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Preclinical activity of NVL-520 in ROS1-driven cancer models with diverse fusion partners and kinase-domain mutations

Anupong Tangpeerachaikul*[1], Clare Keddy*[2], Katelyn Nicholson[2], Monika A. Davare[2], and Henry E. Pelish[1]

Affiliations
[1]Nuvalent Inc., Cambridge, MA, USA
[2]Oregon Health & Science University, Portland, OR
*Authors contributed equally

ADDRESSING A MEDICAL NEED

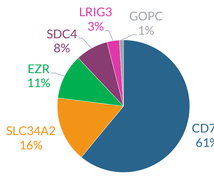
NVL-520
Preclinical features

1. Activity against ROS1, an oncogenic driver
2. Activity against ROS1 resistance mutations including G2032R
3. Activity in the central nervous system (CNS)
4. Sparing TRKB, a key off-target kinase that drives CNS adverse events and dose-limiting toxicities

ROS1 DIVERSITY

FUSION PARTNERS

- In *ROS1*-rearranged cancers, *ROS1* (3') is fused with a partner gene (5') to produce constitutively active ROS1 fusions. Over 20 fusion partners have been discovered.[1]
- Each 5' fusion partner may exert a different effect on subcellular localization, signaling, metastatic capacity, and drug sensitivity of the ROS1 fusion.[2,3,4]



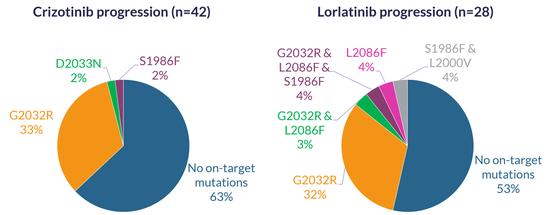
▲ Figure 1 Prevalence of ROS1 fusion partners from the COSMIC dataset as of 2019. Adapted from Neel *et al. Cancer Res* 2019[3]

ROS1 fusion partner	Disease
CD74	Lung adenocarcinoma[5]
CEP85L	Angiosarcoma[6] Glioma & glioblastoma[7]
EZR	Lung adenocarcinoma[8]
GOPC also known as FIG	Cholangiocarcinoma[9] Glioma & glioblastoma[10] Hepatic angiosarcoma[11] Lung adenocarcinoma[12] Ovarian serous carcinoma[13]
LRIG3	Lung adenocarcinoma[14]
SDC4	Lung adenocarcinoma[14]
SLC34A2	Lung adenocarcinoma[5]

▲ Table 1 ROS1 fusions have been identified in various cancers with diverse fusion partners

KINASE-DOMAIN MUTATIONS

- ROS1 kinase-domain mutations (~ residues 1945-2222) have been found to confer resistance to ROS1 inhibitors in both preclinical and clinical settings.[15]
- Resistance mutations observed after progression on FDA-approved ROS1 inhibitor crizotinib and investigational ROS1 inhibitor lorlatinib are shown in Figure 2.
- Clinical resistance mutations to FDA-approved ROS1 inhibitor entrectinib include G2032R and F2004C/I.[17]



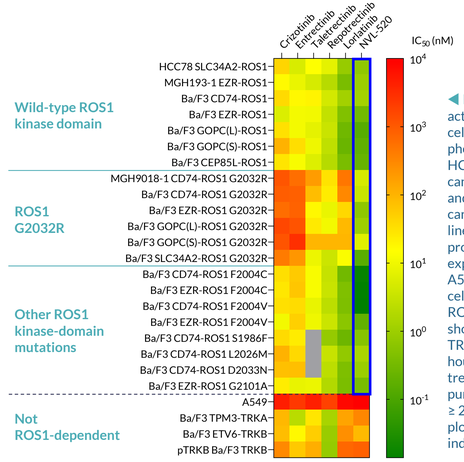
▲ Figure 2 Clinical ROS1 resistance mutations adapted from Lin *et al. Clin Can Res* 2021[16].

Broad coverage of fusion partners and resistance mutations is a beneficial feature for next-generation ROS1 inhibitors.

ACTIVITY IN CELL CULTURE

COMPARATIVE POTENCY ANALYSIS

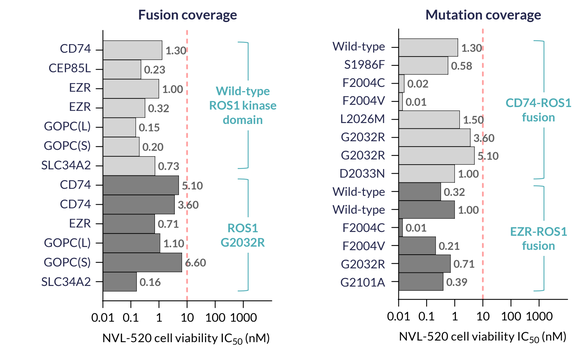
- NVL-520 exhibits IC_{50} < 10 nM activity in all ROS1-fusion models tested in Figure 3 regardless of fusion partner or resistance mutation (blue rectangle).
- For ROS1 G2032R, NVL-520 potency is ≥ 1-order of magnitude higher than crizotinib, entrectinib, and investigational agents taletrectinib, repotrectinib, and lorlatinib.



◀ Figure 3 Heatmap showing activity of ROS1 inhibitors in cell viability assays or TRKB phosphorylation assay. HCC78 is a human lung cancer cell line. MGH193-1 and MGH9018-1 are lung cancer patient-derived cell lines.[18-20] Ba/F3s are mouse pro-B cells engineered to express the indicated genes. A549 is a human lung cancer cell line not dependent on ROS1. pTRKB indicates short-term BDNF-stimulated TRKB phosphorylation (< 2 hours). "IC_{50} <" or "IC_{50} >" are treated as "IC_{50} =" for plotting purposes. All data are from n ≥ 2 repeat testing; average plotted. Grayed-out entries indicate no data available.

FUSION & MUTATION COVERAGE

NVL-520 maintains strong potency (IC_{50} < 10 nM) against every ROS1 fusion partner and resistance mutation tested in Figure 4.



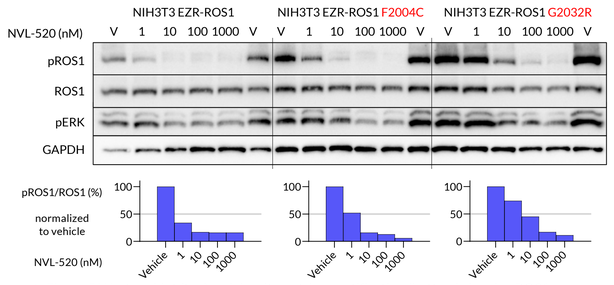
▲ Figure 4 NVL-520 activity against ROS1 fusion partners (left) and resistance mutations (right). IC_{50} of 10 nM is marked with dashed pink line. All data are from n ≥ 2 testing; average plotted. Numerical IC_{50} values are annotated. Duplicate fusions (left) and mutations (right) indicate the same fusions/mutations in different cell models. See complete cell information in Figure 3.

ACTIVITY IN NIH3T3 MODELS

- The mouse embryonic fibroblast cell line NIH3T3 exhibits contact inhibition in monolayer cultures and does not normally form 3-dimensional (3D) colonies.
- Oncogenic transformation of NIH3T3 cells, such as by ROS1 fusions, activates downstream signaling, removes contact inhibition, and enables colony formation.
- Due to its ability to form 3D masses, NIH3T3 is frequently used as a model for testing oncogenic transformation and the effectiveness of kinase inhibitors.

SIGNALING INHIBITION

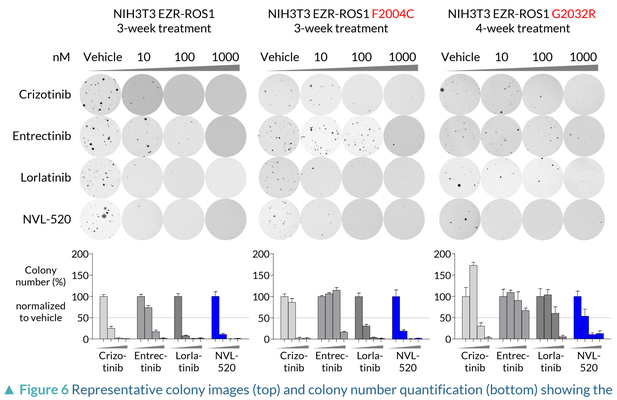
NVL-520 shows potent inhibition (IC_{50} < 10 nM) of ROS1 and ERK phosphorylation in NIH3T3 EZR-ROS1, with wild-type kinase domain or F2004C/G2032R mutations.



▲ Figure 5 Western blot (top) and densitometric quantification (bottom) showing ROS1 phosphorylation inhibition by NVL-520 in NIH3T3 expressing EZR-ROS1 upon 4-hour treatment. V = vehicle; pROS1 = phosphorylated ROS1 at Y2274; n=1 testing.

3D COLONY FORMATION INHIBITION

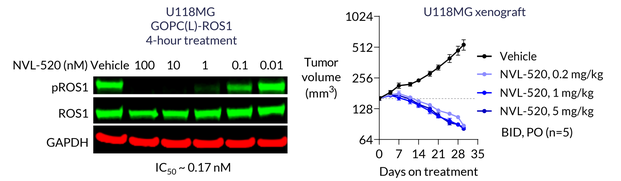
- NVL-520 shows potent inhibition (IC_{50} < 10 nM) of colony formation in NIH3T3 expressing EZR-ROS1, with wild-type kinase domain or F2004C/G2032R mutations.
- The potency of NVL-520 is approximately 1-order of magnitude higher than crizotinib, entrectinib, and lorlatinib for NIH3T3 EZR-ROS1 G2032R



▲ Figure 6 Representative colony images (top) and colony number quantification (bottom) showing the activity of ROS1 inhibitors in suppressing NIH3T3 colonies in soft agar. Average ± SEM (n=3) plotted.

ACTIVITY IN ROS1+ GLIOBLASTOMA

- U118MG is a human glioblastoma cell line driven by GOPC(L)-ROS1 fusion.[10]
- NVL-520 shows potent inhibition (IC_{50} ~ 0.17 nM) of ROS1 phosphorylation in U118MG.
- NVL-520 is efficacious in the U118MG subcutaneous xenograft model and induced maximal regression at all doses tested (0.2 mg/kg – 5 mg/kg BID).



▲ Figure 7 (Left) Western blot showing inhibition of ROS1 activity by NVL-520 in U118MG in cell culture. Densitometry and fitting to 4-parameter logistic equation reveals IC_{50} ~ 0.17 nM. n=1 testing. (Right) NVL-520 induces regression in a U118MG xenograft model subcutaneously implanted in CD17-SCID mice. Vehicle is 20% HP-β-CD and is used to formulate NVL-520. Body weight measurements indicate that all treatments are well tolerated in this study (not shown). Average ± SEM plotted. BID = dosed twice per day; PO = administered orally.

CONCLUSIONS

- NVL-520 shows high activity against diverse ROS1 fusion partners tested including CD74, CEP85L, EZR, GOPC(L), GOPC(S), and SLC34A2. Previous data also indicates potent activity against an SDC4-ROS1 fusion.[21]
- NVL-520 shows high activity against diverse ROS1 kinase-domain mutations tested including S1986F, F2004C/V, L2026M, G2032R, D2033N, and G2101A.
- NVL-520 is active in a model of ROS1-driven glioblastoma.
- Preclinical activity against diverse ROS1 fusion partners and kinase domain mutations suggests broad potential clinical utility of NVL-520.
- NVL-520 is currently under investigation in the Phase 1/2 ARROS-1 study (**NCT05118789**) for advanced ROS1-positive NSCLC and other solid tumors.

	Crizotinib	Entrectinib	Lorlatinib	Repotrectinib	NVL-520 goal
ROS1 activity	Yes	Yes	Yes	Yes	Yes
G2032R activity	No	No	No	Yes	Yes
CNS activity	Not on label	Yes	Yes	Yes	Yes
Sparing TRKB	Limited CNS penetrance	No	Partial at clinical dose	No	Yes

▲ Table 2 Comparative profiles of NVL-520 versus other ROS1 inhibitors.

References

[1]Ou and Nagasaka. *JTO Clin & Res Rep* 2020; 1(3):1
[2]Li et al. JTO 2018; 13(7):987
[3]Neel et al. Cancer Res 2019; 79(3):546
[4]Keddy et al. Mol Can Ther 2022; 21(2):336
[5]Rikova et al. Cell 2007; 131(6):1190
[6]Giacomini et al. PLoS Genet 2013; 9(4):e1003464
[7]Davare et al. Clin Can Res 2018; 24(24):6471
[8]Arai et al. PLoS One 2013; 8(2):e56010
[9]Gu et al. PLoS One 2011; 6(1):e15640
[10]Charest et al. Genes Chrom Cancer 2003; 37(1):58
[11]Marks et al. Oncotarget 2019; 10(2):245
[12]Rimkunas et al. Clin Can Res 2012; 18(16): 4449
[13]Birch et al. PLoS One 2011; 6(12):e28250
[14]Takeuchi et al. Nat Med 2012 12; 18(3):378
[15]Drilon et al. Nat Rev Clin Oncol 2021; 18(1): 35
[16]Lin et al. Clin Can Res 2021; 27(10):2899
[17]Doebele et al. Ann Oncol 2019; 30:v865
[18]Katayama et al. Nat Comm 2019; 10:3604
[19]Lin et al. NPJ Precis Oncol 2020; 4:21
[20]Deshpande et al. Mol Can Ther 2021; 20(12):249
[21]Pelish et al. AACR Annual Meeting 2021

Financial Disclosures
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Disclaimer
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Correspondence: atangpeerachaikul@nuvalent.com