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Preclinical activity of NVL-655 in ALK-driven cancer models beyond non-small cell lung cancer

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ADDRESSING A MEDICAL NEED

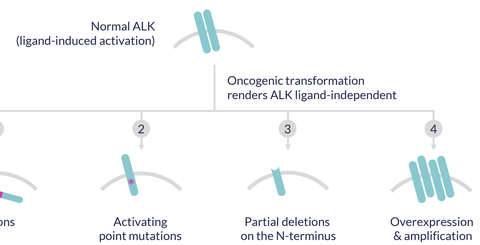
NVL-655
Preclinical Features

1. Activity against ALK, an oncogenic driver
2. Activity against ALK resistance mutations, including G1202R and G1202R-containing compound 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

THE ALK ONCOGENE

MECHANISMS OF ONCOGENESIS

- At least 4 oncogenic activation mechanisms have been identified for ALK: fusions, activating point mutations, partial N-terminal deletions, and overexpression.[1,2]



▲ Figure 1 ALK oncogenic activation mechanisms. Fusions, point mutations, and partial deletions exist in great varieties. Partial deletions may manifest at the genomic[3], transcriptional (including ALK alternative transcription initiation or ATI[4]), or post-translational stages[5].

- Each mechanism may transform cells by altering ALK expression levels, subcellular localization, enzymatic activity, dimerization states, and/or stoichiometry.[2,6,7]
- Fusions, point-mutations, and partial deletions have been targeted with small-molecule ALK inhibitors in clinical settings.[4,8-10]

ALK DRIVES DIVERSE CANCERS

Table 1 summarizes some of the most frequent ALK mutations in diverse cancers.

Mechanism	Disease	Prevalence	Notable examples
Fusions	Lung adenocarcinoma	~5%[11]	EML4-ALK fusion
	Anaplastic large-cell lymphoma	~80%[12]	NPM1-ALK fusion
	Inflammatory myofibroblastic tumor	~50%[13]	TPM3/4-ALK fusion
Point mutations	Neuroblastoma	10-15%[10,14]	R1275Q, F1174X, & F1245X
	Anaplastic thyroid cancer	~11%[15]	L1198F & G1201E
Partial deletions	Melanoma	2-11%[4]	Alternative transcription initiation

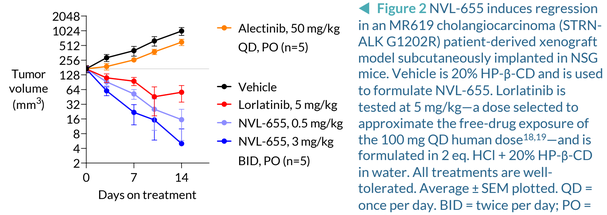
▲ Table 1 ALK activation drives various cancers.

Broad activity across diverse ALK-driven cancers is a beneficial feature for next-generation ALK inhibitors.

ALK MODELS BEYOND NON-SMALL CELL LUNG CANCER

CHOLANGIOCARCINOMA

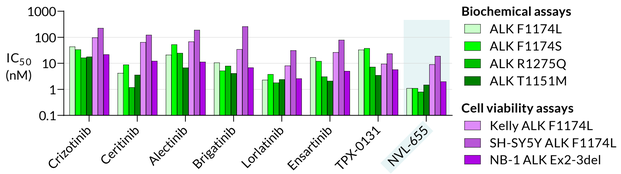
- ALK fusions have been identified in rare cases of cholangiocarcinoma.[16]
- MR619 is an STRN-ALK G1202R xenograft model derived from an ALK-positive cholangiocarcinoma patient after progression on alectinib.[17]
- NVL-655 is efficacious in the MR619 STRN-ALK G1202R patient-derived xenograft model, inducing deep regression at both doses tested (0.5 & 3 mg/kg BID).



◀ Figure 2 NVL-655 induces regression in an MR619 cholangiocarcinoma (STRN-ALK G1202R) patient-derived xenograft model subcutaneously implanted in NSG mice. Vehicle is 20% HP-β-CD and is used to formulate NVL-655. Lorlatinib is tested at 5 mg/kg—a dose selected to approximate the free-drug exposure of the 100 mg QD human dose[18,19]—and is formulated in 2 eq. HCl + 20% HP-β-CD in water. All treatments are well-tolerated. Average ± SEM plotted. QD = once per day. BID = twice per day; PO = administered orally.

NEUROBLASTOMA

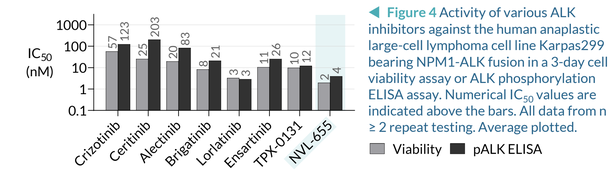
- F1174L, F1174S, and R1275Q are among the most common activating ALK mutations in neuroblastoma.[10,14] In addition, T1151M has been identified.[20]
- NVL-655 exhibits potent activity (IC_{50} = 0.8 – 19 nM) against ALK F1174L/S, R1275Q, T1151M, and partial deletion in biochemical assays and/or in cell viability assays using human neuroblastoma cell lines.



▲ Figure 3 Activity of various inhibitors against ALK activating mutations in neuroblastoma. Biochemical assays were performed using purified ALK kinases and a fluorogenic substrate (PhosphoSens[21]). Cell viability assays (3-day) were performed using Kelly (F1174L), SH-SY5Y (F1174L), and NB-1 (Ex2-3del) human neuroblastoma cell lines. NB-1 is historically known to have ALK-overexpression but was identified to also harbor ALK partial deletion around exons 2-3.[3] All data from n ≥ 2 repeat testing. Average plotted.

ANAPLASTIC LARGE-CELL LYMPHOMA

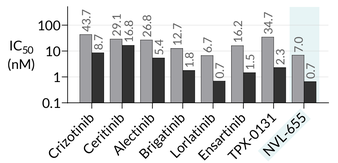
NVL-655 exhibits potent activity (IC_{50} = 2 nM) against the human anaplastic large-cell lymphoma cell line Karpas299 bearing NPM1-ALK fusion.



◀ Figure 4 Activity of various ALK inhibitors against the human anaplastic large-cell lymphoma cell line Karpas299 bearing NPM1-ALK fusion in a 3-day cell viability assay or ALK phosphorylation ELISA assay. Numerical IC_{50} values are indicated above the bars. All data from n ≥ 2 repeat testing. Average plotted.

SOFT-TISSUE SARCOMA

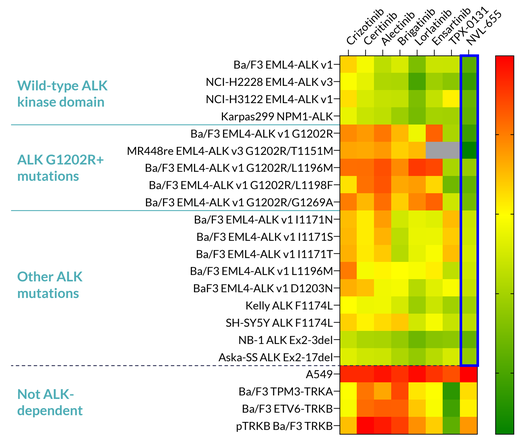
- Soft-tissue sarcomas encompass many types of cancers arising from soft tissues, and some subtypes have been observed to have ALK rearrangements.[22]
- Aska-SS is a human cancer cell line established from synovial sarcoma, a type of soft-tissue sarcoma, and is driven by ALK partial deletion around exons 2-17.[23]
- NVL-655 exhibits potent activity (viability IC_{50} = 7 nM) against Aska-SS.



◀ Figure 5 Activity of various ALK inhibitors against Aska-SS bearing ALK deletion around exons 2-17 in a 3-day cell viability assay and ALK phosphorylation ELISA assay. Numerical IC_{50} values are indicated above the bars. All data from n ≥ 2 repeat testing. Average plotted.

COMPARATIVE POTENCY ANALYSIS

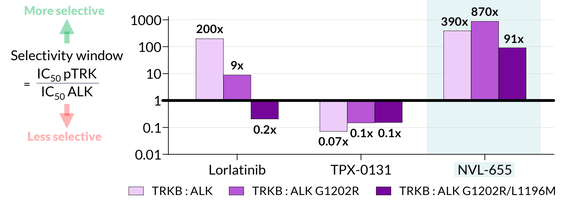
- NVL-655 exhibits high activity (IC_{50} < 50 nM) across all ALK-driven cancer models in Figure 6 regardless of fusion partner, activating mutation, resistance mutation, or disease background (blue rectangle).
- NVL-655 exhibits especially high activity (IC_{50} < 10 nM) against ALK with wild-type kinase domain, ALK G1202R, and ALK G1202R-based compound mutations.
- NVL-655 displays broader activity across diverse ALK oncoproteins than crizotinib, ceritinib, alectinib, brigatinib, lorlatinib, and TPX-0131.



▲ Figure 6 Heatmap showing the activity of ALK inhibitors in 3-day cell viability assays or a TRKB phosphorylation assay. NCI-H2228 and NCI-H3122 are human lung cancer cell lines. Karpas299 is a human lymphoma cell line. Kelly, SH-SY5Y, and NB-1 are human neuroblastoma cell lines. Aska-SS is a human synovial sarcoma cell line. MR448re is a lung cancer cell line derived from a patient who has progressed on lorlatinib. Ba/F3s are mouse pro-B cells engineered to express the indicated genes. A549 is an ALK-independent human lung cancer cell line. pTRKB indicates short-term BDNF-stimulated TRKB phosphorylation (< 2 hours). All data from n ≥ 2 repeat testing. Average plotted. Grayed-out entries indicate no data available. "<" or ">" are ignored for plotting.

COMPARATIVE SELECTIVITY ANALYSIS

- TRKB plays crucial neurological functions. 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.[24-27] Sparing TRKB may be beneficial.[24]
- NVL-655 was designed to selectively inhibit ALK while sparing TRKB, showing 91- to 870-fold selectivity for ALK (with or without resistance mutations) over TRKB.
- By comparison, lorlatinib shows a 9-fold selectivity window for ALK G1202R over TRKB, and TPX-0131[28] shows a < 1-fold selectivity window.



▲ Figure 7 Selectivity window calculated as the ratio between BDNF-stimulated TRKB phosphorylation IC_{50} in Ba/F3 TRKB and cell viability IC_{50} in Ba/F3 ALK (values from Figure 6).

CONCLUSIONS

- NVL-655 shows strong activity in diverse preclinical models of ALK-driven cancers: cholangiocarcinoma, neuroblastoma, lymphoma, and soft-tissue sarcoma.
- Among all inhibitors tested, NVL-655 shows the broadest activity for diverse ALK oncoproteins including fusions, point mutations, and partial N-terminal deletions.
- NVL-655 shows larger ALK-vs-TRK selectivity windows than lorlatinib and TPX-0131.
- Preclinical activity against diverse ALK oncoproteins (fusions, mutations, and partial deletions) in multiple tumor types suggests broad potential clinical utility of NVL-655.

	Crizotinib	2nd gen	Lorlatinib	TPX-0131	NVL-655 goal
ALK activity	Yes	Yes	Yes	Yes	Yes
G1202R activity	No	No	Yes	Yes	Yes
G1202R/L1196M activity	No	No	No	Yes	Yes
CNS activity	Not on label	Yes	Yes	Likely[28]	Yes
Sparing TRKB	Limited CNS penetrance	Yes	Limited for G1202R	No	Yes

▲ Table 2 Comparative profiles of NVL-655 versus other ALK inhibitors. FDA-approved 2nd-generation inhibitors include ceritinib, alectinib, and brigatinib.

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