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Poster #P244

NVL-655 Exhibits Antitumor Activity in Lorlatinib-Resistant Subcutaneous and Intracranial Models of ALK-Rearranged NSCLC

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NVL-655: a Potential Next-Generation ALK Inhibitor

Design goals

1. **Activity against ALK**
   ALK fusion is an oncogenic driver found in up to 5% of non-small cell lung cancers (NSCLC)

2. **Activity against ALK resistance mutations, especially G1202R+ mutations**
   G1202R solvent-front mutation arising from first- and second-generation ALK therapies
   G1202R/L1196M and G1202R/G1269A compound mutations arising from third-generation ALK therapy

3. **Activity in the central nervous system (CNS)**
   for brain metastases found in up to 40% of ALK+ NSCLC patients

4. **Sparing TRKB**
   a key off-target kinase of some ALK and ROS1 inhibitors believed to drive CNS adverse events and dose-limiting toxicities (more details in Poster #P247)

Pelish *et al.* AACR Annual Meeting 2021

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NVL-655 Inhibited ALK, Particularly G1202R+ Mutations, in Biochemical Assays

	Purified ALK kinase domain	NVL-655	Alectinib	Lorlatinib
	Wild-type	0.7	9.2	1.4
G1202R+ mutations	G1202R	0.9	>10000	35
G1202R+ mutations	G1202R/L1196M	2.3	>10000	220
Non-G1202R+ mutations	T1151_L1152insT	1.5	6.9	1.6
Non-G1202R+ mutations	C1156Y	1.0	5.8	0.7
Non-G1202R+ mutations	F1174L	1.0	12	0.5
Non-G1202R+ mutations	L1196M	11	29	20
Non-G1202R+ mutations	S1206R	1.8	29	1.4
Non-G1202R+ mutations	G1269A	16	27	7.8
Non-G1202R+ mutations	G1269S	79	46	67

Potency color legend
IC_{50} < 10 nM
10 nM ≤ IC_{50} < 100 nM
IC_{50} ≥ 100 nM

Table 1 | *In vitro* biochemical IC_{50} (nM) using purified ALK kinase domains and assayed at 1 mM ATP. Data for NVL-655 and lorlatinib were from at least duplicate testing (n=2 or more). Data for alectinib was from a single testing (n=1).

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NVL-655 Inhibited ALK, Particularly G1202R+ Mutations, in Cell Viability Assays

	Cell expressing ALK fusion	NVL-655	Crizotinib	Ceritinib	Alectinib	Brigatinib	Lorlatinib
No kinase domain mutations	NCI-H3122 (EML4-ALK v1)	2.3	180	36	23	21	3.5
	NCI-H2228 (EML4-ALK v3)	0.70	90	55	13	13	< 1.1
	Karpas299 (NPM1-ALK)	2.0	59	25	18	7.8	3.5
	Ba/F3 EML4-ALK v1	1.6	270	90	25	42	< 3.6
G1202R+ mutations	Ba/F3, G1202R	< 0.73	950	570	1600	400	87
	Ba/F3, G1202R/L1196M	7.0	1500	1400	2200	820	3600
	Ba/F3, G1202R/G1269A	3.0	1100	350	1300	240	970
Non-G1202R+ mutations	Ba/F3, L1196M	29	1100	79	120	100	86
	Ba/F3, I1171N	27	320	140	570	30	59
	Ba/F3, I1171S	29	350	140	390	18	59
	Ba/F3, I1171T	35	400	140	260	16	51

Potency color legend
IC_{50} < 10 nM
10 nM ≤ IC_{50} < 100 nM
IC_{50} ≥ 100 nM

Table 2 | Cell viability 3-day IC_{50} (nM) of human cells or of Ba/F3 cells expressing EML4-ALK v1 fusions with indicated mutations. All IC_{50}s are from at least duplicate testing (n=2 or more).

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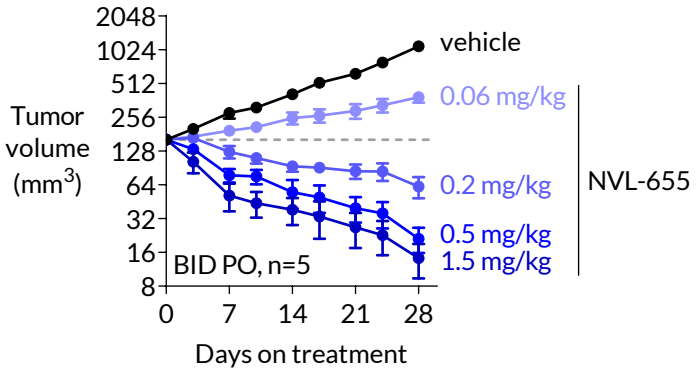
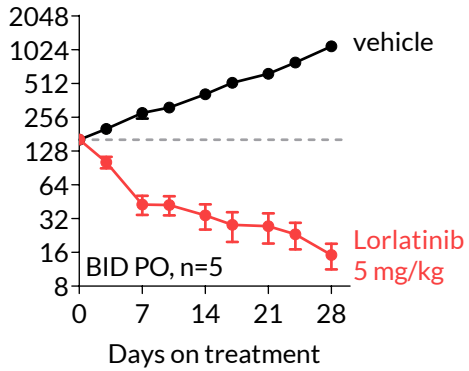
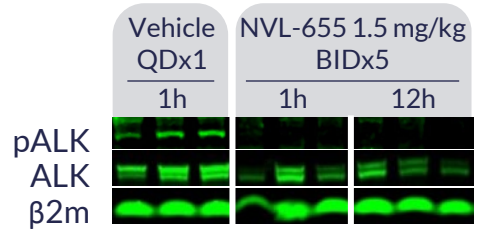
NVL-655 Showed Activity in Tumor Models with ALK Wild-Type Kinase Domain

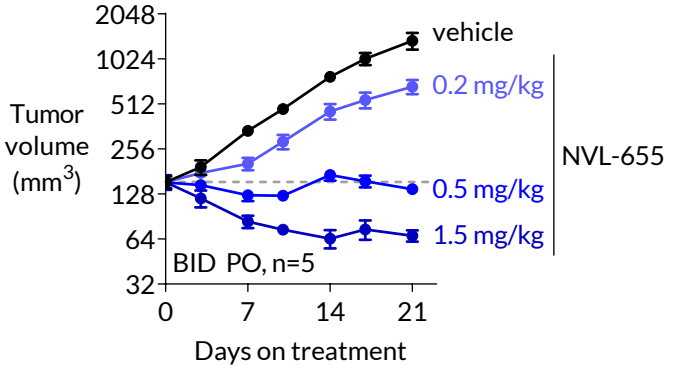
A. PDX
HIP1-ALK

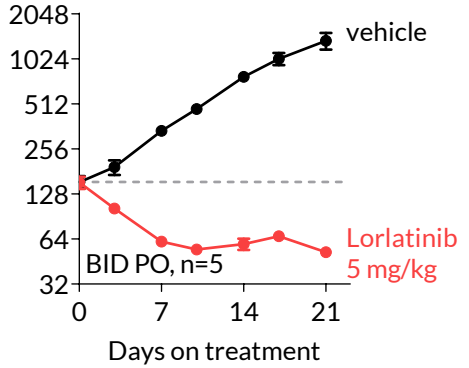






B. Human CDX
NCI-H3122
EML4-ALK v1





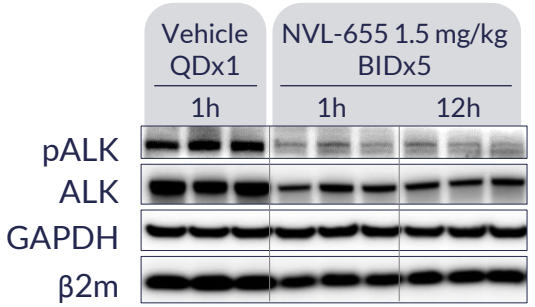


Figure 1 | Activity and pharmacodynamics analysis of NVL-655 in (**A**) a HIP1-ALK patient-derived xenograft (PDX) model and (**B**) an NCI-H3122 (EML4-ALK v1) human cancer cell line-derived xenograft (CDX) model, both subcutaneously implanted in Balb/c nude mice. Lorlatinib dose of 5 mg/kg was selected to approximate the unbound exposure of the human dose of 100 mg QD.[1,2] Vehicle is 20% HP-β-CD. Average ± SEM plotted. NVL-655 and lorlatinib were tested in the same study.

[1]Shaw *et al.*, *Lancet* 2017; 18(12):1590
[2]Yamazaki *et al.*, *J. Pharm. Exp. Ther.* 2014; 351(1):67

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NVL-655 Showed Activity in Tumor Models with ALK Mutant Kinase Domain

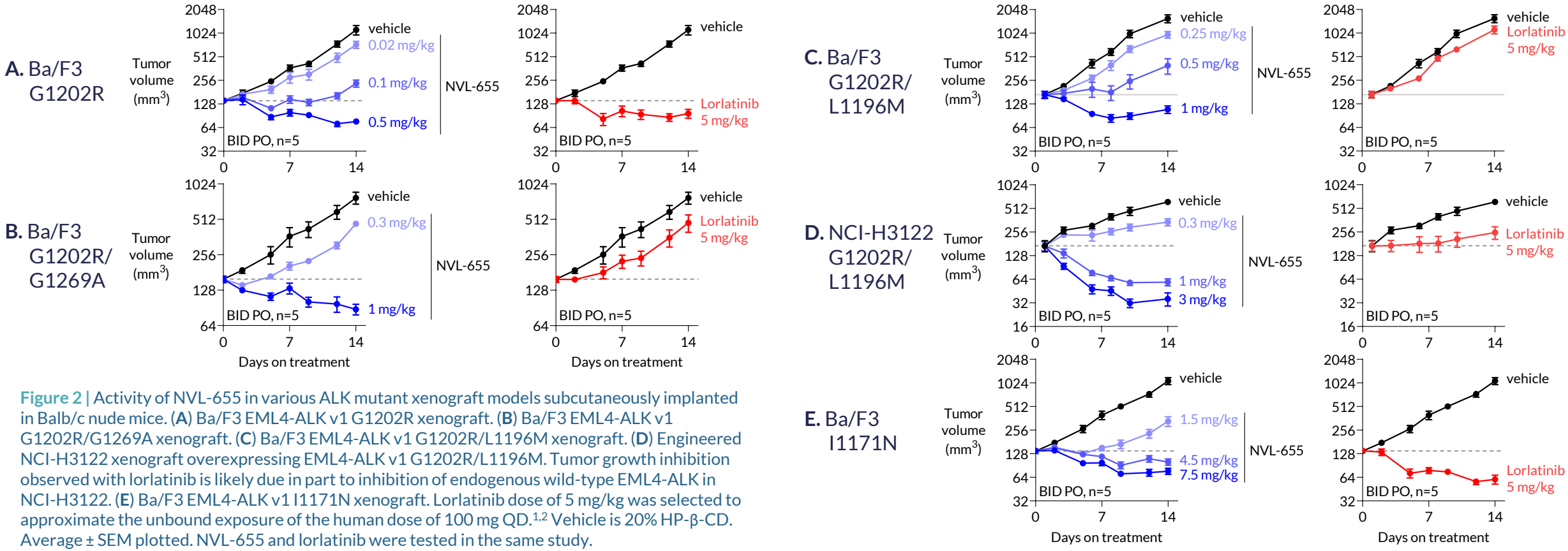

Figure 2 | Activity of NVL-655 in various ALK mutant xenograft models subcutaneously implanted in Balb/c nude mice. (**A**) Ba/F3 EML4-ALK v1 G1202R xenograft. (**B**) Ba/F3 EML4-ALK v1 G1202R/G1269A xenograft. (**C**) Ba/F3 EML4-ALK v1 G1202R/L1196M xenograft. (**D**) Engineered NCI-H3122 xenograft overexpressing EML4-ALK v1 G1202R/L1196M. Tumor growth inhibition observed with lorlatinib is likely due in part to inhibition of endogenous wild-type EML4-ALK in NCI-H3122. (**E**) Ba/F3 EML4-ALK v1 I1171N xenograft. Lorlatinib dose of 5 mg/kg was selected to approximate the unbound exposure of the human dose of 100 mg QD.[1,2] Vehicle is 20% HP-β-CD. Average ± SEM plotted. NVL-655 and lorlatinib were tested in the same study.

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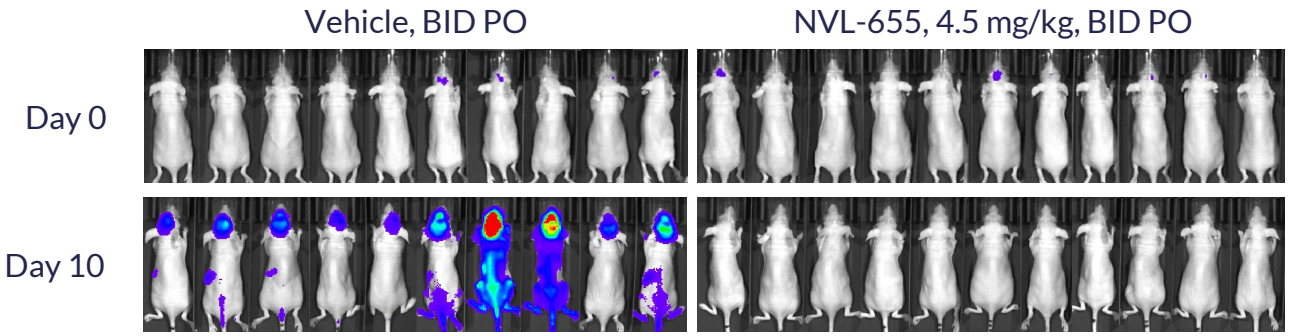
NVL-655 Showed Activity in an Intracranial Mouse Tumor Model

Ba/F3 EML4-ALK v1 G1202R/L1196M luciferase



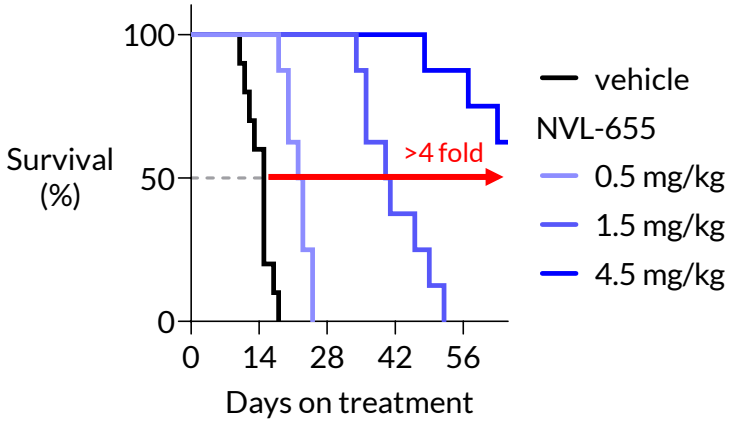


Figure 3 | Activity and survival analysis of NVL-655 in a Ba/F3 EML4-ALK v1 G1202R/L1196M luciferase model intracranially implanted in Balb/c nude mice. (Left) Bioluminescence imaging. Color indicates luminescence, with color scale from blue = 10^6 to red = 10^8 p/sec/cm^2/sr. (Right) Survival analysis. Median survival is 15 days for vehicle group and 22.5, 40.5, and > 65 days for 0.5, 1.5, and 4.5 mg/kg NVL-655 groups, respectively. Vehicle is 20% HP-β-CD.

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Conclusions

- NVL-655 is a potent and brain-penetrant ALK inhibitor as demonstrated by activity in a mouse intracranial tumor model study.
- NVL-655 was observed to be active against a wide variety of ALK mutations, particularly G1202R+ mutations, whether as a single mutation (G1202R) or as compound mutations (G1202R/L1196M and G1202R/G1269A). The activity was observed both *in vitro* and *in vivo* across various contexts:
  - Fusion partners (EML4, NPM1, or HIP1)
  - EML4 breakpoint variants (v1 or v3)
  - Tumor contexts (engineered cells, human cancer cells, or patient-derived tumors).