

Dramatic Response to Lorlatinib in a Patient With *CD74-ROS1*-Positive Lung Adenocarcinoma With Acquired F2004V Mutation

Anastasios Dimou, MD¹; Sai-Hong I. Ou, MD, PhD²; and Robert C. Doebele, MD, PhD¹

INTRODUCTION

A significant subset of patients with non–small-cell lung cancer (NSCLC) harbor activating mutations in key oncogenes like *EGFR*, *ALK*, *ROS1*, *NTRK*, *BRAF*, and others and account for up to one-quarter of the population with this common type of cancer.¹ These oncogene-driven tumors are amenable to treatment with targeted therapies; however, secondary resistance invariably ensues. At the molecular level, resistance depends on activation of bypass pathways or development of secondary mutations in the driver oncogene.^{2,3}

Oncogenic fusions involving the *ROS1* gene are detected in approximately 1% to 2% of patients with NSCLC and the majority of cases occur in tumors of adenocarcinoma histology.⁴ They are generated by the fusion of the 3' end of the *ROS1* gene containing the tyrosine kinase with a variety of partner genes, including *GOPC*, *SLC34A2*, *CD74*, *TPM3*, *SDC4*, *EZR*, *LRIG3*, *KDEL2*, *CCDC6*, among others.⁴ *ROS1* fusions function as primary oncogenic drivers following the oncogene addiction model.⁴ Indeed, inhibition of *ROS1* with the small molecule tyrosine kinase inhibitor (TKI) crizotinib induced durable responses in 72% of the patients with an *ROS1*-positive NSCLC in the PROFILE-1001 study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00585195) identifier: NCT00585195),⁵ leading to approval of crizotinib by regulatory agencies for this patient population. Other TKIs, including ceritinib,⁶ lorlatinib,⁷ and entrectinib,⁸ have shown activity in this patient population as the field evolves.

Our understanding of the landscape of resistance mechanisms for *ROS1*-positive NSCLC is still evolving. Although several TKI options currently exist for patients with *ALK*-positive NSCLC and secondary *ALK* mutations, effective TKIs are limited for the *ROS1*-positive lung cancer population in the postcrizotinib setting.^{9,10} Importantly, the molecular mechanisms of resistance are variable, which poses a barrier for the study of different drugs or drug combinations in molecularly defined cohorts of progressing patients in the form of clinical trials. Here, we describe an unusual case of a *ROS1*-positive patient who developed a *ROS1* F2004V secondary mutation and whose cancer

progressed in the brain and bone sites after 34 months of treatment with entrectinib, followed by dramatic response to lorlatinib. Informed consent for use of the radiology images was obtained by the patient presented.

CASE REPORT

A 22-year-old female patient with no prior medical history was diagnosed with metastatic lung adenocarcinoma after presenting with right-eye vision changes (flashing). An ophthalmologic examination revealed a right amelanotic perifoveal choroidal tumor. Subsequent evaluation with positron emission tomography/computed tomography scanning confirmed metastatic disease with multiple left lung nodules, left hilar lymphadenopathy, and bone lesions. Pathologic review of a core biopsy specimen from a lung lesion showed lung adenocarcinoma with predominant lepidic pattern. Molecular analysis with a commercial next-generation sequencing panel identified a *CD74-ROS1* fusion gene. Interestingly, choroidal metastases have been reported in a number of cases of *ALK*^{11,12} or other oncogene-driven lung cancer.¹³ The patient was treated with radiation therapy to the metastatic lesion in the right eye, followed by first-line crizotinib. Although there was initially a complete response, progression of disease in the brain was documented while she was receiving crizotinib therapy for 10 months. She was then treated with entrectinib, a TKI that penetrates the blood-brain barrier, in the context of the phase I STARTRK-1 clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02097810) identifier NCT02097810). The patient was initially treated at the recommended phase II dose of 600 mg orally once daily for the first eight 28-day cycles, but she experienced asymptomatic progression in multiple, small brain metastases, which led to a decision to escalate the dose to 800 mg orally once daily, resulting in decrease in disease burden in the brain. After another 18 cycles of entrectinib, an increase in the size of the brain lesions was noted. Progressing disease sites in the brain were treated with stereotactic ablative radiation, based on data that demonstrated extension of duration of benefit for TKIs in the setting of oligoprogression.¹⁴ The patient continued receiving entrectinib for a total of 34 months, until further

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on April 11, 2019 and published at ascopubs.org/journal/po on June 3, 2019; DOI <https://doi.org/10.1200/PO.19.00013>


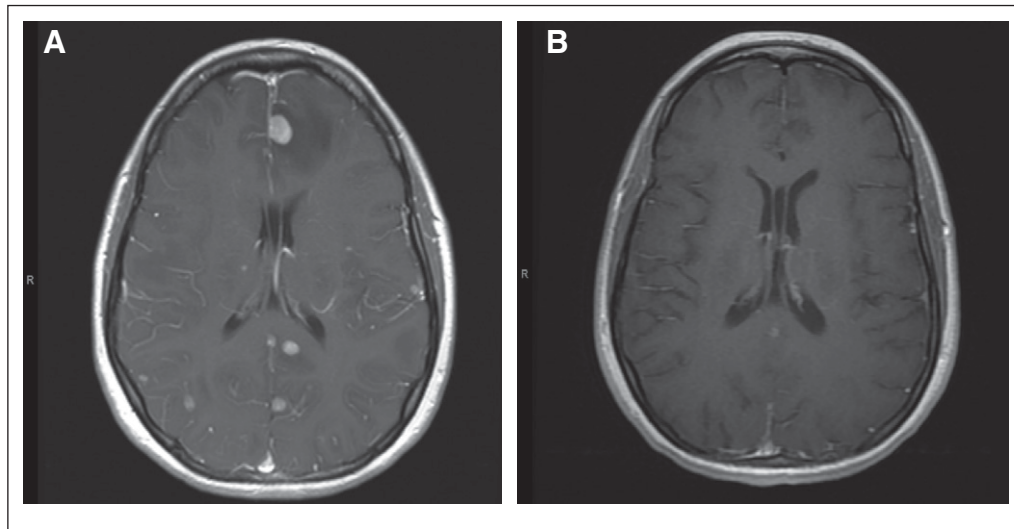
Licensed under the Creative Commons Attribution 4.0 License 

FIG 1. Magnetic resonance images of the brain (T1 axial post-contrast series) (A) before and (B) 1 month after treatment with lorlatinib, showing significant response of all metastatic brain lesions.



progression in the brain developed with concomitant progression in osseous metastases.

At the time of brain and bone progression, a secondary *ROS1* F2004V mutation was detected via circulating cell-free DNA testing using a commercial next-generation sequencing assay. Besides the *CD74-ROS1* fusion and the

F2004V secondary mutation, no other mutations were detected in the *ROS1* kinase domain or other genes. At that time, the patient was removed from the STARTRK-1 study and therapy was begun with brigatinib, a compound approved for the treatment of lung cancers with ALK fusions¹⁵ but also with activity against ROS1.^{16,17} In addition,

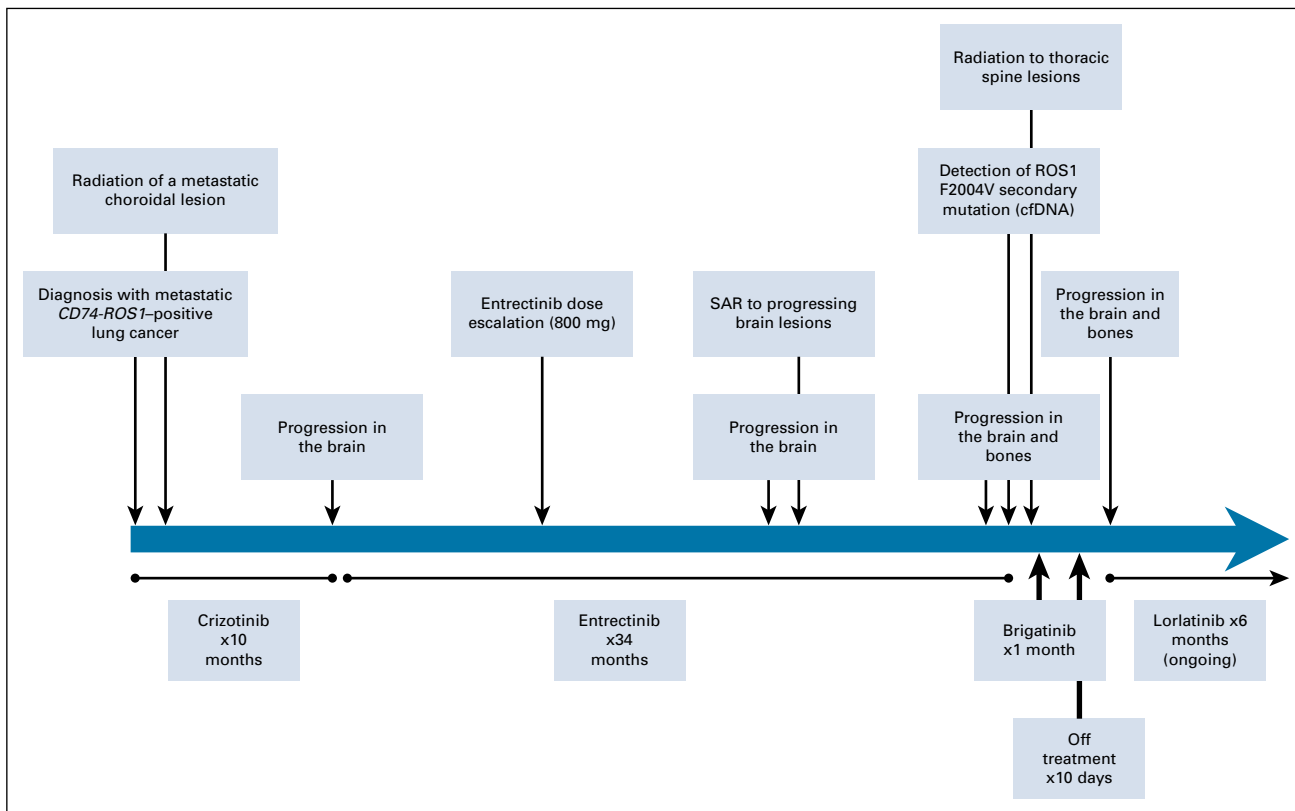


FIG 2. Timeline of the clinical course, highlighting the treatment interventions and the molecular data from the time of diagnosis. cfDNA, circulating cell-free DNA; SAR, stereotactic ablative radiation.

ROS1 Secondary Mutations in Ba/F3 Preclinical Models	Cave/IC ₅₀						ALK analog	Drug	C _{ave} (nmol/Lt)
	Crizotinib	Cabozantinib	Brigatinib	Ceritinib	Lorlatinib	Entrectinib			
WT	35	680.8	90.7	17.2	1219.8	253		Crizotinib	718
L1951R	7.4	108.3	2.4	2			L1122 (no mutation reported in COSMIC)	Cabozantinib	2,243
E1974K	31.7	329.8	140.3	29.1			P1144 (no mutation reported in COSMIC)	Brigatinib	1,447
L1982F	104.5	763		45			L1152R	Ceritinib	1,232
S1986F	6.2			41.1	1158		C1156F	Lorlatinib	579
S1986Y	5.7			12.4	579		C1156Y	Entrectinib	2,088
E1990G	178.6			55.7			D1160H		
F1994L	216.9			63.6			F1164 (no mutation reported in COSMIC)		
F2004C	17.7	39.5	71.6	17.9			F1174L*	Color	Cave/IC ₅₀
F2004V		79.3					F1174V		≥ 15
E2020K	17.5	213.8	58.1	12.6			L1190M		≥ 10, < 15
L2026M	5.6	376.4	14.2	23.9	373.5	0.6	L1196M		≥ 5, < 10
G2032R	0.7	135.7	2.5	1	2.1	1	G1202R		< 5
D2033N	4.3		14.7	2.6	315	12.3	D1203N		No data
F2075C	42.8	149.1	159	17.9			F1245C*		
F2075V	31.1	71.4	102.8	13.4			F1245V*		
V2089M	46.1	298	216	28.8			C1259Y		
G2101A	26.5						G1269A		
D2113G	28.3		60.8	7.6			S1281 (no mutation reported in COSMIC)		
D2113N	24.3	146.7	61.3	10.4			S1281 (no mutation reported in COSMIC)		
M2134I	50.4	517.3	129.8	22.1			M1302 (no mutation reported in COSMIC)		
L2155S	1.8						F1323 (no mutation reported in COSMIC)		

FIG 3. Secondary mutations in *ROS1* fusions reported in in vitro experiments in the literature and differential sensitivity to drugs with known *ROS1* activity. The numbers in the boxes correspond to the average concentration (C_{ave}) for the drug in steady state in patients, divided by the drug IC₅₀ values calculated in Ba/F3 cells expressing the mutant *ROS1* molecules. The half maximal inhibitory concentration (IC₅₀) values were obtained from published reports. Colors represent relative potencies of the drugs compared with achievable drug exposure. The average IC₅₀ was used if there was more than one publication for a certain mutation-drug combination. Repotrectinib is another potent *ROS1* inhibitor with activity against many of these secondary mutations; it is not shown in the figure because the RP2D is not known yet for this drug.²⁹ C_{ave} values were calculated as the ratio of the area under the curve for 0 to 24 hours to 24 hours of the drug at steady state in patients.^{35,36} Secondary mutations that have been reported in clinical cases are highlighted in red and the *ROS1* F2004V mutation described clinically for the first time in this report is highlighted in purple. The last column shows the homolog amino acid positions in ALK, estimated with the blastp program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>), and any *ALK* mutations at those positions reported in the literature or the Catalog of Somatic Mutations in Cancer (COSMIC) database. (*) These *ALK* mutations also are found in neuroblastoma.

brigatinib had activity against *CD74-ROS1* with F2004L in vitro.¹⁸ The patient also underwent radiation therapy for several thoracic spine progressing lesions. Unfortunately, follow-up scans showed disease progression in the brain and several osseous disease sites (outside of the radiated field).

Although, to our knowledge, the *ROS1* F2004V has not been described before in patients, it is analogous to the *ALK* F1174L mutation described in patients with neuroblastoma¹⁹ and confers paradoxical apoptosis to Ba/F3 cells, a result of excessive oncogenic signaling through *ROS1* and p38MAPK.²⁰ Also, a secondary F1174V mutation has been described in *ALK*-positive lung cancer with resistance to crizotinib.²¹ Instead, Ba/F3 cells expressing *ROS1* F2004V survive in the presence of low-dose *ROS1* inhibitor.²⁰ However, the patient in this report did not respond to withdrawal of brigatinib, and symptoms, namely headaches, developed from brain metastases. Disease burden in the brain was not amenable to stereotactic ablative radiation, and to avoid whole-brain radiation therapy, a decision was made to enroll the patient in a clinical trial

(ClinicalTrials.gov identifier NCT03178071) of lorlatinib, another TKI with *ROS1* activity.²² Lorlatinib induced a dramatic response in the brain and the systemic disease sites, evident in the first disease evaluation with imaging studies (Fig 1). Disease remains in control 6 months later, while the patient continues receiving lorlatinib (Fig 2).

DISCUSSION

In the case presented, we describe a *ROS1* F2004V mutation in a patient with *ROS1* fusion-positive lung adenocarcinoma after progression while receiving entrectinib. This mutation, along with F2075C, emerged as a possible mechanism of resistance to cabozantinib, a multikinase inhibitor with *ROS1* activity in vitro.²⁰ Although the patient in that study did not receive cabozantinib, it is likely that F2004V is a mechanism of secondary resistance to *ROS1* inhibition with entrectinib. In that work, Ogura et al²⁰ established Ba/F3 cells expressing *CD74-ROS1* and resistance to cabozantinib by exposure to *N*-ethyl-*N*-nitrosourea, a known mutagen. Interestingly, the resistant cell lines were TKI addicted, because they required

the presence of low-dose cabozantinib to survive. Withdrawal of the TKI induced apoptosis, which was driven by excessive ROS1 signaling. Despite these experimental data, we did not observe clinical response in the patient upon withdrawal of brigatinib. Given that the concept of “TKI addiction” is suggested in preclinical models only,²³⁻²⁵ the approach of TKI withdrawal cannot be recommended unless there is additional clinical validation.

The molecular spectrum of secondary resistance to crizotinib and other ROS1 inhibitors in ROS1 fusion–positive patients with lung cancer is not yet well established. The two largest published cohorts include 16 patients from Massachusetts General Hospital²⁶ and 12 patients from the University of Colorado.²⁷ In the Massachusetts General Hospital cohort, nine of 16 patients had a *ROS1* secondary mutation identified at the time of progression. Seven of these patients harbored the G2032R mutation and none had the F2004V mutation. Conversely, a *ROS1* compound secondary mutation (L2026M/L1951R) was identified in one of 12 patients in the University of Colorado cohort. In addition, in this cohort, there was also activation of the HER2 axis, an activating *KIT* D816G mutation and a *CTNNB1* S45F mutation, each case a possible mechanism of resistance to ROS1 inhibition. In three patients, there were copy-number gains in oncogenes, which might also represent mechanisms of resistance. Figure 3 summarizes the in vitro^{10,16,20,28-33} and clinical^{10,27,30,34} data of *ROS1* secondary mutations reported in the literature.

Molecular testing during the course of treatment with a targeted therapy often identifies molecular mechanisms of resistance to the inhibition of the driver oncogene.¹ In the best-characterized cases of secondary resistance, there are data from large cohorts of patients and available effective TKIs, as is the case with *EGFR* T790M secondary mutation. Nevertheless, predicting response to a TKI in the presence of a secondary mutation in the driver oncogene might rely on less extensive clinical data or on preclinical data only. Patient-derived cell lines,²⁷ Ba/F3 cells engineered to express and depend on the driver oncogene of interest with and without the secondary mutation,¹⁰ and xenograft tumors in mice²⁹ serve as resistance models in many of these scenarios. In vitro development of resistance in cell-line models is possible by exposure to increasing doses of TKIs for several months.³⁷ Alternatively, brief exposure to the mutagen *N*-ethyl-*N*-nitrosourea, followed by drug screening, can select for resistant clones.^{20,28} Finally, it is possible for a clinician to encounter a case of resistance for which molecular testing might identify resistance mechanisms not described before. Reports of such “n of 1” cases, including outcomes, is an important resource that can guide treatment of cases with rare acquired resistance mutations.

In conclusion, we show that *ROS1* F2004V secondary mutation confers resistance to entrectinib and brigatinib, and possibly other ROS1 inhibitors, but is sensitive to lorlatinib. This knowledge will help guide treatment of prospective patients with the same molecular background.

AFFILIATIONS

¹University of Colorado School of Medicine, Aurora, CO

²University of California School of Medicine, Irvine, Orange, CA

CORRESPONDING AUTHOR

Robert C. Doebele, University of Colorado Cancer Center, MS 8105, 12801 E 17th Ave, Aurora, CO 80045; e-mail: robert.doebele@ucdenver.edu.

SUPPORT

Supported by the Specialized Programs of Research Excellence in Lung Cancer (Grant 5P50CA058187).

AUTHOR CONTRIBUTIONS

Conception and design: All authors

Provision of study material or patients: Anastatios Dimou, Sai-Hong I. Ou

Collection and assembly of data: All authors

Data analysis and interpretation: Anastatios Dimou, Robert C. Doebele

Manuscript writing: All authors

Final approval of manuscript: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated.

Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of

this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Sai-Hong I. Ou

Stock and Other Ownership Interests: Turning Point Therapeutics

Honoraria: Pfizer, Roche Pharma AG, Roche, ARIAD/Takeda, AstraZeneca, Foundation Medicine, Merck

Consulting or Advisory Role: Pfizer, Roche, AstraZeneca, Takeda, Foundation Medicine, Turning Point Therapeutics, Ignyta

Speakers' Bureau: Genentech, AstraZeneca, Takeda

Research Funding: Pfizer (Inst), Roche Pharma AG (Inst), AstraZeneca/MedImmune (Inst), AstraZeneca (Inst), ARIAD (Inst), Ignyta (Inst), Astellas Pharma (Inst), Chugai Pharma (Inst), Revolution Medicines (Inst)

Robert C. Doebele

Stock and Other Ownership Interests: Rain Therapeutics

Consulting or Advisory Role: OncoMed, Ignyta, GreenPeptide, AstraZeneca, Roche, Bayer, Takeda, Rain Therapeutics

Research Funding: Ignyta (Inst)

Patents, Royalties, Other Intellectual Property: Licensing fees from Abbott Molecular for Patent PCT/US2013/057495, licensing fees from Ignyta for biologic materials (Inst), licensing fees for biologic materials from Genentech (Inst), licensing fees for patent from Rain Therapeutics

Travel, Accommodations, Expenses: Ignyta, Rain Therapeutics, Roche

No other potential conflicts of interest were reported.

ACKNOWLEDGMENT

Lorlatinib was obtained by the Pfizer Expanded Access Program (ClinicalTrials.gov identifier: NCT03178071).

REFERENCES

- Pakkala S, Ramalingam SS: Personalized therapy for lung cancer: Striking a moving target. *JCI Insight* 3:e120858, 2018
- Pao W, Miller VA, Politi KA, et al: Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2:e73, 2005
- Turke AB, Zejnullahu K, Wu YL, et al: Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. *Cancer Cell* 17:77-88, 2010
- Davies KD, Doebele RC: Molecular pathways: ROS1 fusion proteins in cancer. *Clin Cancer Res* 19:4040-4045, 2013
- Shaw AT, Ou SH, Bang YJ, et al: Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med* 371:1963-1971, 2014
- Lim SM, Kim HR, Lee JS, et al: Open-label, multicenter, phase II study of ceritinib in patients with non-small-cell lung cancer harboring ROS1 rearrangement. *J Clin Oncol* 35:2613-2618, 2017
- Shaw AT, Felip E, Bauer TM, et al: Lorlatinib in non-small-cell lung cancer with ALK or ROS1 rearrangement: An international, multicentre, open-label, single-arm first-in-man phase I trial. *Lancet Oncol* 18:1590-1599, 2017
- Drilon A, Siena S, Ou SI, et al: Safety and antitumor activity of the multitargeted pan-TRK, ROS1, and ALK inhibitor entrectinib: Combined results from two phase I trials (ALKA-372-001 and STARTRK-1). *Cancer Discov* 7:400-409, 2017
- Lin JJ, Shaw AT: Recent advances in targeting ROS1 in lung cancer. *J Thorac Oncol* 12:1611-1625, 2017
- Facchinetti F, Lorient Y, Kuo MS, et al: Crizotinib-resistant ROS1 mutations reveal a predictive kinase inhibitor sensitivity model for ROS1- and ALK-rearranged lung cancers. *Clin Cancer Res* 22:5983-5991, 2016
- Jiang K, Brownstein S, Sekhon HS, et al: Ocular metastasis of lung adenocarcinoma with ELM4-ALK translocation: A case report with a review of the literature. *Saudi J Ophthalmol* 27:187-192, 2013
- Okuma Y, Tanaka Y, Kamei T, et al: Alectinib for choroidal metastasis in a patient with crizotinib-resistant ALK rearranged positive non-small cell lung cancer. *OncoTargets Ther* 8:1321-1325, 2015
- Shimomura I, Tada Y, Miura G, et al: Choroidal metastasis of non-small cell lung cancer that responded to gefitinib. *Case Rep Ophthalmol Med* 2013:213124, 2013
- Weickhardt AJ, Scheier B, Burke JM, et al: Local ablative therapy of oligoprogressive disease prolongs disease control by tyrosine kinase inhibitors in oncogene-addicted non-small-cell lung cancer. *J Thorac Oncol* 7:1807-1814, 2012
- Markham A: Brigatinib: First global approval. *Drugs* 77:1131-1135, 2017
- Chong CR, Bahcall M, Capelletti M, et al: Identification of existing drugs that effectively target NTRK1 and ROS1 rearrangements in lung cancer. *Clin Cancer Res* 23:204-213, 2017
- Gettinger SN, Bazhenova LA, Langer CJ, et al: Activity and safety of brigatinib in ALK-rearranged non-small-cell lung cancer and other malignancies: A single-arm, open-label, phase 1/2 trial. *Lancet Oncol* 17:1683-1696, 2016
- Anjum R, Vodala S, Kohlmann A, et al: An in vitro mutagenesis screen identifies L1951R and G2032R as drug-resistant mutants of CD74-ROS1. *Mol Cancer Ther* 12, 2013 (suppl; abstr A98)
- Debruyne DN, Bhatnagar N, Sharma B, et al: ALK inhibitor resistance in ALK(F1174L)-driven neuroblastoma is associated with AXL activation and induction of EMT. *Oncogene* 35:3681-3691, 2016
- Ogura H, Nagatake-Kobayashi Y, Adachi J, et al: TKI-addicted ROS1-rearranged cells are destined to survival or death by the intensity of ROS1 kinase activity. *Sci Rep* 7:5519, 2017
- Ou SH, Milliken JC, Azada MC, et al: ALK F1174V mutation confers sensitivity while ALK I1171 mutation confers resistance to alectinib. The importance of serial biopsy post progression. *Lung Cancer* 91:70-72, 2016
- Facchinetti F, Friboulet L: Lorlatinib in ALK- and ROS1-positive NSCLC: The future has a start. *Transl Lung Cancer Res* 7:S103-S106, 2018 (suppl 2)
- Amin AD, Rajan SS, Liang WS, et al: Evidence suggesting that discontinuous dosing of ALK kinase inhibitors may prolong control of ALK+ tumors. *Cancer Res* 75:2916-2927, 2015
- Das Thakur M, Salangsang F, Landman AS, et al: Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. *Nature* 494:251-255, 2013
- Suda K, Tomizawa K, Osada H, et al: Conversion from the "oncogene addiction" to "drug addiction" by intensive inhibition of the EGFR and MET in lung cancer with activating EGFR mutation. *Lung Cancer* 76:292-299, 2012
- Gainor JF, Tseng D, Yoda S, et al: Patterns of metastatic spread and mechanisms of resistance to crizotinib in ROS1-positive non-small-cell lung cancer. *JCO Precis Oncol* 10.1200/PO.17.00063 2017
- McCoach CE, Le AT, Gowan K, et al: Resistance mechanisms to targeted therapies in ROS1+ and ALK+ non-small cell lung cancer. *Clin Cancer Res* 24:3334-3347, 2018
- Davare MA, Vellore NA, Wagner JP, et al: Structural insight into selectivity and resistance profiles of ROS1 tyrosine kinase inhibitors. *Proc Natl Acad Sci USA* 112:E5381-E5390, 2015
- Drilon A, Ou SI, Cho BC, et al: Repotrectinib (TPX-0005) is a next-generation ROS1/TRK/ALK inhibitor that potently inhibits ROS1/TRK/ALK solvent-front mutations. *Cancer Discov* 8:1227-1236, 2018
- Drilon A, Somwar R, Wagner JP, et al: A novel crizotinib-resistant solvent-front mutation responsive to cabozantinib therapy in a patient with ROS1-rearranged lung cancer. *Clin Cancer Res* 22:2351-2358, 2016
- Katayama R, Kobayashi Y, Friboulet L, et al: Cabozantinib overcomes crizotinib resistance in ROS1 fusion-positive cancer. *Clin Cancer Res* 21:166-174, 2015
- Song A, Kim TM, Kim DW, et al: Molecular changes associated with acquired resistance to crizotinib in ROS1-rearranged non-small cell lung cancer. *Clin Cancer Res* 21:2379-2387, 2015

33. Zou HY, Li Q, Engstrom LD, et al: PF-06463922 is a potent and selective next-generation ROS1/ALK inhibitor capable of blocking crizotinib-resistant ROS1 mutations. *Proc Natl Acad Sci USA* 112:3493-3498, 2015
34. Awad MM, Katayama R, McTigue M, et al: Acquired resistance to crizotinib from a mutation in CD74-ROS1. *N Engl J Med* 368:2395-2401, 2013
35. Lacy S, Nielsen J, Yang B, et al: Population exposure-response analysis of cabozantinib efficacy and safety endpoints in patients with renal cell carcinoma. *Cancer Chemother Pharmacol* 81:1061-1070, 2018
36. Zhang S, Anjum R, Squillace R, et al: The potent ALK inhibitor brigatinib (AP26113) overcomes mechanisms of resistance to first- and second-generation ALK inhibitors in preclinical models. *Clin Cancer Res* 22:5527-5538, 2016
37. Nelson-Taylor SK, Le AT, Yoo M, et al: Resistance to RET-inhibition in RET-rearranged NSCLC is mediated by reactivation of RAS/MAPK signaling. *Mol Cancer Ther* 16:1623-1633, 2017

