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Poster #P247

Evaluating TRKB Activity of Novel Preclinical Brain-Penetrant ROS1 and ALK Inhibitors

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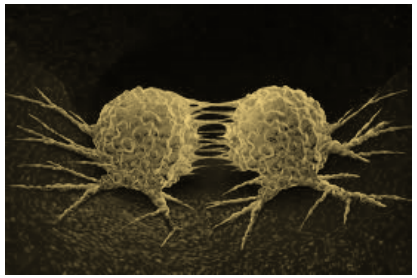
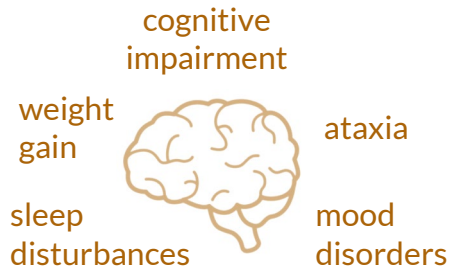
TRKB-related CNS adverse events present a key challenge for the development of next-generation ROS1 & ALK therapies



Kinase domain similarity to TRKB	
ROS1	73%
ALK	71%

Aligned by Clustal Omega on uniport.org



Up to 20% of ROS1+ and 40% of ALK+ NSCLC have brain metastasis[1,2]

↓

Newer-generation ROS1 and ALK inhibitors must be active in the CNS

TRKB is structurally similar to ROS1 and ALK

↓

Brain-penetrant ROS1 and ALK inhibitors may also inhibit TRKB in the CNS

TRKB inhibition in CNS is implicated in the adverse events of entrectinib & lorlatinib[3,4,5,6]

↓

Next-gen ROS1 and ALK inhibitors should spare TRKB

Abbreviations
CNS = central nervous system
NSCLC = non-small cell lung cancer

[1]Ou and Zhu, *Lung Cancer* 2019; 130:207
[2]Gainor *et al.*, *JCO Precis. Oncol.* 2017; 2017:PO.17.00063
[3]Cocco *et al.*, *Nat. Rev. Clin. Oncol.* 2018; 15(12):731
[4]Shaw *et al.*, *NEJM* 2020; 383:2018
[5]Entrectinib Prescribing Information (FDA)
[6]Lorlatinib Prescribing Information (FDA)

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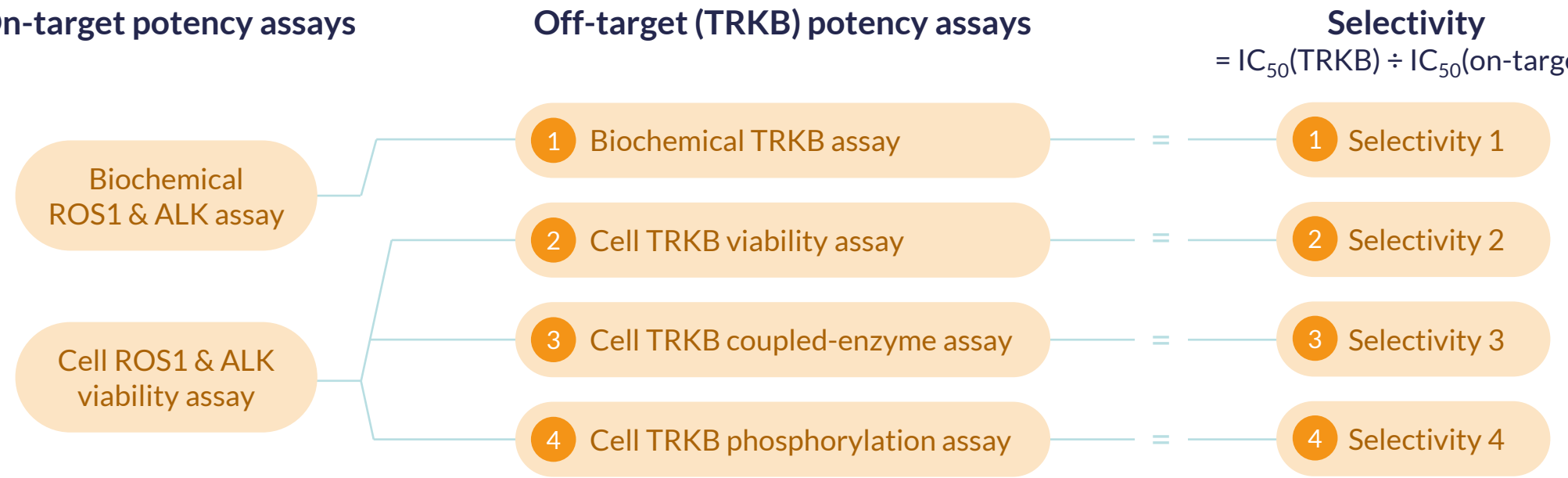
TRKB Assays Guide Selectivity Optimization for ROS1 & ALK Inhibitors

- We previously reported NVL-520 and NVL-655: preclinical inhibitors of ROS1 and ALK, respectively.
- An important part of the discovery process was generating reliable and relevant on-target (ROS1 & ALK) and off-target (TRKB) potency assays to support structure-activity relationship (SAR) studies.



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4 Assays Cover Various Biological Contexts of TRKB Signaling

1 BIOCHEMICAL TRKB ASSAY

Figure 1 | Phosphorylation of the PhosphoSens peptide® (AssayQuant Technologies) by TRKB results in fluorescence. Inhibition of TRKB kinase activity causes a dose-dependent reduction in fluorescence, which can be used to calculate potency (IC_{50}).

Shults and Imperiali., *J. Am. Chem. Soc.* 2003; 125(47): 14248-14249

2 CELL TRKB VIABILITY ASSAY

Figure 2 | In the absence of IL-3 cytokine, Ba/F3 viability is dependent on the ETV6-TRKB fusion oncogene. Inhibition of TRKB kinase activity causes a dose-dependent reduction in viability as measured by the CellTiter-Glo reagent, which can be used to calculate potency (IC_{50}).

Taylor *et al.*, *J Clin Invest.* 2018; 128(9): 3819–3825.

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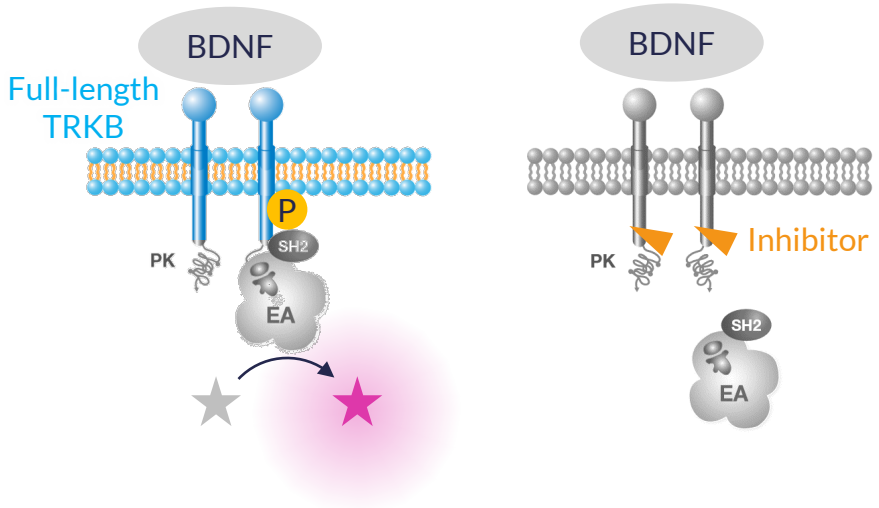
4 Assays Cover Various Biological Contexts of TRKB Signaling

3 CELL TRKB COUPLED-ENZYME ASSAY

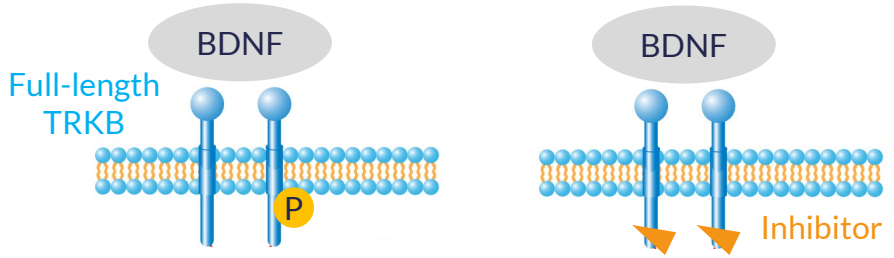


TRKB coupled with β-galactosidase produce luminescence in U2OS cells

TRKB inhibition suppresses luminescence

Figure 3 | PathHunter assay® (Eurofins) uses a split β-galactosidase (PK-EA) system in U2OS cells to measure TRKB kinase activity. BDNF (bone-derived neurotrophic factor) stimulates TRKB-PK phosphorylation, docking of SH2-EA, and PK-EA complementation to reconstitute fully active β-galactosidase, which catalyzes conversion of a fluorogenic probe. Inhibition of TRKB results in a dose-dependent loss of luminescence, which can be used to calculate potency (IC_{50}). Figure adopted from Eurofins.

4 CELL TRKB PHOSPHORYLATION ASSAY



Phospho-TRKB in Ba/F3 cells is measured using AlphaLISA kit

TRKB inhibition suppresses AlphaLISA signal

Figure 4 | Full-length TRKB is expressed in Ba/F3 cells. Stimulation with BDNF results in phosphorylation, which can be quantified using the AlphaLISA® phospho-TRK kit. Inhibition of TRKB causes dose-dependent reduction of AlphaLISA signal, which can be used to calculate potency (IC_{50}). Figure adopted from Eurofins.

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4 Assays Cover Various Biological Contexts of TRKB Signaling

	Advantages	Limitations
1 BIOCHEMICAL TRKB ASSAY	• Unaffected by cellular complexities • Kinetic readout rather than endpoint readout	• Least physiologically relevant • Kinase domain only
2 CELL TRKB VIABILITY ASSAY	• Biologically relevant cellular environment	• Oncogenic TRKB fusion, not full-length • Viability may not reflect kinase inhibition
3 CELL TRKB COUPLED-ENZYME ASSAY	• Biologically relevant cellular environment • Full-length and ligand-stimulated TRKB	• Serum condition requires adjustment • Indirect readout (β-gal, not TRKB)
4 CELL TRKB PHOSPHORYLATION ASSAY	• Biologically relevant cellular environment • Full-length and ligand-stimulated TRKB • Direct phosphorylation measurement	• Human TRKB expressed in a non-human cell

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NVL-520 Was Selective for ROS1 & ROS1 G2032R over TRKB across 4 Assays

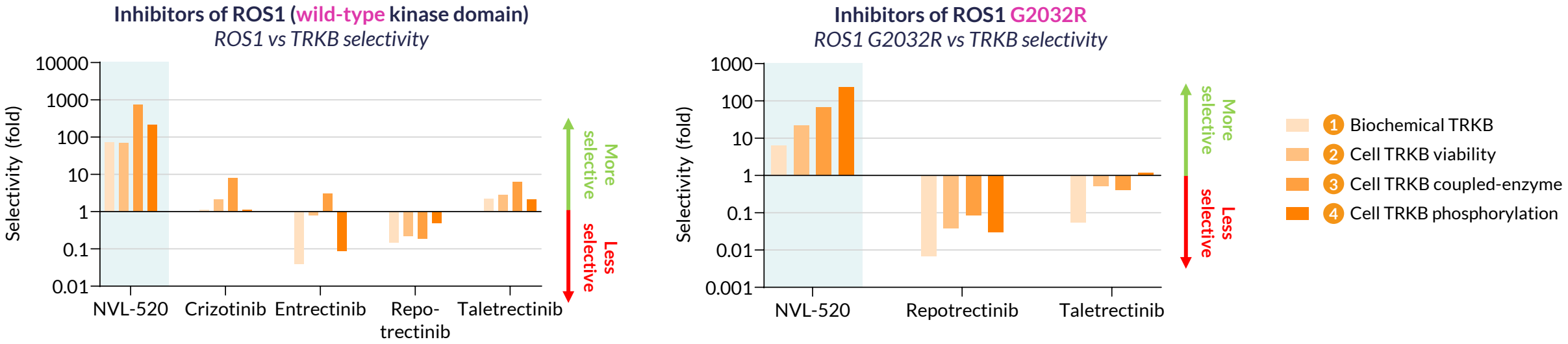

Figure 5 | Selectivity profiles of ROS1 inhibitors across 4 assays. Y-axis denotes selectivity for ROS1 (left) or ROS1 G2032R mutation (right) over TRKB, expressed as fold. Selectivity is calculated from IC_{50}(TRKB) ÷ IC_{50}(on-target). Inequality symbols (< or >) are ignored for plotting. Some data may be from n=1 testing.

Selectivity of NVL-520	Assay 1	Assay 2	Assay 3	Assay 4
versus repotrectinib	970x higher	580x higher	800x higher	>8000x higher
versus taletrectinib	120x higher	42x higher	170x higher	200x higher

Table 1 | Comparing the selectivity (ROS1 G2032R vs TRKB) of NVL-520 against repotrectinib or taletrectinib. Higher selectivity was observed in all 4 assays.

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NVL-655 Was Selective for ALK & ALK G1202R+ over TRKB across 4 Assays

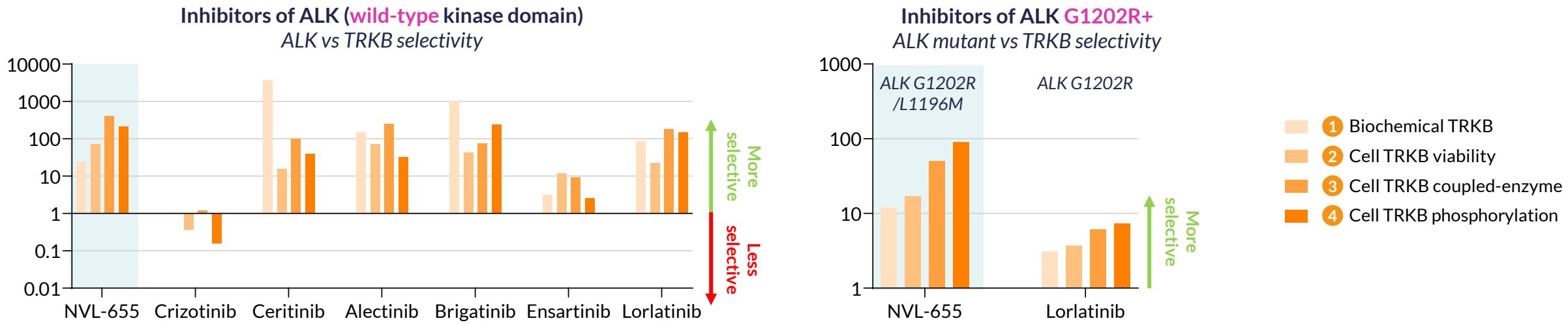

Figure 6 | Selectivity profiles of ALK inhibitors across 4 assays. Y-axis denotes selectivity for ALK (left) or ALK G1202R+ mutations (right, as indicated above the bars) over TRKB, expressed as fold. Selectivity is calculated from IC_{50}(TRKB) ÷ IC_{50}(on-target). Inequality symbols (< or >) are ignored for plotting. Some data may be from n=1 testing.

Selectivity of NVL-655	Assay 1	Assay 2	Assay 3	Assay 4
versus lorlatinib	3.9x higher	4.6x higher	8.2x higher	12x higher

Table 2 | Comparing the selectivity of NVL-655 (ALK G1202R/L1196M vs TRKB) against lorlatinib (ALK G1202R vs TRKB). Higher selectivity was observed in all 4 assays.

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Conclusions

- We have devised a multi-assay approach to evaluate TRKB inhibition and guide discovery of selective ROS1 and ALK inhibitors, with the goal of minimizing adverse events and driving durable responses for patients.
- Leveraging multiple assays increases the confidence that results are reproducible across various biological contexts.
- Results from 4 TRKB assays may differ in absolute numbers but are consistent in relative terms. Compounds that showed greater selectivity in one assay also showed greater selectivity in all other assays.
- All examined assays indicated that NVL-520 (a novel ROS1-selective inhibitor) and NVL-655 (a novel ALK-selective inhibitor) were highly selective for their wild-type and treatment-resistant oncogenic targets over off-target kinase TRKB.