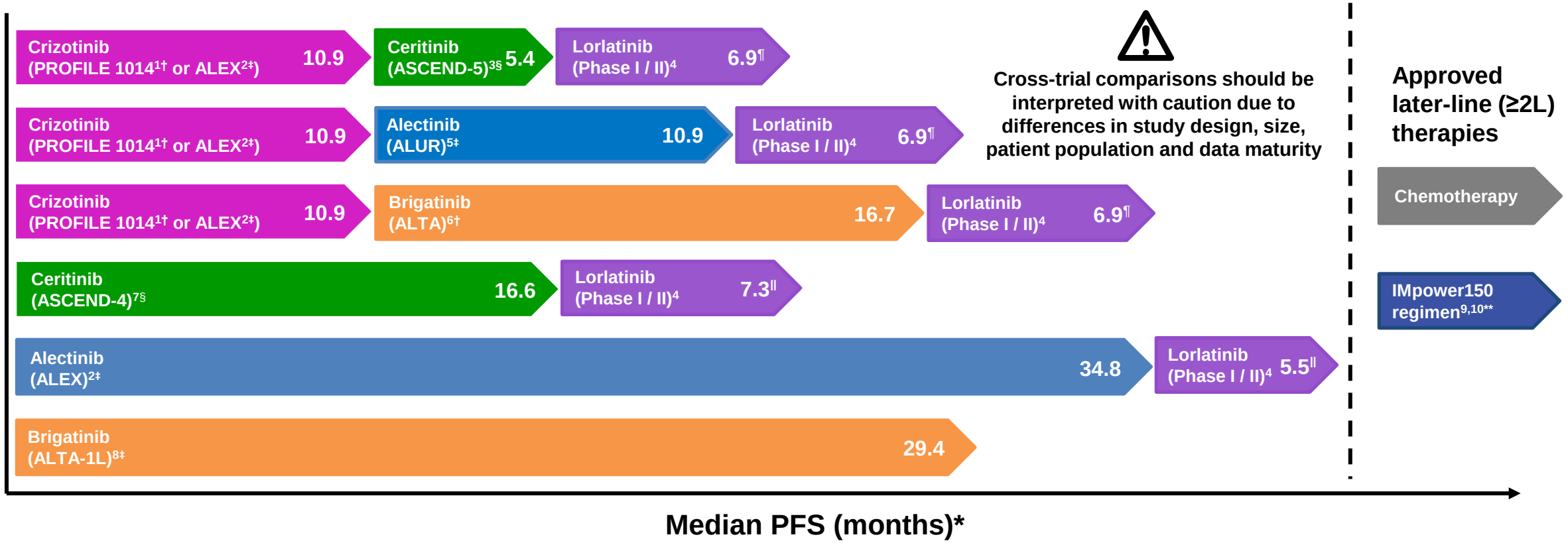


Figure 1 Recommended treatment algorithm for disease progression on ALK tyrosine kinase. CNS, central nervous system; ALK, anaplastic lymphoma kinase; TKI, tyrosine kinase inhibitors.

There are now multiple treatment sequence options for patients with advanced *ALK*+ NSCLC



Treating patients in the 1L setting with approved ALK TKIs such as crizotinib or ceritinib increases the risk that patients will progress sooner than if treated with 1L alectinib

*Adapted and updated from Ferrara et al, 2018.[11] For illustration purposes only. Median PFS for ALK TKIs that are currently approved in the 1L or ≥2L setting are shown; †Median PFS by IRC; ‡Median PFS by INV; §Median PFS by BIRC; ¶Data from the EXP4 + EXP5 group (two or three prior ALK TKIs ± CT) ‖Lorlatinib PFS data following ceritinib or alectinib in **any line**; **EMA-approved only (the IMpower150 regimen is not FDA-approved for use in pretreated, advanced *ALK*+ NSCLC)

1L = first line; 2L = second line; *ALK* = anaplastic lymphoma kinase BIRC = blinded independent review committee; CT = chemotherapy
EMA = European Medicines Agency; FDA = Food and Drug Administration; INV = investigator IRC = independent review committee
NSCLC = non-small cell lung cancer; PFS = progression-free survival; TKI = tyrosine kinase inhibitor

1. Solomon, et al. N Engl J Med 2014; 2. Mok, et al. Ann Oncol 2020
3. Shaw, et al. Lancet Oncol 2017; 4. Besse, et al. ASCO 2018
5. Wolf, et al. WCLC 2019; 6. Huber, et al. J Thorac Oncol 2020
7. Soria, et al. Lancet 2017; 8. Camidge, et al. J Clin Oncol 2020
9. Socinski, et al. ASCO 2018; 10. Atezolizumab SmPC
11. Ferrara, et al. J Thorac Oncol 2018

Thank You!