



A Novel Sequentially Evolved *EML4-ALK* Variant 3 G1202R/S1206Y Double Mutation In *Cis* Confers Resistance to Lorlatinib: A Brief Report and Literature Review

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ABSTRACT

Lorlatinib is a third-generation ALK inhibitor that can overcome the largest number of acquired ALK resistance mutations, including the solvent-front mutation G1202R. Here, we report, for the first time, a novel, sequentially-evolved *EML4-ALK* variant 3 G1202R/S1206Y double mutation in *cis* detected in a patient with ALK-positive NSCLC after disease progression on sequential crizotinib, alectinib, and then lorlatinib. Three-dimensional computer modeling of this double mutation and other G1202R-based double mutations with lorlatinib (*ALK* G1202R/L1196M, *ALK* G1202R/F1174C, *ALK* G1202R/I1198F, *ALK* G1202R/G1269A) were provided to reveal how these double mutations may confer resistance to lorlatinib through diverse steric hindrances in the ALK kinase domain. In addition, we performed a comprehensive literature review on published acquired double or triple ALK mutations that are resistant to lorlatinib from both patient samples and in vitro mutagenesis experiments.

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Keywords: G1202R; S1206Y; Double mutation; Lorlatinib resistance; ALK+ NSCLC

Introduction

Lorlatinib is a third-generation ALK tyrosine kinase inhibitor (TKI) that can overcome the largest number of

acquired ALK resistance mutations^{1,2} regardless of whether they emerge in the background of the two major *EML4-ALK* fusion variants (variants 1 and 3).² On the basis of a phase 2 study,³ lorlatinib is approved both in

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the United States (November 2, 2018) and by the European Medical Agency (February 28, 2019) for the treatment of patients with *ALK*-positive NSCLC who have progressed on either first-line (1L) ceritinib or 1L alectinib or crizotinib and at least another *ALK* TKI. Sequential use of *ALK* TKIs from the first generation to second generation (2G) and then to lorlatinib has led to acquired double or triple resistance mutations.⁴⁻⁷ Here, we report a novel acquired *ALK* double mutation in *cis* that is resistant to lorlatinib after several years of lorlatinib use following sequential treatment with crizotinib and alectinib.

Methods and Results

Case Description

The patient is a never-smoking Asian woman who was diagnosed with stage IV NSCLC with malignant pleural effusion in June 2010 at age 57 years (Fig. 1A). She received carboplatin/nab-paclitaxel/bevacizumab for four cycles, followed by bevacizumab/pemetrexed maintenance for three cycles. Her tumor was profiled at the University of Colorado as part of the Lung Cancer Mutation Consortium and found to be positive for *ALK* by fluorescence in-situ hybridization (FISH). The patient was referred to the crizotinib phase 2 trial (PROFILE1005, NCT00932451), but tested negative for *ALK* by FISH by the trial-designated central laboratory, so she was enrolled into the “*ALK*-negative” cohort of the crizotinib phase 1 trial (PROFILE1001, NCT00585195). The false-negative result by the central laboratory in the early days of crizotinib clinical trial was likely owing to the steep learning curve of analyzing *ALK* FISH results because of the complexity of interpreting break-apart FISH (break-apart probes have to be separated by at least two probe sizes to be considered positive; interpreting 3' isolated *ALK* signals; counting 15% of tumor cells with break-apart signals based on at least 50 unique tumors cells). She received crizotinib at 250 mg twice daily from February 2011 to May 2013 with confirmed partial response as the best response. On disease progression, she was enrolled in the alectinib phase 1/2 study (NCT01871805) from May 2013 to October 2013 with progressive disease as the best response. Next-generation sequencing of a metastatic right axillary lymph node revealed the presence of *EML4-ALK* variant 3 fusion and *ALK* G1202R mutation conferring resistance to alectinib.

She was then retreated with crizotinib with the addition of carboplatin/pemetrexed plus bevacizumab followed by maintenance pemetrexed plus bevacizumab from October 2013 to September 2015 before enrollment into the three previous *ALK* TKIs cohort of phase 2 lorlatinib study (NCT01970865). She achieved confirmed partial response as the best response with

lorlatinib at 100 mg daily until December 2019, when she developed headache and jaw pain with brain and cervical spine magnetic resonance imaging revealing four subcentimeter brain lesions and a soft tissue mass around the odontoid. This was the first evidence of brain metastasis during her almost 10-year course of *ALK*+ NSCLC. Circulating tumor DNA obtained as previously described⁵ at that time revealed *EML4-ALK* variant 3 plus the emergence of an on-target *ALK* S1206Y resistance mutation (mutant allele frequency [MAF]: 0.49%) in addition to original *ALK* G1202R resistance mutation (MAF: 0.39%) in *cis* (Fig. 1B). This double *ALK* G1202R/S1206Y mutation in *cis* was identified about 40 months of lorlatinib treatment. After stereotactic radiation to the brain metastases and the odontoid mass, she received a commercial supply of lorlatinib at 100 mg daily in combination with chemotherapy carboplatin/pemetrexed/bevacizumab for three cycles and then pemetrexed/bevacizumab maintenance with stable disease. The patient subsequently underwent occiput to C3 fusion in June 2020 while on lorlatinib. Pemetrexed/bevacizumab chemotherapy was resumed post-operatively with a steady decrease of carcinoembryonic antigen levels from 357.7 ng/mL immediately after the procedure to 127.2 ng/mL at the last record. Repeat circulating tumor DNA testing revealed decreased but continual presence of *ALK* G1202R (MAF: 0.19%) and *ALK* S1206Y (MAF: 0.34%) (Fig. 1B). Currently, the patient remains on the combination of lorlatinib and chemotherapy. The patient provided written consent to have her treatment history published.

Review of Literature

We identified multiple double and triple mutations from both patient samples and in vitro mutagenesis experiments that confer resistance to lorlatinib after a review of the literature (Table 1). Both G1202R-paired or non-G1202R-paired mutations are identified, indicating multiple pathways leading to a common on-target phenotypic resistance to lorlatinib.⁶⁻¹² Indeed, even without a preexisting solvent-front mutation but with a common preexisting acquired resistance mutation to alectinib, such as I1171X,¹³ subsequent treatment with lorlatinib despite its efficacy may lead to an increased risk of double mutations. Other less common acquired resistance mutations to crizotinib can also lead to double mutations when subsequently treated with lorlatinib.

Three-Dimensional Modeling of Lorlatinib Resistance

Given that *ALK* G1202R is one of the most frequently acquired resistance mutation to 2G *ALK*

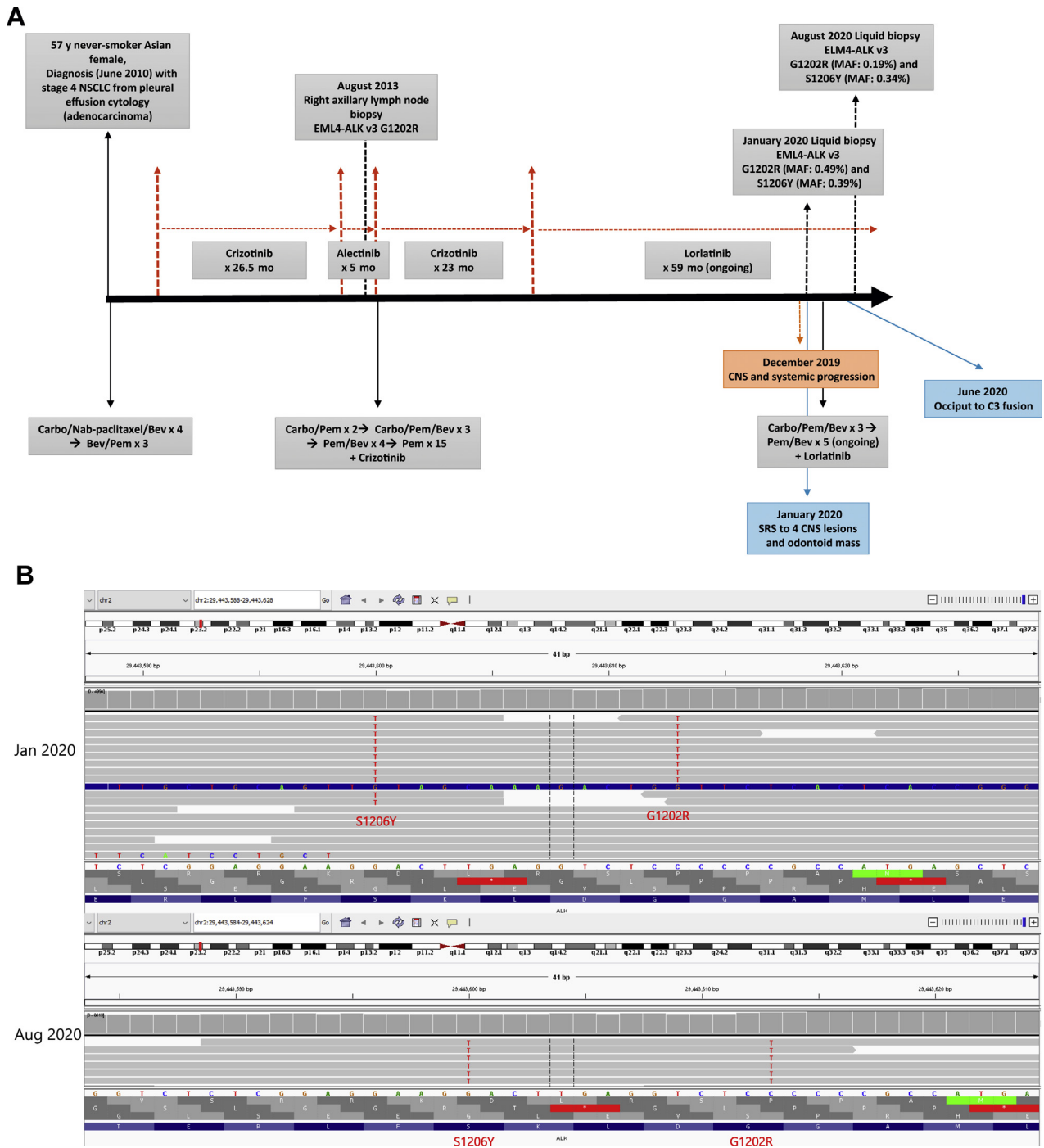


Figure 1. (A) Schematic summary of the treatment course. (B) IGV of the G1202R/S1206Y in cis double mutation over the duration of treatment. Bev, bevacizumab; Carbo, carboplatin; CNS, central nervous system; IGV, integrated genome view; Pem, pemetrexed; SRS, stereotactic radiosurgery; MAF, mutant allele frequency.

TKI and often sets the stage for subsequently acquired second-site resistance mutations to emerge after treatment with lorlatinib on the basis of its currently approved indications, we have modeled all the reported *ALK* G1202R-based double mutations in the presence of lorlatinib (Fig. 2A–H). Although the

flexible *ALK* G1202R residue provides steric hindrance to lorlatinib in certain rotamers (Fig. 2B), the basic *ALK* G1202R residue could adopt a “pulled forward” rotamer to avoid steric hindrance to lorlatinib. This G1202R rotamer can be stabilized by interaction with the acidic *ALK* E1210 residue and

Table 1. List of Compound Mutations Resistant to Lorlatinib From Both Clinical Samples and In Vitro Mutagenesis Experiments

Existing Pre-Lorlatinib Mutation(s)	Post-Lorlatinib Mutations	Clinical Response to ALK TKI	Reference
Clinical samples			
G1202R	G1202R/L1196M	NA	Yoda, 2018 ⁶
	G1202R/L1196M (EML4-ALK v3, tumor)	NA	Dagogo-Jack, 2019 ⁷
G1202R/L1196M (plasma)	Intrinsic resistance	NA	Sharma, 2019 ¹⁰
G1202R	G1202R/F1174L (tumor)	NA	Pailler, 2019 ⁹
	G1202R/F1174C (CTC-1)		
	G1202R/T1151M (CTC-10)		
NA	G1202R/G1269A (EML4-ALK v3, tumor)	NA	Dagogo-Jack, 2019 ⁷
S1206F/G1202R ^a	S1206F/G1202R/G1269A ^a (EML4-ALK v3, tumor)	NA	Dagogo-Jack, 2019 ⁷
	G1202R/G1269A/L1204V (EML4-ALK v3, tumor)	NA	Dagogo-Jack, 2019 ⁷
G1202R	G1202R/L1204V/G1269A	NA	Yoda, 2018 ⁶
	I1171N/T + D1203N (EML4-ALK v3, tumor)	NA	Dagogo-Jack, 2019 ⁷
	I1171N/T + L1198F (EML4-ALK v2, tumor)	NA	Dagogo-Jack, 2019 ⁷
G1202R/I1171N	I1171N/D1203N (plasma)	NA	Dagogo-Jack, 2019 ⁷
G1202R/E1145K	G1202R/F1174L	NA	Recondo, 2020 ¹¹
D1203N	D1203N/G1123D (plasma)	NA	Dagogo-Jack, 2019 ⁷
L1196M	L1196M/G1202R (plasma)	NA	Dagogo-Jack, 2019 ⁷
L1196M	L1196M/D1203N	NA	Dagogo-Jack, 2019 ⁷
I1171N/L1196M (EML4-ALK v3)	L1196M/G1202R (cis) + I1171N (plasma)	NA	Hu, 2020 ¹²
I1171S/F1174L	I1171S/G1269A (plasma)	NA	Dagogo-Jack, 2019 ⁷
E1210K/D1203N	E1210K/D1203N/G1269A (plasma)	NA	Dagogo-Jack, 2019 ⁷
G1202R/L1196M/V1801L ^b	G1202R/L1196M/C1156Y ^b (plasma)	NA	Dagogo-Jack, 2019 ⁷
C1156Y	C1156Y/L1198F	Crizotinib	Shaw, 2016 ⁴
ENU mutagenesis screen			
Parent mutation	Acquired second mutation	In vitro sensitivity ^c	
C1156Y (EML4-ALK v1)	I1171T	NA	Yoda, 2018 ⁶
	F1174C/I/V	NA	Yoda, 2018 ⁶
	L1196M	NA	Yoda, 2018 ⁶
	L1198F	NA	Yoda, 2018 ⁶
	D1203N	NA	Yoda, 2018 ⁶
	S1256F	NA	Yoda, 2018 ⁶
	G1269A	NA	Yoda, 2018 ⁶
I1171N (EML4-ALK v3)	F1174L	None	Okada, 2019 ⁸
	F1174I	None	Okada, 2019 ⁸
	L1196M	Ceritinib [(11 ± 0.8) nM] Brigatinib [(32 ± 6.1) nM]	Okada, 2019 ⁸
	L1198H	None	Okada 2019 ⁸
	L1198F	Brigatinib [(49 ± 3.9) nM]	Okada, 2019 ⁸
	L1256F	Brigatinib [(42 ± 6.3) nM]	Okada 2019 ⁸
	G1269A	Ceritinib [(14 ± 2.9) nM] Brigatinib [(7.1 ± 0.4) nM]	Okada, 2019 ⁸
F1174C (EML4-ALK v1)	L1196M	NA	Yoda, 2018 ⁶
	G1269A	NA	Yoda, 2018 ⁶
G1269A (EML4-ALK v1)	I1171N/T	NA	Yoda, 2018 ⁶
	L1196M	NA	Yoda, 2018 ⁶
L1196M (EML4-ALK v1)	I1171S	NA	Yoda, 2018 ⁶
	F1174C/L/V	NA	Yoda, 2018 ⁶
	L1179V	NA	Yoda, 2018 ⁶
	L1198F/H	NA	Yoda, 2018 ⁶
	L1256F	NA	Yoda, 2018 ⁶
G1202R (EML4-ALK v1)	L1196M	NA	Yoda, 2018 ⁶
	L1198F	NA	Yoda, 2018 ⁶

(continued)

Table 1. Continued

Existing Pre-Lorlatinib Mutation(s)	Post-Lorlatinib Mutations	Clinical Response to ALK TKI	Reference
G1202R (EML4-ALK v3)	F1174C	None	Okada, 2019 ⁸
	F1174L	None	Okada, 2019 ⁸
	L1196M	None	Okada, 2019 ⁸
	L1198F	None	Okada, 2019 ⁸
	G1269A ^d	None	Okada, 2019 ⁸

^aCase presented at WCLC 2019 by Professor Alice T. Shaw.¹⁴

^bG1202R was the dominant resistance mutation after brigatinib, and G1202R/C1156Y was the dominant resistance mutation after lorlatinib.

^cSensitivity is considered as IC₅₀ less than or equal to 50 nM (based on ENU clonal cells).

^dPatient derived cell lines.

CTC, circulating tumor cell; ENU, N-ethyl-N-nitrosourea; IC₅₀, concentration that inhibits 50%; NA, not available; TKI, tyrosine kinase inhibitor; v1, variant 1; v2, variant 2; v3, variant 3.

also *ALK* D1203 and *ALK* S1206 residues (Fig. 2C). Therefore, although the *ALK* G1202R mutation reduced lorlatinib binding affinity to *ALK* considerably, the activity of lorlatinib would still be potent enough to achieve clinical benefit.¹ However, *cis* mutations on top of *ALK* G1202R will further reduce the binding affinity of lorlatinib with *ALK* leading to clinical resistance. The S1206Y mutation destabilizes the *ALK* D1203-S1206-E1210-G1202R quaternary complex and limits G1202R movement space to create a more steric hindrance to lorlatinib (Fig. 2D). Potential resistance mechanisms owing to *ALK* G1202R/L1196M (Fig. 2E), *ALK* G1202R/F1174C (Fig. 2F), *ALK* G1202R/L1198F (Fig. 2G), and *ALK* G1202R/G1269A (Fig. 2H) are illustrated.

Discussion

Here we report for the first time (to our knowledge), a novel, sequentially acquired, dual *ALK* mutation (G1202R/S1206Y) in *cis* that confers resistance to lorlatinib. Considering *EML4-ALK* variant 3 G1202R is already a recalcitrant *ALK* mutation, we hypothesize that this novel G1202R/S1206Y double mutation could be highly resistant to lorlatinib. This *ALK* G1202R/S1206Y double mutation not only has not been reported from patient samples from the literature review but also has not been generated even under artificial N-ethyl-N-nitrosourea-accelerated mutagenesis and high-dose lorlatinib selection pressure at 1000 nM in the presence of either *EML4-ALK* variant 1 G1202R⁵ or *EML4-ALK* variant 3 G1202R.⁷ In vitro inhibition assays will be necessary to compare the resistance of this novel double mutation to other *ALK* G1202R-containing double mutations for this hypothesis-generating observation. One patient case containing a triple mutation S1206F/G1202R/G1269A has previously been reported.¹⁴ The clinical history was that the patient sequentially developed

ALK S1206F mutation after crizotinib treatment, *ALK* G1202R mutation in the absence of *ALK* S1206F mutation after ceritinib, and then a triple mutation *ALK* S1206F/G1202R/G1269A after lorlatinib treatment.¹⁴

ALK G1202R is one of the most frequently acquired resistance mutations to 2G *ALK* TKIs.^{15,16} In fact, the more potent an *ALK* TKI against the wild-type *ALK* is, the higher the incidence of *ALK* G1202R as the acquired on-target resistance mechanism.¹⁵ Owing to the pattern of use of lorlatinib on the basis of its approved indications (post-1L alectinib, post-1L ceritinib, and post-crizotinib and at least one other *ALK* TKI), most of the compound mutations that are resistant to lorlatinib have evolved from an existing solvent-front mutation *ALK* G1202R, with *ALK* G1202R plus *ALK* L1196M being the most frequent, followed by *ALK* G1202R plus *ALK* F1174L/C, *ALK* G1202R plus *ALK* L1198F, and *ALK* G1202R plus *ALK* G1269A.⁶⁻¹² A real-world study of lorlatinib revealed a decreasing median progression-free survival (PFS) with an increasing line of previous *ALK* TKI usage.¹⁷ From three-dimensional modeling, we postulate two resistance mechanisms of *ALK* G1202R-based double mutations to lorlatinib. The first mechanism involves destabilization of the quaternary complex of D1203-S1206-E1210-G1202R that moves *ALK* G1202R forward to allow lorlatinib to fit better with an existing G1202R mutation. The second mechanism involves a second-site steric hindrance that interferes with the binding of lorlatinib to *ALK* G1202R despite the presence of the intact *ALK* D1203-S1206-E1210-G1202R quaternary complex.

We kept this patient on lorlatinib to provide continuous central nervous system coverage. In addition, we continued lorlatinib given that *EML4-ALK* variant 3 was still detectable from the liquid biopsy, and the *ALK* G1202R/S1206Y is likely a resistance subclone. Lin et al.¹⁸ have previously reported that continuation of AKI TKIs with the addition of

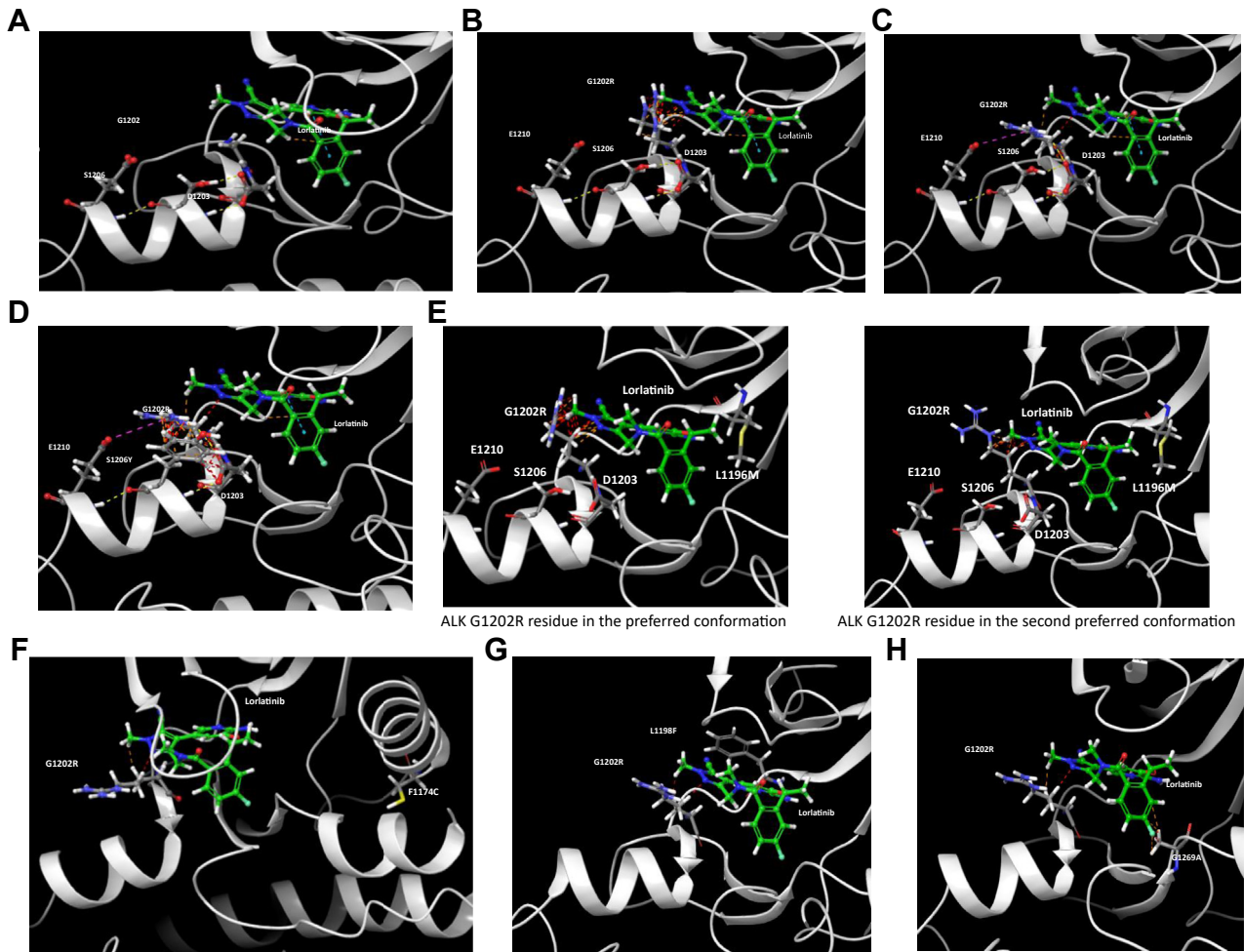


Figure 2. (A) Lorlatinib in complex with ALK kinase. ALK G1202, ALK D1203, ALK S1206, and ALK E1210 are solvent-front residues (PDB ID 4CLI). (B) ALK G1202R residue in the preferred conformation. It will provide a steric hindrance to lorlatinib. (C) ALK G1202R residue in the less preferred conformation with minimized steric hindrance to lorlatinib. In this “pulled forward” conformation, the basic ALK G1202R will interact with the acidic ALK E1210 residue and form a ALK D1203-S1206-E1210-G1202R quaternary complex with ALK D1203 and ALK S1206. Therefore, lorlatinib remains active against ALK G1202R, although with an approximately 50-fold reduction in activity against nonmutated ALK.¹ (D) ALK S1206 is located in the center of the quaternary complex D1203-S1206-E1210-G1202R that is involved in stabilizing the “forward movement” of G1202R. The ALK S1206Y mutation will destabilize this complex and move ALK G1202R back to its normal position to confer steric hindrance to lorlatinib. (E) ALK L1196M mutation makes G1202R residue to clash with lorlatinib even at the “pulled-forward” conformation. Therefore, lorlatinib loses its potency against ALK G1202R/L1196M compound mutation (PDB ID 4CLJ). On the left, ALK G1202R residue in the preferred conformation. On the right, ALK G1202R residue in the second preferred conformation. (F) ALK F1174C mutation does not have direct interaction with Lorlatinib. It will shift ALK kinase to favor its active conformation to have a tighter binding with ATP. Therefore, ALK G1202R/F1174C further reduces the binding affinity of lorlatinib with ALK in comparison with ALK G1202R mutation (PDB ID 4CLI). (G) ALK L1198F mutation does not favor the interaction with the polar cyano group on lorlatinib, which will further reduce the already unfavored interaction with lorlatinib after ALK G1202R mutation (PDB ID 5AA9). (H) ALK G1269 residue is adjacent to the F-element on the phenyl ring of lorlatinib. ALK G1269A mutation will induce a steric hindrance to lorlatinib to further reduce the binding affinity of lorlatinib with ALK G1202R, which is already 50-fold less potent in comparison to the wild-type ALK at the kinase domain. ATP, adenosine triphosphate.

chemotherapy can result in a longer median PFS than just switching to chemotherapy alone, although the magnitude of increase was only 3.6 months. Notably, there was a mild decrease in the double mutation alleles ALK G1202R (from MAF of 0.39% to 0.19%) and ALK S1206Y (from MAF of 0.49% to 0.34%) after the administration of chemotherapy. Currently, a fourth-generation ALK TKI, TPX-0131, designed to overcome

many of the aforementioned ALK double mutations, could potentially be the next treatment strategy for this patient when it is available for clinical trial.¹⁹

Recently, in a randomized phase 3 trial (CROWN), lorlatinib compared with crizotinib achieved an impressive blinded independent review committee (BIRC)-assessed hazard ratio (HR) of 0.28 (95% confidence interval [CI]: 0.19–0.41) for PFS in all patients, HR

of 0.20 (95% CI: 0.10–0.43) for patients baseline brain metastasis, and HR of 0.32 (95% CI: 0.20–0.49) for patients without baseline brain metastasis, with all subgroup analyses favoring lorlatinib over crizotinib.²⁰ Given the propensity for the accumulation of sequential mutations leading to resistance to all ALK TKIs and based on the CROWN results, the frontline use of lorlatinib will be an important strategy to circumvent these recalcitrant compound mutations.

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