ROS1-rearranged NSCLC With Secondary Resistance Mutation: Case Report and Current Perspectives

Florian Guisier,[1,2,3] Nicolas Piton,[4] Mathieu Salaun,[1,2,3] Luc Thiberville[1,2,3]

Clinical Practice Points

- Several ROS1 inhibitors have been developed that exhibit various potency against secondary resistance *ROS1* mutations.
- Despite in vitro efficacy against G2032R-mutated *ROS1*-rearranged non–small-cell lung cancer cell lines, cabozantinib treatment failed in our patient.
- Discrepancies between in silico predictions, in vitro data, and clinical outcomes are discussed based on our patient's case.
- Repotrectinib is the only ROS1 inhibitor with reported favorable clinical outcome in G2032R-mutated *ROS1*-rearranged non–small-cell lung cancer.

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Introduction

ROS1 rearrangement has been described as oncogenic in non–small-cell lung cancer (NSCLC) and is found in approximately 1% of non-squamous NSCLC.[1,2] The resulting fusion protein can be targeted by several tyrosine kinase inhibitors (TKIs), including crizotinib, which was the first to demonstrate a clinical benefit.[3] Cohorts of patients with *ROS1*-rearranged NSCLC treated with ceritinib or lorlatinib have also been reported.[4-7]

Several *ROS1* mutations have been described as resistance mechanisms in the setting of crizotinib treatment, from post-progression tumor biopsy, in vitro data, and in silico analysis (Table 1). The G2032R mutation is the most frequently identified.[8] It was described in 2 preclinical reports as potentially sensitive to cabozantinib.[9,10] Nevertheless, no clinical observation in this setting has been reported to date. Here, we report the case of a crizotinib-resistant patient with *ROS1*-rearranged NSCLC with G2032R mutation, for which cabozantinib treatment failed, and review the current knowledge about ROS1 TKI resistance mutations and their management.

Case Presentation

A 59-year-old woman was diagnosed with stage IV NSCLC in January 2017. Immunohistochemistry for *ROS1* fusion was highly positive, as assessed by the D4D6 ROS1 antibody (Cell Signaling Technology, Danvers, MA). *ROS1* rearrangement was confirmed by fluorescence in situ hybridization, using the ZytoLight assay (ZytoVision, Bremerhaven, Germany). *ROS1* fusion partner was *SDC4* as assessed by ligation-dependent reverse transcription polymerase chain reaction.[11] The patient received first-line crizotinib, with dramatic partial response. In September 2017, we observed reoccurence of a left pleural effusion and growth of lung nodules. Lorlatinib was initiated without previous biopsy. After an initial partial tumor response, a new pleural progression was observed in January 2018. Pleural biopsies were performed to look for resistance mechanisms. The histology remained adenocarcinoma, and the *ROS1* fusion was still observed. Additionally, a G2032R *ROS1* mutation was identified using the Oncomine Comprehensive Assay V3 (ThermoFisher, Waltham, MA). A tumor sample was also analyzed with the Tumor Hotspot MASTR Plus panel (Agilent, Santa Clara, CA). No bypass mutation was found with neither of the 2 panels, assessing previously reported bypass resistance mechanisms to ALK, ROS1, or EGFR inhibitors, including mutations in *AXL, BIM, BRAF, EGFR, HER2, IGFR1, KIT, KRAS, MET, NRAS,* or *PIK3CA* and copy number alteration in *AKT1, EGFR, FGFR1,*

[1]Service de Pneumologie, Oncologie Thoracique, et Soins Intensifs Respiratoires, CHU Charles Nicolle, Rouen, France
[2]LITIS QuantIF EA4108, Normandie University, Rouen, France
[3]INSERM CIC 1404
[4]Service d'Anatomie et Cytologie Pathologiques, CHU Charles Nicolle, Rouen, France

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Address for correspondence: Florian Guisier, MD, Service de Pneumologie, Oncologie Thoracique, et Soins Intensifs Respiratoires, CHU Charles Nicolle, 1 Rue de Germont, 76000 Rouen, France
E-mail contact: florian.guisier@chu-rouen.fr

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Table 1 Pharmacodynamic Characteristics and Efficacy Profile of ROS1 Inhibitors

	Crizotinib	Ceritinib	Lorlatinib	Brigatinib	Cabozantinib	Foretinib	Entrectinib	AZD3463	Repotrectinib	Ensartinib
Cthrough (ng/mL)	237 to 800	1400		552	1080	46 to 900				
Cthrough (nM)	530 to 913					70	1330		425	
CSF/plasma conc.	0.001 to 0.003						0.4		0.04	
WT CD74-ROS	2 to 44	11 to 230	0.05 to 1	2.7 to 30	0.5 to 9	1.8 to 14	5.3 to 10	10	0.2	39
WT SLC34A2-ROS1	21	506	1.15			19				
WT FIG-ROS	41 to 60	304 to 488	0.2			6				
E1935G	350					6.6				
L1947R	1420					17.9				
L1951R	8.8 to 97	76.4 to 611		611	0.18 to 20.7					
G1971E	605					8.7				
E1974K	23	42.3		10.3	6.8	6.2		30.9		
V1979A	b	b								
V1979M	b									
1981 Tins[c]	d	d	d							
L1982F	4.7 to 6.9	23 to 27			0.08 to 2.94	15.3				
S1986 Y/F[a]	116 to 125	73 to 99	1 to 1.6							
E1990G	4.0	22.1			0.34					
F1994L	3.3	19.4			0.01					
M2001T[c]	d	d	d							
K2003I	1.4 to 1.5	4.5 to 11.7			0.28 to 0.32					
F2004C	40.5	68.7		20.2	56.8	23.5		19.9		
E2020K	41.1	97.8		24.9	10.5	9.7		53.2		
F2024 C/V[c]	d	d	d							
L2026Ma	22.4 to 259	4.6 to 90	1.1 to 2	3.5 to 200	0.92 to 11	3.2 to 8.6	3500	1800		
L2028-[c]										
G2032Ra	254 to 2700	276 to 2200	160 to 508	170 to 1172	1.4 to 26	39.7 to 90	1813 to 2200		8.4	372
D2033Na	140 to 200	306 to 535	0.38 to 3.3	69.1 to 128	0.2 to 0.65	2.2	169		1.3	402
T2036-[c]										
C2060G	690					13.6				
F2075 V/C	17 to 23	68.7 to 92		9.1 to 14.1	4.9 to 31.4	9.7 to 16		6.2, 41.6		
V2089M	15.6	42.8		6.7	7.5	2.9		16.1		
V2098I	10.9 to 901	44.4		9.0	1.7	2.5 to 9.9		12.5		
G2101Aa	27.1	0.06	d			0.004				
D2113 N/G	25.4 to 29.6	118 to 163		24	15.3 to 41.2	8.5 to 25.4		40 to 42.5		
M2134I	14.2	55.7		11.1	4.3	5.4		15.2		
L2155S	405	185				534				
L2223S	1.13	11.2			0.07					
L2223X		b								

Abbreviations: CSF = cerebrospinal fluid; IC50 = half maximal inhibitory concentration; WT = wild type.
Where available, the IC50 of ROS1 inhibitors for engineered cells harbouring WT or mutated *ROS1* fusion is given (from references[6,8-19]).
Green: high in vitro potency, expected clinical efficacy. Yellow: medium in vitro potency. Red: low in vitro potency, expected clinical failure.
[a]These mutations have been observed in patients.
[b]These mutations have emerged in vitro, in cells treated with crizotinib or ceritinib, but IC50 is unknown.
[c]These mutations have been predicted in silico, and not reported in cell lines or patients to date. The in silico prediction is based on the homology between *ROS1* and *ALK* and on the known *ALK* resistance mutations to crizotinib.
[d]Expected efficacy based on in silico simulations.

IGF1R, MET, or *ALK*. Cabozantinib was started at 60 mg daily. The patient experienced grade 2 nausea and myalgia, preventing us from increasing the cabozantinib dosage. We observed a continuous tumor progression, with onset of a left axillary adenomegaly and growth of lung nodules after 1 month of treatment. Chemotherapy with carboplatin and pemetrexed was initiated, resulting in a partial tumor response after 2 cycles. The patient received 2 more cycles and was then treated with pemetrexed as maintenance chemotherapy. A liver metastasis appeared after 3 cycles of pemetrexed and was biopsied. The G2032R *ROS1* mutation was still found on this sample. No bypass mutation was found using the Tumor Hotspot MASTR Plus panel. She then received chemotherapy with carboplatin and paclitaxel and experienced progressive disease after 2 cycles.

Discussion

ROS1 inhibitors with reported clinical efficacy are crizotinib, ceritinib, entrectinib, lorlatinib, and repotrectinb. Additionally, preclinical data showing some level of potency are available for brigatinib, cabozantinib, foretinib, AZD3463, and ensartinib. A major pitfall in managing patients with *ROS1*-rearranged NSCLC is the onset of secondary resistance mutations during crizotinib treatment, notably, the solvent-front mutation G2032R. None of the routinely available TKIs has clinical efficacy against this mutation.

Preclinical data show that cabozantinib is a potent inhibitor of the G2032R mutated CD74-ROS1 fusion protein. In fact, the half maximal inhibitory concentration (IC50) of cabozantinib against engineered BaF3 cells with *CD74-ROS1*^{G2032R} rearrangement was reported to stand between 13 and 37nM.[9-12,20] Nevertheless, the IC50 was higher when tested against the MGH047 patient-derived cell line.[20]

In a case report of D2033N-mutated *ROS1*-rearranged NSCLC,[6] treatment with cabozantinib 60 mg daily resulted in a dramatic tumor shrinkage and near complete response for at least 8 months. Nevertheless, in vitro data show that cabozantinib is more active against the D2033N mutation than the G2032R mutation (IC50, 0.8nM vs. 15.3nM, respectively).[6,9]

To our knowledge, there is no clinical report of cabozantinib treatment for G2032R-mutated *ROS1*-rearranged NSCLC. Our

Table 2 Double ROS1 Mutations and Their Sensitivity to Inhibitors

	Crizotinib	Ceritinib	Brigatinib	Cabozantinib	Foretinib	AZD3463
V1979A + K2003I		X				
V1979A + L2223S		X				
V1979M + K2111T		X				
L1982F + K2003I		X				
L1982F + M2128V	X					
L1982V + K2111T		X				
L1982W + K2003I	X					
E1990G + M2128V	7.7	64.9		<0.01		
F1994L + M2128V		X				
K2003I + L2223S		X				
K2003I + L2026M	18.4	9.95		0.21		
G2032R + K2003I	X					
G2032R + F2004C	1452	1495	627	889	948	1325
G2032R + E2020K	1785	1952	434	47.8	110	1190
G2032R + F2075C/V	1256 to 1757	1539 to 1644	234 to 416	220 to 239	393 to 543	750 to 1036
G2032R + V2089M	1200	1250	155	23.1	48.4	625
G2032R + V2098I	1618	1550	602	22.5	27.7	2000
G2032R + D2113G/N	1228 to 2216	1649 to 1900	921 to 1396	238 to 256	476 to 576	1179 to 1315
G2032R + M2134I	1313	1768	452	16.7	42.7	887

Double *ROS1* mutations that have been reported from in vitro accelerated mutagenesis screening are described.
Where available, the half maximal inhibitory concentration (IC50) of ROS1 inhibitors for engineered cells harboring the corresponding mutated *ROS1* fusion is given (from references [9,20]).
Green: high in vitro potency, expected clinical efficacy. Yellow: medium in vitro potency. Red: low in vitro potency, expected clinical failure.
X: This composite mutation has been scribeded but no IC50 was reported.

patient experienced rapid tumor progression. Besides the intrinsic potency of cabozantinib, other mechanisms could have led to treatment failure in our patient: (1) In our patient, the G2032R *ROS1* mutation has been identified on a pleural biopsy. No bypass alteration has been found. We cannot exclude that another undetected alteration occurred. (2) A second hypothesis would be tumor heterogeneity between pleural metastasis and the primary tumor. Nonetheless, progression under cabozantinib treatment occurred concomitantly on the primary tumor site and with pleural effusion, suggesting a common resistance mechanism. Moreover, the G2032R mutation was also found on a subsequent liver metastasis biopsy. (3) Cabozantinib was given at 60 mg once daily and the dose was not increased, owing to grade 2 limiting toxicity. A higher dosage may have been more efficient. In fact, in other malignancies, including medullary thyroid carcinoma and glioblastoma, cabozantinib has been studied with a higher dosage. In medullary thyroid carcinoma, the 140 mg daily dose was used in clinical trials.[21,22] Nevertheless, this regimen resulted in a high toxicity, with dose reduction in 79% and 82% of patients and treatment discontinuation in 16% and 22% of patients in these 2 trials, respectively. Grade 3 to 4 adverse events occurred in 69% of patients. Similarly, in a glioblastoma clinical trial, the treatment initiation dose was amended from 140 to 100 mg daily because of limiting toxicity (100% grade 3 to 4 adverse events at 140 mg/d vs. 72% at 100 mg/d).[23] As a result, many experts recommend to start cabozantinib treatment at 80 or 60 mg/d.[24,25] The 60 mg dose is, in fact, the recommended dose in renal cell carcinoma, and has been used in all NSCLC cohorts treated with cabozantinib, including the Eastern Cooperative Oncology Group and American College of Radiology Imaging Network (ECOG-ACRIN) 1512 study[26] and the phase II study in *RET*-rearranged NSCLC.[13] Even with this dose, treatment discontinuation and dose reduction were frequent, and grade 3 to 5 toxicities occurred in 71% of patients.[26]

Prior to cabozantinib treatment, our patient received lorlatinib for 4 months. Lorlatinib is an ALK/ROS1 inhibitor. In a cohort of 12 patients with *ROS1*-rearranged NSCLC, the objective response rate was 50%, including 4 of 5 treatment-naive patients and 2 of 7 crizotinib-resistant patients.[7] No data was shown regarding the presence of resistance mutations in these patients. Potency against the S1986Y/F *ROS1* mutation has been reported in 1 patient.[27] In a recent communication, Besse et al reported a series of 6 patients with G2032R-mutated *ROS1*-rearranged NSCLC treated with lorlatinib.[14] No tumor response was observed; 5 of 6 patients had stability as best tumor response. In preclinical studies, the IC50 of lorlatinib on BaF3 G2032R-mutated *ROS1*-rearranged cells was 508, 203, and 177 nM.[10,27] In a mouse tumor xenograft model, inhibition of tumor growth was only achieved for doses of 10 mg/kg/d or more.[10] Hence, the G2032R mutation is likely a resistance mutation to lorlatinib.

Other resistance mutations in *ROS1*-rearranged cells have been described either in the clinic (the L2026M and G2101A mutations) or from in vitro and in silico studies (Table 1).[6,8-12,15-20,27,28] Interestingly, both the L2026M and G2101A mutations were predicted in silico before their observation in patients. Composite mutations arising from in vitro accelerated mutagenesis screening have also been described and are presented in Table 2 together with their putative sensitivity to several TKIs.[9,20]

Inhibitory potency of several TKIs against engineered Ba/F3 *ROS1-CD74*-mutated cell lines has been reported. These data may help in patients' management when a *ROS1* mutation is identified; nevertheless, physicians have to keep in mind that they suffer some limitations: (1) The toxicity profile of TKIs may limit their efficacy in the clinic, despite high in vitro potency. In our patient's case, we were unable to increase cabozantinib dosage over 60 mg/d. (2) Other cell signaling pathways may be activated in patients' tumor cells, which represent bypass resistance

mechanisms.[29] In our patient, we detected no such mechanism. (3) Tumor development often gives rise to several subclonal cell populations. Some of these clones may contribute to treatment failure, either because they acquired another resistance mechanism (eg, a second undetected *ROS1* mutation), a bypass resistance, or a histology transformation. This tumor heterogeneity is not integrated in the in vitro cell proliferation assay. (4) *ROS1* fusion partner is not always *CD74*. Whether or not this can modulate the potency of a given TKI needs further studies. Nevertheless, as shown in Table 1, ceritinib may not be active against *FIG-ROS1* and *SLC34-ROS1*-rearranged NSCLC.

Besides these considerations, using second- or third-generation ROS1 inhibitors such as lorlatinib or brigatinib may be useful to reach enhanced cerebrospinal fluid penetration. In fact, central nervous system metastasis is a major mode of progression while on long term ROS1 TKI treatment. Lorlatinib showed better intracranial efficacy than crizotinib in *ROS1*-rearranged NSCLC.[7,8] Brigatinib has intracranial efficacy similar to lorlatinib in ALK-rearranged NSCLC, but clinical data are limited in *ROS1*-rearranged NSCLC.

According to current data, the G2032R mutation confers resistance to all investigated TKIs but cabozantinib, foretinib, and repotrectinib. As discussed above, the toxicity profile of cabozantinib may limit its use in this indication. Foretinib and repotrectinib were not available at the time of this report, and no clinical data has been reported using foretinib in this setting. Noteworthy, repotrectinib was reported to be effective in a patient with G2032R-mutated *ROS1*-rearranged NSCLC, resulting in 7 months partial response, including on cerebral metastasis.[16]

Conclusion

In conclusion, this is the first report of cabozantinib treatment in G2032R-mutated *ROS1*-rearranged NSCLC. In our patient, this treatment failed, with cabozantinib given at 60 mg once daily and limiting toxicity. Other strategies for the treatment of in G2032R-mutated *ROS1*-rearranged NSCLC are needed. Repotrectinib is the only TKI with a reported favorable outcome in this setting and warrants further investigations.

Disclosure

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