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NUV-520 is a brain-penetrant and highly selective ROS1 inhibitor with antitumor activity against the G2032R solvent front mutation

Henry E. Pelish[1], Anupong Tangpeerachaikul[1], Nancy E. Kohl[1], James R. Porter[1], Matthew D. Shair[1], Joshua C. Horan[1]

Affiliations
[1]Nuvalent, Cambridge, MA

Nuvalent

ADDRESSING A MEDICAL NEED

NUV-520 Design goals

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

1 ACTIVITY AGAINST ROS1

- ROS1 is a receptor tyrosine kinase.
- ROS1 fusions, such as CD74-ROS1, are oncogenic drivers detected in 1-3% of non-small cell lung cancer (NSCLC).[1]
- Crizotinib and entrectinib are approved tyrosine kinase inhibitors (TKIs) for the treatment of ROS1+ NSCLC.

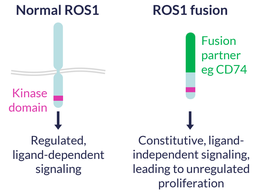
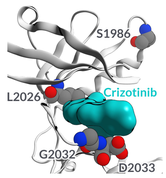


Figure 1 Oncogenesis of ROS1 fusions.

2 ACTIVITY AGAINST ROS1 G2032R



- After crizotinib treatment, ~40% of patients acquire the G2032R "solvent front" mutation, which confers resistance to crizotinib, entrectinib, and lorlatinib.[1]
- Other clinically observed resistance mutations include S1986F, L2026M, and D2033N.[1,2]

Figure 2 Crizotinib-ROS1 crystal structure[3]. Residues with resistance mutations are highlighted.
PDB:3ZBF; Awad *et al.*, *NEJM* 2013; 368:2395

3 ACTIVITY IN THE CNS

- ~30% of ROS1+ NSCLC patients have CNS metastases at diagnosis.[3]
- Crizotinib is not efficacious for CNS metastases. Approximately 55% of patients who have progressive disease following crizotinib treatment show CNS metastases.[3]

4 SPARING TRKB

- TRK-family kinases (TRKA/B/C) play crucial neurological functions in humans.[4]
- Sparing TRKB may be beneficial. TRKB-related adverse events have been reported for CNS-penetrant TRK inhibitors and include cognitive impairment, mood disorders, sleep disturbances, dizziness, ataxia, and weight gain.[4,5,6,7]
- TRKB is structurally similar to ROS1, making it challenging to achieve selectivity.

In summary, NUV-520 is a novel ROS1 inhibitor designed to address the gaps identified by earlier-generation ROS1 inhibitors:

	Crizotinib	Entrectinib	Lorlatinib	Repotrectinib	NUV-520
ROS1 activity	Yes	Yes	Yes	Yes	**Yes**
G2032R activity	No	No	No	Trial ongoing	**Yes**
CNS activity	No	Yes	Yes	Yes	**Yes**
Sparing TRKB	Limited CNS penetrance	No	Partial	No	**Yes**

▲ Table 1 Comparative profiles of NUV-520 and other ROS1 inhibitors.

IN VITRO ACTIVITY

BIOCHEMICAL ACTIVITY

- NUV-520 potently inhibits both ROS1 and ROS1 G2032R in *in vitro* assays.
- ROS1 G2032R shows strong resistance against crizotinib, entrectinib, and lorlatinib.

ROS1	NUV-520	Crizotinib	Entrectinib	Lorlatinib	Repotrectinib
Wild-type	0.7 nM	12 nM	5.2 nM	0.4 nM	1.4 nM
G2032R	7.9 nM	5700 nM	>1100 nM	950 nM	30 nM

▲ Table 2 In vitro biochemical IC_{50} using purified ROS1 kinase domains (assayed at 1 mM ATP).

KINASE SELECTIVITY SCREEN

- NUV-520 is highly selective for ROS1.
- Across a panel of 335 wild-type kinases, only ALK is inhibited with an IC_{50} ≤ 10-fold of ROS1 and 5 other kinases with IC_{50}s ≤ 50-fold of ROS1.

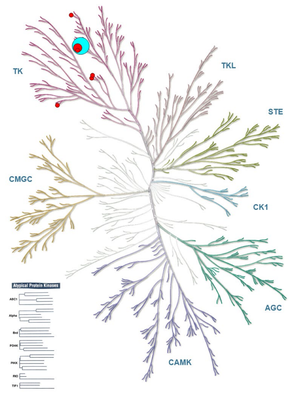


Figure 3 Results of kinase selectivity screen for NUV-520 (Wild Type Kinase Panel, Reaction Biology, Germany), displayed on a kinome tree. The cyan circle denotes ROS1. Other kinases are plotted with red circles whose size corresponds to the IC_{50} relative to ROS1. Kinases with IC_{50} > 50-fold of ROS1 IC_{50} are not plotted.
Eid S. *et al.*, ***Bioinformatics*** 2017. Illustration reproduced courtesy of Cell Signaling Technologies, Inc. (www.cellsignal.com).

Fold ROS1 IC_{50}	Kinase
1x	ROS1
1 – 10x	ALK
10 – 50x	LTK, FAK, PYK2, TRKB, FER
>50x	328 other kinases

CELLULAR ACTIVITY

- NUV-520 potently inhibits Ba/F3 cells expressing CD74-ROS1 fusions.
- NUV-520 exhibits potency against clinically relevant drug-resistant mutations.
- ROS1 G2032R shows strong resistance to crizotinib, entrectinib, and lorlatinib.

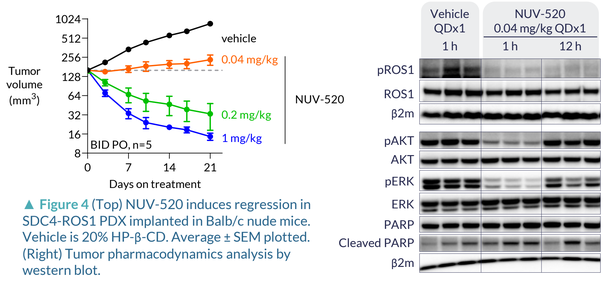
ROS1	NUV-520	Crizotinib	Entrectinib	Lorlatinib	Repotrectinib
Wild-type	1.2 nM	40 nM	23 nM	1.3 nM	4.4 nM
G2032R	3.5 nM	960 nM	1500 nM	300 nM	25 nM
S1986F	< 0.58 nM	39 nM	26 nM	< 0.27 nM	0.84 nM
L2026M	1.5 nM	110 nM	41 nM	0.77 nM	3.3 nM
D2033N	1.0 nM	77 nM	79 nM	0.44 nM	2.5 nM

▲ Table 3 Cell viability 3-day IC_{50} of engineered Ba/F3 cells expressing CD74-ROS1 fusions.

IN VIVO ANTITUMOR ACTIVITY

NSCLC PDX: SDC4-ROS1

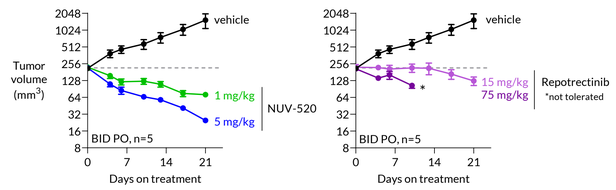
NUV-520 is efficacious in the NSCLC patient-derived xenograft (PDX) model LU-01-0414 harboring SDC4-ROS1 (Wuxi AppTec). Western blot of tumor tissues confirms *in vivo* ROS1 inhibition through reduced phosphorylation of ROS1, ERK, and AKT, and indicates apoptosis (PARP cleavage).



▲ Figure 4 (Top) NUV-520 induces regression in SDC4-ROS1 PDX implanted in Balb/c nude mice. Vehicle is 20% HP-β-CD. Average ± SEM plotted. (Right) Tumor pharmacodynamics analysis by western blot.

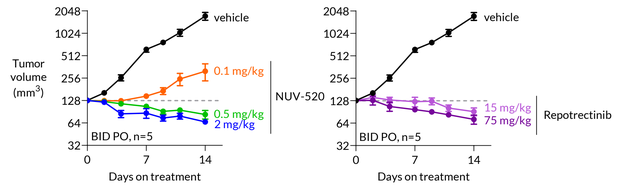
NSCLC PDX: CD74-ROS1 G2032R

NUV-520 is efficacious in the NSCLC PDX model CTG-2532 harboring CD74-ROS1 G2032R (Champions Oncology). In the same study, repotrectinib gives limited regression at 15 mg/kg and is not tolerated at 75 mg/kg.



▲ Figure 5 NUV-520 induces regression in CD74-ROS1 G2032R PDX implanted in Nude-*Foxn1*^{nu} mice. Repotrectinib is tested in the same study and dosed as a suspension in 0.5% CMC/1% Tween-80.[8] Vehicle is 20% HP-β-CD and is used to formulate NUV-520. Average ± SEM plotted.

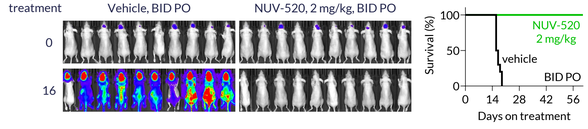
BA/F3 XENOGRAFT: CD74-ROS1 G2032R



▲ Figure 6 NUV-520 induces regression in a xenograft model of Ba/F3 CD74-ROS1 G2032R cells implanted in Balb/c nude mice. Repotrectinib is tested in the same study and dosed as a suspension in 0.5% CMC/1% Tween-80.[8] Vehicle is 20% HP-β-CD and is used to formulate NUV-520. Average ± SEM plotted. In this model, both compounds are well tolerated.

CNS ANTITUMOR ACTIVITY

NUV-520 is efficacious in a mouse intracranial tumor model of Ba/F3 CD74-ROS1 G2032R luciferase, reducing brain tumors and extending median survival by > 3-fold.



▲ Figure 7 (Left) Bioluminescence imaging of Balb/c nude mice intracranially implanted with Ba/F3 CD74-ROS1 G2032R luciferase. Vehicle is 20% HP-β-CD. Color indicates luminescence, with color scale from blue = 10^6 to red = 10^8 p/sec/cm^2/sr. (Right) Survival analysis. Median survival is 16.5 days for vehicle group and >61 days for NUV-520-treated group.

SPARING TRKB

- TRK-family kinases (TRKA/B/C) play crucial neurological functions in humans.[4]
- Sparing TRKB may be beneficial. TRKB-related adverse events have been reported for CNS-penetrant TRK inhibitors and include cognitive impairment, mood disorders, sleep disturbances, dizziness, ataxia, and weight gain.[4,5,6,7]
- NUV-520 was designed to selectively inhibit ROS1 while sparing TRKB, in contrast to dual TRK/ROS1 inhibitors entrectinib and repotrectinib. NUV-520 shows large selectivity windows for ROS1 (208-fold) and ROS1 G2032R (68-fold) vs TRKB.

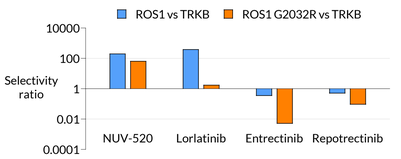


Figure 8 ROS1 vs TRKB (blue) and ROS1 G2032R vs TRKB (orange) selectivity. Cellular BNDF-stimulated TRKB phosphorylation IC_{50} was quantified using PathHunter assay (Eurofins) and compared to Ba/F3 CD74-ROS1 3-day viability IC_{50}. IC_{50}s were adjusted for serum binding in each assay.

CONCLUSIONS

- NUV-520 is a potent, highly selective, and brain-penetrant ROS1 inhibitor as demonstrated by *in vitro* and *in vivo* studies.
- NUV-520 has broad activity against ROS1 resistance mutations, including G2032R, and multiple ROS1 fusions.
- NUV-520 is highly selective for ROS1 and ROS1 G2032R over TRKB, indicating the potential to minimize TRK-related CNS adverse events seen with dual TRK/ROS1 inhibitors and drive more durable responses for patients with ROS1 mutations.

References
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Financial Disclosures
HEP, AT, JRP, and JCH are employees and shareholders of Nuvalent. NEK is a consultant of Nuvalent. JRP and MDS are on the Nuvalent Board of Directors.

Disclaimer
Compounds other than NUV-520 were purchased from commercial sources.

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Correspondence: hpelish@nuvalent.com