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Mutagenesis Screens Support Potential Best-in-Class Profile for Neladalkib (NVL-655), a Brain-Penetrant and TRK-Sparing ALK Inhibitor

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NELADALKIB DEVELOPMENT RATIONALE

- Neladalkib (NVL-655) is an ALK tyrosine kinase inhibitor (TKI) created with the aim to overcome limitations of currently available ALK TKIs, including by maintaining activity against ALK single and compound resistance mutations, having brain penetrance, and avoiding TRK inhibition (Figure 1)[1,2].
- In *ALK*-positive advanced NSCLC, alectinib is frequently used in the first line. Lorlatinib has demonstrated activity in patients with ALK G1202R, which confers resistance to 2G ALK TKIs including alectinib.

TKI:	Crizotinib (1G)	Alectinib (2G)*	Lorlatinib (3G)	Neladalkib
Status for *ALK*+ NSCLC:	Approved	Approved	Approved	Investigational

Neladalkib Design Goals

1	Activity against ALK	ALK fusions are oncogenic drivers in various cancers, including ~5% of NSCLC[3]
2	Activity against ALK mutations	Including single mutants (e.g., G1202R, which develops in ~50% of patients after progression on 2G ALK TKIs[4–6]) and compound mutants (e.g., G1202R/L1196M, which develops after sequential 2G ALK TKI–lorlatinib treatment[4]
3	Brain penetrance	~40% of patients present with brain metastases[7]
4	Avoiding TRK-related neurotoxicities	TRK inhibition in CNS is linked to neurologic adverse events and dose-limiting toxicities[8]

▲ **Figure 1 | Neladalkib development rationale.** Also see Disclaimer. *Other 2G ALK TKIs include ceritinib, brigatinib, and ensartinib.

PLASMA EXPOSURE & IN VITRO EFFICACY

PLASMA EXPOSURE

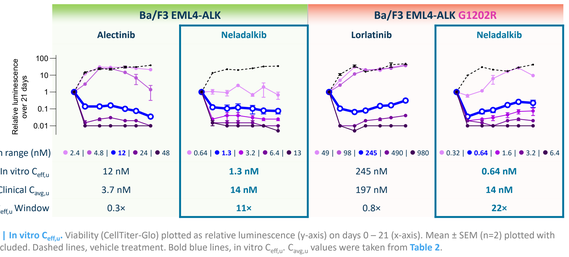
ROS1 TKI	Dosage	Clinical $C_{avg,u}$
Alectinib	600 mg BID	**3.7 nM**[9,*]
Lorlatinib	100 mg QD	**197 nM**[10]
Neladalkib	150 mg QD	**14 nM***

- Table 1 lists the average unbound plasma exposure ($C_{avg,u}$) of ALK TKIs, calculated from reported human PK data. $C_{avg,u}$ provides a balanced parameter between C_{max} and C_{min} for interpreting preclinical findings in this study.
- Concentrations in preclinical cell-based assays were corrected to unbound concentrations for comparison to clinical $C_{avg,u}$ using Fu(10% FBS) for alectinib (0.12), lorlatinib (0.98), and neladalkib (0.32).

▲ **Table 1 | Clinical $C_{avg,u}$.** $C_{avg,u}$ for alectinib and lorlatinib were calculated by dividing steady-state AUC, by measurement period τ from human PK studies, adjusting for molecular weight and Fu(human). Data were obtained from indicated references. Asterisks (*) indicate data obtained by Nuvalent, including Fu(human) for alectinib and neladalkib, and human PK for neladalkib as of the August 8, 2023 data cut[11].

EFFICACIOUS IN VITRO CONCENTRATION

- We determined the efficacious unbound concentration ($C_{eff,u}$) of each TKI that would suppress Ba/F3 growth over 21 days in vitro (Table 2). This was used to inform TKI concentrations in the subsequent ENU mutagenesis study.
- For Ba/F3 EML4-ALK
  - **Neladalkib was 9× more potent than alectinib** ($C_{eff,u}$ = 1.3 versus 12 nM).
  - Neladalkib provided average clinical exposure 11-fold above in vitro efficacy ($C_{avg,u}$ = 11 × $C_{eff,u}$), whereas alectinib provided $C_{avg,u}$ = 0.3 × $C_{eff,u}$.
- For Ba/F3 EML4-ALK G1202R
  - **Neladalkib was 383× more potent than lorlatinib** ($C_{eff,u}$ = 0.64 versus 245 nM).
  - Neladalkib provided $C_{avg,u}$ = 22 × $C_{eff,u}$, whereas lorlatinib provided $C_{avg,u}$ = 0.8 × $C_{eff,u}$.



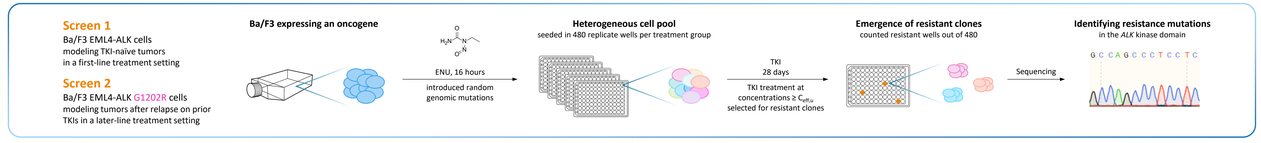
▲ **Table 2 | In vitro $C_{eff,u}$.** Viability (CellTiter-Glo) plotted as relative luminescence (y-axis) on days 0 – 21 (x-axis). Mean ± SEM (n=2) plotted with outliers excluded. Dashed lines, vehicle treatment. Bold blue lines, in vitro $C_{eff,u}$. $C_{avg,u}$ values were taken from Table 2.

The large windows between neladalkib's clinical exposure and preclinical efficacy suggest a potential for deep and sustained inhibition of ALK and ALK G1202R, including in the CNS.
By contrast, the narrower windows observed with alectinib (against ALK) and lorlatinib (against ALK G1202R) suggest greater challenges to maintaining efficacious concentrations, peripherally or in the CNS.

PRECLINICAL ENU SCREENING TO DISCOVER ON-TARGET RESISTANCE TO ALK TKIs

- *N*-ethyl-*N*-nitrosourea (ENU) screening in Ba/F3 cells is often used to predict on-target resistance to TKIs[12–14]. It has been shown to produce clinically relevant predictions[15] despite limitations such as mutational bias and being an engineered in vitro model.
- ENU induces random mutations, creating a heterogeneous cell pool that may contain resistant clones. TKI treatment selects for these clones, and resistance mutations can be identified by sequencing (Figure 2).
- TKI concentrations were designed to prevent long-term growth. Any outgrowth indicated presence of TKI-resistant subpopulations.
- **We performed 2 ENU screens to discover potential on-target resistance to ALK TKIs in a first-line setting and in a post-TKI setting.**

▼ **Figure 2 | Using ENU screens to discover ALK TKI resistance.** Ba/F3 cells were exposed to ENU, seeded in 480 replicate wells per treatment group, and treated with TKIs for 28 days. Resistant wells were counted, and select clones were subject to Sanger sequencing in the *ALK* kinase domain (encoding amino acids 1116 – 1392, Uniprot ID: Q9UM73[16]). **This work focused on on-target resistance as Ba/F3 cells (mouse blood lineage) are not optimal for studying bypass resistance in human cancers.**



SCREEN 1: RESISTANCE IN FIRST-LINE SETTING

Using Ba/F3 EML4-ALK cells, modeling TKI-naïve tumors in a first-line treatment setting
Comparator: Alectinib, frequently used in the first line for *ALK*-positive advanced NSCLC

◀ **Figure 3 | ENU screening to discover ALK TKI resistance in a first-line (EML4-ALK) setting.**
(**Top**) Resistant wells on day 28. The number of clones that emerged for each treatment group is indicated and includes all resistance (on- and off-target). $C_{avg,u}$ markers (Table 2) are positioned relative to the unbound TKI concentration in the screen (x-axis).
(**Bottom**) Some resistant clones were sequenced in the *ALK* kinase domain. The number of clones sequenced is indicated. Mutations are grouped by amino acid residue. In some cases, 2 mutations were identified in the same well. * indicates premature termination.

- **On-target resistance is predicted to be likely for alectinib as a first-line ALK TKI.**
  - Alectinib's $C_{avg,u}$ (3.7 nM) is below 12 nM used in the screen, at which 480/480 (100%) resistant wells emerged.
  - Alectinib's major metabolite M4 has similar potency and protein binding as alectinib but less than half of the exposure[10]. The combined exposure of alectinib and M4 is still expected to be below 12 nM.
  - ALK I1171N/S/T and V1180L emerged as alectinib-resistant mutations, consistent with prior reports[6].
  - ALK G1202R, expected to be resistant to alectinib, was not identified in this screen. Prior reports indicated an absence or low frequency of ALK G1202R following ENU mutagenesis [17–19], representing a potential limitation of this assay.
- **On-target resistance is predicted to be unlikely for neladalkib as a first-line ALK TKI.**
  - Neladalkib's $C_{avg,u}$ (14 nM) is above 8.2 nM used in the screen, at which only 9/480 (1.9%) resistant wells emerged.

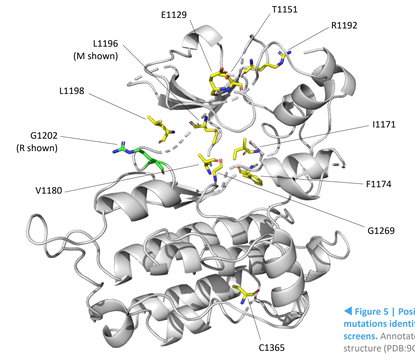
SCREEN 2: RESISTANCE IN POST-TKI SETTING

Using Ba/F3 EML4-ALK **G1202R** cells, modeling TKI-relapsed tumors in a later-line treatment setting
Comparator: Lorlatinib, designed to inhibit ALK G1202R, which confers resistance to 2G ALK TKIs

◀ **Figure 4 | ENU screening to discover ALK TKI resistance in a later-line (EML4-ALK G1202R) setting.** All new mutations identified co-existed with G1202R. See full description in Figure 2.

- **On-target compound-mutant resistance is predicted to be likely for lorlatinib as a later-line ALK TKI.**
  - Lorlatinib's $C_{avg,u}$ (197 nM) is below 245 nM used in the screen, at which 480/480 (100%) resistant wells emerged.
  - ALK G1202R/L1196M and G1202R/L1198F emerged as lorlatinib-resistant mutations, consistent with a prior report[18].
- **On-target compound-mutant resistance is predicted to be unlikely for neladalkib as a later-line ALK TKI.**
  - Neladalkib's $C_{avg,u}$ (14 nM) is above 3.2 nM used in the screen, at which only 3/480 (0.6%) resistant wells emerged.

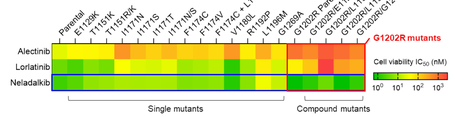
PRECLINICAL PROFILING AGAINST ALK-MUTANT CLONES



◀ **Figure 5 | Positions of residues with mutations identified in mutagenesis screens.** Annotated on ALK G1202R/L1196M structure (PDB:9GBE)[20].

We isolated 14 resistant clones with single ALK mutations and 4 with compound ALK mutations.

- **Neladalkib was the only TKI to achieve IC_{50} ≤ 10 nM potency or better against all clones**, indicating broader preclinical activity against ALK resistance mutations than alectinib or lorlatinib (Figure 6, blue box).
- Neladalkib was highly potent against the clones harboring ALK I1171X, F1174X, and V1180L (IC_{50} range = 1.1 – 10 nM), exhibiting >10× more potency than alectinib (Figure 6).
- Neladalkib was highly potent against the clones harboring ALK compound mutations G1202R/L1196M, G1202R/L1198F, and G1202R/G1269A (IC_{50} range = 0.6 – 3.5 nM), exhibiting >110× more potent activity than lorlatinib (Figure 6, red box).



◀ **Figure 6 | Potency heatmap of ALK TKIs against 18 resistant clones from ENU screen.** Parental refers to Ba/F3 EML4-ALK or EML4-ALK G1202R. Resistant clones from the ENU screen harbor indicated ALK mutations. Cell viability was measured after 3 days. Data represent an average IC_{50} (nM) from n ≥ 2 testing.

CONCLUSIONS

- On-target mutation is a common mechanism of resistance to TKIs. TKIs that maintain activity against on-target mutations have delayed or prevented development of resistance, which may contribute to treatment durability when used in earlier lines of therapy[21–23].
- Comparison of the clinical concentration of neladalkib to its efficacious in vitro concentration suggests a potential for deep and sustained inhibition of ALK and ALK G1202R fusions in humans, including in the CNS.
- Neladalkib effectively suppressed on-target resistance in ENU mutagenesis screens with both ALK and ALK G1202R fusions, predicting that on-target resistance is unlikely when used as either a first-line or a later-line therapy. By contrast, on-target resistance is predicted to be more likely for alectinib as a first-line therapy (inducing single mutants) and lorlatinib as a later-line therapy (inducing compound mutants).
- **Preclinical data have demonstrated that neladalkib has a differentiated profile that combines activity against ALK resistance mutations, brain penetrance, and TRK avoidance [1,2].** The mutagenesis screens reported here further support this potential by showing that on-target resistance is unlikely following treatment with neladalkib, which may contribute to deep and durable responses for patients.

› **Abbreviations**

AUC = area under the curve
BID = twice daily
$C_{avg,u}$ = average unbound concentration
$C_{eff,u}$ = efficacious unbound concentration
C_{max} = maximum concentration
C_{min} = concentration at dosing trough
CNS = central nervous system
ENU = *N*-ethyl-*N*-nitrosourea
FBS = fetal bovine serum
FDA = Food and Drug Administration
Fu = fraction unbound
G = generation
NGS = next-generation sequencing
NSCLC = non-small cell lung cancer
PK = pharmacokinetics
QD = once daily
SEM = standard error of the mean
τ = dosing period
TKI = tyrosine kinase inhibitor

› **Disclaimer**

As of March 2025, crizotinib, ceritinib, alectinib, brigatinib, ensartinib, and lorlatinib have been approved by the FDA for the treatment of patients with *ALK*+ metastatic NSCLC. **Neladalkib is being investigated in a Phase 1/2 trial for patients with advanced *ALK*+ NSCLC and other solid tumors (ALKOVE-1, NCT05336426) and a Phase 3 trial for patients with advanced *ALK*+ NSCLC in a head-to-head comparison against alectinib (ALKAZAR, NCT06765109).** Preclinical experiments are not powered to determine the statistical significance of differences in measurements between any of the inhibitors tested.

› **References**

1. Lin et al. Cancer Discovery 2024; 14(12):2367
2. Drilon et al. Annals of Onc 2024; 35suppl2:S802 (ESMO)
3. Ettinger et al. NCCN Guidelines for NSCLC 2024
4. Dagogo-Jack et al. Clin Can Res 2019; 25(22):6662
5. Gainor et al. Cancer Discovery 2016; 6(10):1118
6. Lin et al. Cancer Discovery 2017; 7(2):137
7. Gainor et al. JCO Precis Onc 2017; 2017:PO.17.00063
8. Cocco et al. Nat Rev Clin Onc 2018; 15(12):731
9. Alectinib prescribing information (FDA)
10. Lorlatinib prescribing information (FDA)
11. Lin et al. Mol Can Ther 2023;22(12supp):B154 (ENA)
12. Bradeen et al. Blood 2006; 108(7):2332
13. Davare et al. PNAS 2013; 110(48):19519
14. Katayama et al. Clin Can Res 2015; 21(1):166
15. Koga et al. Cancer Sci 2022; 113(3):815
16. https://www.uniprot.org
17. Zhang et al. Chem Biol Drug Des 2011; 78(6):999
18. Yoda et al. Cancer Discovery 2018; 8(6):714
19. Okada et al. EBioMedicine 2019; 41:105
20. Lin et al. Cancer Discovery 2024; 14(12):2367
21. Drilon et al. NEJM 2024; 390:118
22. Shaw et al. NEJM 2020; 383:2018
23. Soria et al. NEJM 2018; 378:113

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