#1468

NUV-655 is a selective, brain-penetrant ALK inhibitor with antitumor activity against the lorlatinib-resistant G1202R/L1196M compound mutation

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ADDRESSING A MEDICAL NEED

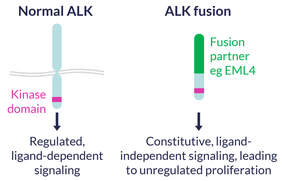
NUV-655 Design goals

1. Activity against ALK, an oncogenic driver
2. Activity against ALK resistance mutations including G1202R, G1202R/L1196M, and G1202R/G1269A (collectively, G1202R+ mutations)
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

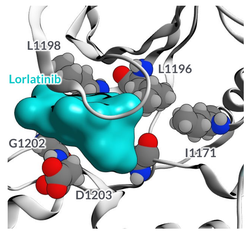
1 ACTIVITY AGAINST ALK

- ALK is a receptor tyrosine kinase.
- ALK fusions, such as EML4-ALK, are oncogenic drivers detected in 3-5% of non-small cell lung cancer (NSCLC).[1]
- There have been 5 tyrosine kinase inhibitors (TKIs) approved for the treatment of ALK+ NSCLC. They are categorized into 3 generations: *1st generation* (crizotinib); *2nd generation* (ceritinib, alectinib, and brigatinib); and *3rd generation* (lorlatinib).

Figure 1 ❯
Oncogenesis of ALK fusions.



2 ACTIVITY AGAINST ALK G1202R+ MUTATIONS



- Following treatment with 1st and/or 2nd generation ALK therapies, ~40% of patients develop the G1202R "solvent front" mutation, which confers resistance to crizotinib, ceritinib, alectinib, and brigatinib.
- Lorlatinib has been approved for treating patients who have previously received 1st and/or 2nd generation ALK therapies. However, compound mutations, including G1202R/L1196M and G1202R/G1269A, have been observed in these patients after progressive disease following lorlatinib.[2] The G1202R/L1198F compound mutation has also been observed to be resistant to lorlatinib in mutagenesis experiments.[3]

❮ Figure 2 Lorlatinib-ALK crystal structure[3]. Residues with known resistance mutations are highlighted. PDB:4CLI; Johnson *et al., J. Med. Chem.* 2013; 57:4720

3 ACTIVITY IN THE CNS

- 30-40% of ALK+ NSCLC patients have CNS metastases at diagnosis.[4,5]
- Advanced patients with a history of ALK TKI treatments show a high incidence of CNS metastases (>60%).[4,5]

4 SPARING TRKB

- TRK-family kinases (TRKA/B/C) play crucial neurological functions in humans.[6]
- 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.[6,7,8,9]
- TRKB is structurally similar to ALK, making it challenging to achieve selectivity.

In summary, NUV-655 is a novel ALK inhibitor designed to address the gaps identified by earlier-generation TKIs:

	Crizotinib	Ceritinib	Alectinib	Brigatinib	Lorlatinib	NUV-655
ALK activity	Yes	Yes	Yes	Yes	Yes	Yes
G1202R activity	No	No	No	No	Yes	Yes
G1202R/L1196M activity	No	No	No	No	No	Yes
CNS activity	No	Yes	Yes	Yes	Yes	Yes
Sparing TRKB	Limited CNS penetrance	Yes	Yes	Yes	5-fold for G1202R	Yes

▲ Table 1 Comparative profiles of NUV-655 and other ALK inhibitors.

IN VITRO ACTIVITY

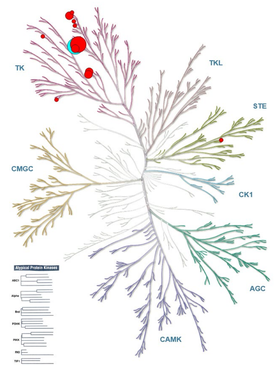
BIOCHEMICAL ACTIVITY

- NUV-655 potently inhibits both ALK and ALK G1202R/L1196M in *in vitro* assays.
- ALK G1202R/L1196M shows strong resistance against crizotinib, ceritinib, alectinib, and lorlatinib.

ALK	NUV-655	Crizotinib	Ceritinib	Alectinib	Brigatinib	Lorlatinib
Wild-type	1.2	16	4.0	2.3	3.0	2.2
G1202R/L1196M	2.5	3800	130	>10000	61	570

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

KINASE SELECTIVITY SCREEN



- NUV-655 is selective for ALK.
- Across a panel of 335 wild-type kinases, 5 kinases are inhibited with IC_{50}s ≤ 10-fold of ALK, which include ROS1, LTK, PYK2, TRKB, and FAK; and 6 other kinases are inhibited with IC_{50}s ≤ 50-fold of ALK.
- In a more physiologically relevant assay, NUV-655 shows >43-fold selectivity over TRKB (see Figure 5).

❮ Figure 3 Results of kinase selectivity screen for NUV-655 (Wild Type Kinase Panel, Reaction Biology, Germany), displayed on a kinome tree. The cyan circle denotes ALK. Other kinases are plotted with red circles whose size corresponds to the IC_{50} relative to ALK. Kinases with IC_{50} > 50-fold of ALK IC_{50} are not plotted.

Eid S. *et al., Bioinformatics* 2017. Illustration reproduced courtesy of Cell Signaling Technologies, Inc. (www.cellsignal.com).

Fold ALK IC_{50}	Kinase
1x	ALK, ROS1
1 – 10x	LTK, PYK2, TRKB, FAK
10 – 50x	SLK, TRKA, FER, MUSK, EPHA6, TRKC
>50x	323 other kinases

CELLULAR ACTIVITY

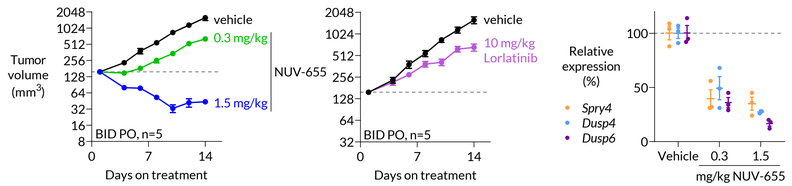
- NUV-655 potently inhibits human cell lines and Ba/F3 cells expressing EML4-ALK fusions, v1 or v3.
- NUV-655 is potent against G1202R+ drug-resistant mutations including G1202R, G1202R/L1196M, G1202R/G1269A, and G1202R/L1198F, which confer resistance to earlier-generation ALK therapies.

	Cell with ALK fusion	NUV-655	Crizotinib	Ceritinib	Alectinib	Brigatinib	Lorlatinib
No kinase domain mutations	NCI-H2228 (EML4-ALK v3)	0.70	90	55	13	13	< 1.1
	NCI-H3122 (EML4-ALK v1)	2.0	180	48	22	22	3.5
	Wild-type	1.6	270	90	25	42	4.2
G1202R+ mutations	G1202R	< 0.73	950	570	1600	400	120
	G1202R/L1196M	7.0	1500	1400	2200	820	3900
	G1202R/G1269A	3.0	1100	350	1300	240	970
	G1202R/L1198F	2.0	170	1300	2200	470	720

▲ Table 3 Cell viability 3-day IC_{50} (nM) of human cell lines (NCI-H2228, NCI-H3122) or of Ba/F3 cells expressing EML4-ALK v1 fusions.

IN VIVO ANTITUMOR ACTIVITY

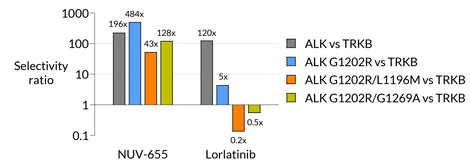
- NUV-655 is efficacious in a Ba/F3 xenograft model harboring EML4-ALK v1 G1202R/L1196M. In the same study, lorlatinib only modestly inhibited tumor growth, consistent with the detection of the G1202R/L1196M compound mutation in patients at progression on lorlatinib.[2] Both compounds were well-tolerated in this study.
- Quantitative PCR supports ALK inhibition in tumor tissues through reduced expression of MAP kinase pathway transcripts *Spry4*, *Dusp4*, and *Dusp6*.



▲ Figure 4 (Left and middle) NUV-655 induces regression in a xenograft model of Ba/F3 EML4-ALK v1 G1202R/L1196M cells implanted in Balb/c nude mice. Lorlatinib is tested in the same study at 10 mg/kg, a dose selected to approximate the exposure of the human dose of 100 mg QD.[10,11] Vehicle is 20% HP-β-CD. Average ± SEM plotted. (Right) Tumor pharmacodynamics analysis by qPCR showing expression of 3 genes (*Spry4*, *Dusp4*, *Dusp6*) relative to *Gapdh*.

CNS PENETRANCE & SPARING TRKB

- NUV-655 shows a high unbound brain-to-plasma partition coefficient (Kp,uu = 0.16 at 1 h) and a high CSF-to-unbound plasma partition coefficient (1.2 at 1 h) after a single oral dose of 10 mg/kg in Wistar Han rats. The values are comparable to that of lorlatinib (0.11 and 0.47, respectively) in a similar experiment performed in parallel.
- TRKB plays crucial neurological functions, and sparing TRKB may be beneficial.[6] 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.[6,7,8,9]
- NUV-655 was designed to selectively inhibit ALK while sparing TRKB, showing 43- to 484-fold selectivity windows depending on ALK mutation. By comparison, lorlatinib shows only a 5-fold selectivity window for ALK G1202R vs TRKB.



❮ Figure 5 ALK vs TRKB selectivity. Cellular BDNF-stimulated TRKB phosphorylation IC_{50} was quantified using PathHunter assay (Eurofins) and compared to 3-day viability IC_{50} of Ba/F3 EML4-ALK v1. IC_{50}s were adjusted for serum binding in each assay. The PathHunter assay is more physiologically relevant (ligand-stimulate full-length TRKB on the cell membrane) than the biochemical screen in Figure 3.

CONCLUSIONS

- NUV-655 is a potent, selective, and brain-penetrant ALK inhibitor as demonstrated by *in vitro* and *in vivo* studies.
- NUV-655 is active against G1202R+ mutations including compound mutations G1202R/L1196M, G1202R/G1269A, and G1202R/L1198F, which confer resistance to all approved ALK therapies.
- NUV-655 is selective for ALK and ALK G1202R+ mutations over TRKB, indicating the potential to minimize TRK-related CNS adverse events and drive more durable responses for patients.

❯ References
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❯ Financial Disclosures
HEP, AT, JRP, and JCH are employees of Nuvalent.
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JRP and MDS are on the Nuvalent Board of Directors.

❯ Disclaimer
Compounds other than NUV-655 were purchased from commercial sources.

❯ Acknowledgements
We thank Alice T. Shaw, Aaron Hata, and Jessica J. Lin for helpful advice.