REVIEW ARTICLE

A Catalog of 5' Fusion Partners in *ROS1*-Positive NSCLC Circa 2020

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ABSTRACT

ROS1 fusion–positive (*ROS1*+) NSCLC was discovered in 2007, the same year as the discovery of *ALK*-positive (*ALK*+) NSCLC but has trailed *ALK*+ NSCLC in terms of development. There seems to be a differential response to ROS1 inhibitors, which depend on fusion partners (CD74, SLC34A2, or SDC4); thus, knowledge of the fusion partners in *ROS1*+ NSCLC is important. To date (end of February 2020), we have identified 24 unique 5' fusion partners of *ROS1* in *ROS1*+ NSCLC from published literature and congress proceedings. Thus, we published this catalog for easy reference.

Keywords: *ROS1* fusion partner; Next-generation sequencing; *ROS1*-positive NSCLC

Introduction

ROS1 fusion–positive (*ROS1*+) NSCLC was discovered in 2007,—the same year as *ALK* fusion–positive (*ALK*+) NSCLC.[1] It constitutes about 2.9% of all adenocarcinomas of the lung.[2] The development of ROS1 TKIs has followed the development of ALK TKIs; but to date, there are only two U.S. Food and Drug Administration–approved ROS1 TKIs (crizotinib and entrectinib).[3,4] Neel et al.[5] reported that different *ROS1* fusion partners determine the subcellular localization of the *ROS1* fusion variant and the subsequent oncogenic potency of that *ROS1* fusion variant. In addition, Li et al.[6] suggested that *ROS1* fusion partners (*CD74-ROS1* versus non–*CD74-ROS1*) have a differential response to crizotinib, and, more importantly, have a predilection for central nervous system metastasis. Thus, it is important to have a catalog of fusion partners of *ROS1* in *ROS1*+ NSCLC.

Methods and Results

We extensively searched publications in PubMed, conference abstracts and presentations, and the cBioPortal for Cancer Genomics website to identify novel *ROS1* fusion partners (including noncoding RNAs). We included only 5' fusion partners that retained the 3'-ROS1 kinase domain. Overall, a total of 24 distinct *ROS1* fusion partners were identified in the literature by the end of February 2020 (Table 1). We did not include one case report, in which the *ROS1* fusion variant arose as a resistance mechanism to EGFR TKI, but the fusion partner to *ROS1* was a 3' fusion partner (*ROS1-ADGRG6*). In that *ROS1* fusion variant, the *ROS1-ADGRG6* fusion

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Table 1. Catalog of Fusion Partners in *ROS1*-Positive NSCLC

No.	Fusion Partner	Year Published in Print/Presented	Chromosomal Location	Fusion Breakpoint	Response to ROS1 TKI at the Time of Publication	Tumor Source	Method of Detection	Variant Frequency in Tumor (%)	FISH/ IHC	References
1	CD74	2007	5q33.1	(C6, R34)	Not treated with ROS1 TKI	FFPE	5' RACE RT-PCR	NR	+/NR	Rikova et al.[1]
2	SLC34A2	2007	4p15.2	(S4, R34)	Not treated with ROS1 TKI	HCC78 cell line	5' RACE RT-PCR	NR	+/NR	Rikova et al.[1]
3	EZR	2012	6q25.3	(E10, R34)	Not treated with ROS1 TKI	FFPE	5' RACE RT-PCR	NR	+/NR	Takeuchi et al.[16]
4	LRIG3	2012	12q14.1	(L16, R35)	Not treated with ROS1 TKI	FFPE	5' RACE RT-PCR	NR	+/NR	Takeuchi et al.[16]
5	SDC4	2012	20q13.12	(S2, R32) (S4, R32) (S4, R34)	Not treated with ROS1 TKI	FFPE	5' RACE RT-PCR	NR	+/NR	Takeuchi et al.[16]
6	TPM3	2012	1q21.3	(T8, R35)	Not treated with ROS1 TKI	FFPE	5' RACE RT-PCR	NR	+/NR	Takeuchi et al.[16]
7	GOPC (FIG)	2012	6q22.1	NR	Not treated with ROS1 TKI	FFPE	RT-PCR	NR	+/+	Rimkunas et al.[17]
		2012	6q22.1	(G7, R35)	Not treated with ROS1 TKI	FFPE	RT-PCR,	NR	NR/NR	Suehara et al.[18]
8	KDREL2	2012	7p22.1	NR	Not treated with ROS1 TKI	PPFE	DNA NGS	NR	NR/NR	Govindan et al.[19]
9	CCDC6	2012	10q21.2	(C6, R34)	Not treated with ROS1 TKI	FFPE	DNA NGS	NR	NR/NR	Seo et al.[20]
10	LIMA1[a]	2012	12q13.12	NR	Response to crizotinib	FFPE	NR	NR	+/NR	Shaw et al.[21]
11	MSN[a]	2012	Xq12	(M9, R34)	NR	FFPE	Targeted RNA sequencing	NR	+/NR	Zheng et al. [22]
		2012	Xq12	NR	Response to crizotinib	FFPE	Targeted RNA sequencing	NR	+/NR	Shaw et al.[21]
12	CLTC	2014	17q23.1	(C31, R35)	Not treated with ROS1 TKI	FFPE	RNA sequencing	NR	NR/NR	TCGA[23]
13	TMEM106B	2015	7p21.3	(T3, R35)	Not treated with ROS1 TKI	FFPE	DNA NGS	NR	NR/NR	Ou et al.[24]
14	TPD52L1	2016	6q22.31	(T3, R33)	Not treated with ROS1 TKI	FFPE	DNA NGS	NR	NR/NR	Zhu et al.[25]
15	SLC6A17	2017	1p13.3	NR	NR	FFPE	NGS	NR	NR/NR	Zehir[26] www.cbioportal.org[9]
16	CEP72	2018	5p15.33	(C11, R23)	Not treated with ROS1 TKI	FFPE	DNA NGS	NR	NR/NR	Zhu et al.[27]
17	ZCCHC8	2018	12q24.31	NR	Not treated with ROS1 TKI	FFPE	NGS	NR	NR/NR	Park et al.[28]
		2018	12q24.31	(Z2, R36)	Response to crizotinib	FFPE	NGS	NR	+/NR	Hicks et al.[29]
		2018	12q24.31	(Z2, R36)	Response to crizotinib[b]	FFPE	NGS	NR	NR/NR	Zhu et al.[30]
18	SLMAP	2018	3p14.3	(S?, R35)	Not treated with ROS1 TKI	FFPE	NGS	NR	NR/NR	Park et al.[28]
19	MYO5C	2018	15q21.2	(M?, R35)	Not treated with ROS1 TKI	FFPE	NGS	NR	NR/NR	Park et al.[28]
20	TFG	2018	3q12.2	NR	Not treated with ROS1 TKI	FFPE	NGS	NR	NR/NR	Park et al.[28]
21	WNK1	2019	12p13.33	(W25, R34)	PR to crizotinib	FFPE	NGS	19.3	NR/NR	Liu et al.[31]

(continued)

Table 1. Continued

No.	Fusion Partner	Year Published in Print/Presented	Chromosomal Location	Fusion Breakpoint	Response to ROS1 TKI at the Time of Publication	Tumor Source	Method of Detection	Variant Frequency in Tumor (%)	FISH/ IHC	References
22	MLL3 (KMT2C)	2019	7q36.1	NR	NR	Plasma	NGS	NR	NR/NR	Dagogo-Jack et al.[32]
23	CTD-2021J15.1 (LINC00973)	2019	3	NR	NR	Plasma	NGS	NR	NR/NR	Dagogo-Jack et al.[32]
24	RBPMS	2020	8p12	(R1, R32)	Response to crizotinib	FFPE	NGS	23.7	NR/NR	Zhang et al.[33]

[a]Both fusions were detected and treated in the crizotinib phase 2 trial. The *MSN-ROS1* fusion identified in the 2 reports was likely the same identical fusion variant. One report described the technique of its identification while the other report reported its response to crizotinib in the expand crizotinib phase 1 trial.
[b]With concurrent de novo *MET* amplification.
5' RACE RT-PCR, 5' rapid amplification of CDA ends reverse transcription polymerase chain reaction; CCDC6, coiled-coil domain containing 6; CD74, cluster of differentiation 74; CEP72, centrosomal protein 72; CLTC, clathrin heavy chain; DCBLD1, discoidin, CUB and LCCL domain containing 1; EZR, ezrin; FFPE, formalin-fixed paraffin embedded; FISH, fluorescence in situ hybridization; GOPC (FIG), golgi associated PDZ and coiled-coil motif containing; IHC, immunohistochemistry; KMT2C (MLL3), lysine methyltransferase 2C; LIMA1, LIM domain and actin binding 1; LINC00973 (CTD-2021J15.1), long intergenic nonprotein coding RNA 973; LRIG3, leucine rich repeats and immunoglobulin-like domains 3; MSN, moesin; MYO5C, myosin VC; NGS, next-generation sequencing; NR, not reported; TFG, trafficking from ER to golgi regulator, TMEM106B, transmembrane protein 106B; TPM3, tropomyosin 3; PR, partial response; TKI, tyrosine kinase inhibitor; WNK1, WNK lysine deficient protein kinase 1; ZCCHC8; zinc finger CCHC-type containing 8.

variant was generated by the fusion of exons 1 to 33 of *ROS1*, which did not contain the ROS1 kinase domain to exons 2 to 26 of *ADGRG6*. However, as the patient responded to crizotinib treatment, there was likely a potential presence of a 3' *ROS1* fusion variant.[7] Another case reported FAM135B as a fusion partner in *ROS1*+ NSCLC.[8] However, on verification of the data in the cBioPortal for Cancer Genomics,[9] it was noted that the patient sample (P-0006921-T01-IM5) contained an *SLC34A2-ROS1* and a *ROS1-FAM135B* variant. In addition, the fusion breakpoint of *ROS1-FAM135B* was not recorded in the cBioPortal for Cancer Genomics. Given the nomenclature listed on the said portal, we interpreted, with the limited information available, that FAM135B would be a 3' fusion partner generated from a nonreciprocal translocation rather than a true 5' *ROS1* fusion partner. Only one intergenic rearrangement has been reported in *ROS1*+ NSCLC (Table 2).

Discussion

The number of *ROS1* fusion partners identified in *ROS1*+ NSCLC as of February 2020 is approximately 24, which is lower than that reported for *ALK*+ and *RET*+ NSCLC.[10,11] It is quite surprising, given the fact that *ROS1*+ NSCLC was discovered in 2007, whereas *RET*+ NSCLC was discovered only in 2012, although *RET* fusions have been identified in other solid tumors, especially in thyroid cancer. The *ROS1* gene is located on chromosome 6q22.1 and only two fusion partners are located near *ROS1* (GOPC, TPD52L1), and one fusion partner, ERZ, is located on 6q25.3. Unlike *ALK*+ and *RET*+ NSCLC, only one intergenic rearrangement has been reported in *ROS1*+ NSCLC (Table 2).

Another unique feature of *ROS1*+ NSCLC is the high incidence of venous thromboembolic events.[12-14] Given the potential role of fusion partners in affecting different oncogenic potencies on the *ROS1* fusion variant,[5] the potential differential response to crizotinib, and the predilection for central nervous system metastasis,[6] identifying *ROS1* fusion partners is essential to further advance the science and management of *ROS1*+ NSCLC. Although five fusion partners (CD74, SLC34A2, SDC4, ERZ, TPM3) made up most of the *ROS1*+ patients with NSCLC who were enrolled in the entrectinib trials, 23% of the patients diagnosed with *ROS1*+ NSCLC had unknown fusion partners.[4] Thus, it is important for future prospective studies of ROS1 TKIs to identify the fusion partners as much as possible, so that future translational studies can be performed from hypotheses generated from the subgroup analysis of these trials.

Table 2. List of Chromosomal Location of Intergenic Translocations With Potential *ROS1* Fusion Partners

No.	Year Published in Print/Presented	Chromosomal Location	Potential Fusion Partner Gene	*RET* Exon Fusion	Response to ALK TKI At the Time of Publication	Tumor Source	Method of Detection	Variant Frequency in Tumor	FISH/ IHC	References
1	2019	6q22.1	*DCBLD1*[c]	R35	NR	FFPE	DNA NGS	NR	NR/ NR	Xu et al.[34]

[c]*DCBLD1* intergenic rearrangement-ROS1 was identified as a potential resistance RTK fusion to osimertinib in an EGFR+ patient with NSCLC (Del 19, T790M) in addition to RP11-565P22.6-NTRK1 fusion.
FFPE, formalin-fixed paraffin embedded; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing; NR, not reported; TKI, tyrosine kinase inhibitor.

Conclusions

1. *ROS1*+ NSCLC is a heterogeneous disease with at least 24 distinct fusion partners identified in the literature up until February 2020; but fewer fusion partners were identified compared with *ALK*+ and *RET*+ NSCLC.
2. It is likely that many more fusion partners and intergenic rearrangements will be identified with the ever-increasing adoption of targeted RNA sequencing and whole transcriptome sequencing owing to the increasing demands of identifying rare, actionable fusions, such as *NTRK* and *NRG1* fusions.
3. We recommend clinicians worldwide to continue to report these novel fusions/intergenic rearrangements, with information on exon breakpoints/fusions, response to ROS1 TKI and allele frequency, and, if possible, whether the tumor is *ROS1*-positive on fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC).
4. In this *ROS1* fusion partner catalog, most of the *ROS1*+ NSCLC did not undergo any FISH or IHC testing. Currently, the companion diagnostic test for *ROS1* rearrangement approved by the U.S. Food and Drug Administration is next-generation sequencing (Oncomine Dx Target test, PMA numberP160045).[15] But given that FISH and IHC are still routinely used to detect *ROS1* fusion, we continue to encourage clinicians when they report novel 5' *ROS1* fusion partners to describe the FISH or IHC results if they had been performed.

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