NX-019, A Brain Penetrant, Mutation Selective EGFR Inhibitor With Broad Mutant EGFR Activity

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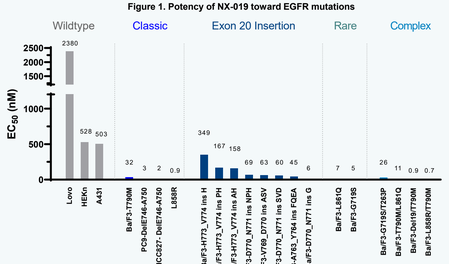
INTRODUCTION

- Currently approved targeted EGFR therapies have limited efficacy toward many of the EGFR common driver mutations, such as L7858R, exon 19 mutations, and exon 20 insertion mutations.[1]
- ~20-50% of NSCLC patients develop brain metastases (BMs) during the course of the disease[2], but BMs are still poorly addressed by current TKIs.
- NX-019 is a potent, orally bioavailable, CNS penetrant, mutant selective EGFR inhibitor targeting a broad range of EGFR mutations including common activation mutations (e.g., exon 19 deletions and L858R), T790M, exon 20 insertions and other rare mutations.
- Preclinical *in vitro* and *in vivo* studies have revealed that:
  - NX-019 displays high potency across a broad range of mutant EGFR models, indicating the potential of NX-019 to treat patients with mutations not currently addressed by, or resistant to, current EGFR TKI therapies.
  - NX-019 is highly selective for mutant EGFR over wild-type EGFR, and exhibits greater potency in cell lines with classic, exon 20 insertion, and other rare EGFR mutations compared to cells expressing wildtype EGFR. Classical EGFR dermatologic adverse events were not observed in preclinical animal models, which is in agreement with the improved selectivity profile of NX-019.
- Studies to measure the efficacy of NX-019 toward CNS lesions in xenograft brain models demonstrate tumor regressions and prolonged PD effects.
- CNS exposure studies in multiple preclinical species show a Kp,uu approaching 1, indicating excellent CNS exposure.
  - Moreover, NX-019 was readily detectable in the CSF of rat, dog and cynomolgous monkeys.

RESULTS

NX-019 Shows Selective Potency In A Broad Range of EGFR Mutations

Figure 1. Potency of NX-019 toward EGFR mutations



- Ba/F3 lines were engineered with specific EGFR mutations using standard protocols. Ba/F3 or cancer cell lines, expressing wildtype or mutant EGFR, were treated with titrating concentrations of NX-019 and cell viability was measured after 3 days.
- NX-019 demonstrated enhanced potency in cell lines expressing all classes of EGFR mutations compared to cell lines with wildtype EGFR.

ABBREVIATIONS

BID, twice daily; *BM*, brain metastases; *CNS*, central nervous system; *CSF*, cerebrospinal fluid; *ctDNA*, circulating tumor DNA; *EGFR*, epidermal growth factor receptor; *KP,uu*, concentration ratio of unbound drug in brain to plasma; *MTD*, maximum tolerated dose; *NSCLC*, non-small cell lung cancer; *p-EGFR*, phosphorylated EGFR; *PD*, pharmacodynamic; *PK*, pharmacokinetic; *ORR*, objective response rate; *PDX*, patient derived xenograft; *PO*, orally; *RP2D*, recommended phase 2 dose; *SoC*, standard of care; *TKI*, tyrosine kinase inhibitor; *WT*, wildtype.

NX-019 Shows Selective Inhibition of Mutant EGFR Over WT EGFR

Figure 2A. Western blot analysis of normal skin and Exon 20 ins (D770_N771insSVD) tumors

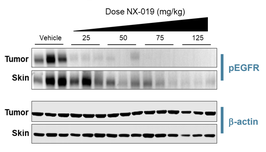
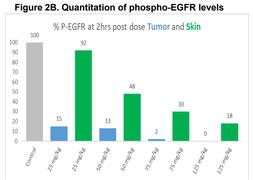
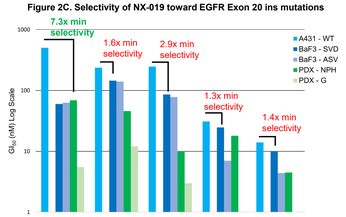


Figure 2B. Quantitation of phospho-EGFR levels



- Ba/F3 EGFR Exon 20 ins (D770_N771insSVD) expressing cells were subcutaneously implanted into BALB/c nude mice. When tumors reached an average of 310 mm^3, mice were dosed with 25-125 mg/kg NX-019 PO BID x 1.
- Tumor and skin samples were collected 2 hours post dosing and analyzed by western blot to detect levels of phosphorylated EGFR and β-actin – **Fig 2A**. Phosphorylated EGFR levels were quantitated and normalized to total EGFR and β-actin protein levels – **Fig 2B**.
- NX-019 demonstrated enhanced inhibition of, and selectivity for, mutant EGFR in tumors compared to wildtype EGFR in skin samples.

Figure 2C. Selectivity of NX-019 toward EGFR Exon 20 ins mutations



- Cell lines expressing wildtype EGFR or specific EGFR exon 20 insertion mutants were treated with titrating concentrations of NX-019 or other EGFR inhibitors and cell viability was measured – **Fig 2C**.
- NX-019 demonstrated improved EGFR exon 20 insertion mutation selectivity in comparison to other compounds tested.

NX-019 Shows Tumor Regressions in EGFR Exon 20 ins Xenografts

Figure 3A. Efficacy of NX-019 in PDX model of Exon 20 ins (LU0387 - H773_V774insNPH)

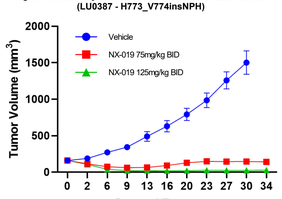
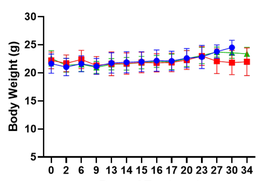


Figure 3B. Body weight of treated mice



- PDX model Exon 20 ins (LU0387 - H773_V774insNPH) cells were implanted subcutaneously in BALB/c nude mice.
- When tumors reached an average of 160 mm^3, NX-019 was dosed at 75 and 125 mg/kg in mice PO BID.
- Tumor volume was measured and graphed – **Fig 3A**.
- The body weight of the mice was measured and graphed – **Fig 3B.**
- NX-019 shows tumor regressions and cures at both tested doses with no body weight effects.

NX-019 Shows Significant CNS/Brain Exposure in Animal Models

Figure 4A. NX-019 brain and plasma exposure ratio

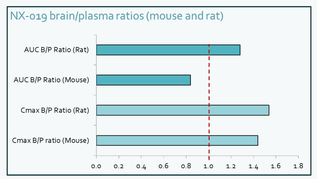
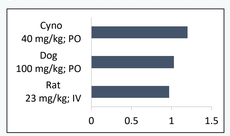


Figure 4B. NX-019 Kp,uu values in mouse and rat

Species	Route	Dose (mg/kg)	Kp,uu brain
Mouse	PO	100	0.68
Rat	IV	22.7	0.73

Kp,uu is the ratio of unbound drug in brain versus plasma

Figure 4C. Calculated Plasma Free Fraction/CSF Ratio



- PK exposure levels of NX-019 in plasma and brain were measured – **Fig 4A**.
- Kp,uu values of brain exposure levels of NX-019 were calculated for mouse and rat – **Fig 4B**.
- The free fraction of NX-019 was measured in plasma and CSF in cynomolgus monkey, dog and rat – **Fig 4C**.
- NX-019 has similar levels of exposure in the CNS and plasma compartments which indicate that NX-019 is brain penetrant with very good CNS exposure levels.

NX-019 Shows Tumor Clearance in Orthotopic CNS Models of Mutant EGFR

Figure 5A. Efficacy of NX-019 in brain orthoptic PC-9 xenograft model

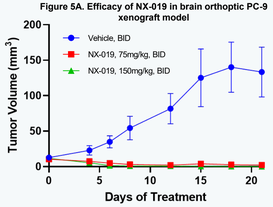


Figure 5B. Body weight of treated mice

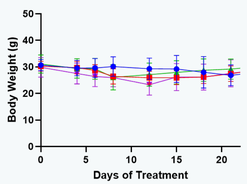


Figure 5C. Tumor images at Day 22

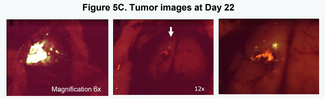
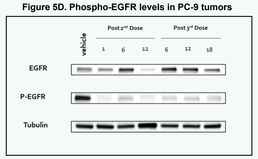


Figure 5D. Phospho-EGFR levels in PC-9 tumors



- PC-9 GFP tumor cells (EGFR ex 19 del mutant) cells were implanted orthotopically in the brain of NCr nu/nu mice.
- NX-019 was dosed at 75 and 125 mg/kg in mice PO BID.
- Tumor volume was measured and graphed – **Fig 5A.**
- The body weight of the mice were measured and graphed – **Fig 5B.**
- Fluorescence images of tumors post dose 22 were taken during autopsy procedure. Representative images are shown – **Fig 5C.**
- NX-019 shows tumor regressions and cures at both tested doses with no body weight effects.
- A companion study was completed looking at the PD effects of NX-019 in PC-9 CNS showing sustained inhibition of EGFR phosphorylation at time points post dose (175 mg/kg) – **Fig 5D**. Western blot images of tumors probed with EGFR, P-EGFR and Tubulin antibodies.

NT-019-101: First In Human Study for Mutant EGFR Cancer Is Enrolling

NCT05514496: A Study of NX-019 in Patients With Advanced, EGFR Mutant Cancer

- This is a **2-part, first-in-human**, open-label study to determine the safety and tolerability of NX-019 and preliminary efficacy in patients with locally advanced or metastatic EGFR mutant cancer.
- **Part 1**: The primary objective of Part 1 of this study is to evaluate the safety and tolerability of NX-019 and to determine the maximum tolerated dose (MTD)/Recommended Phase 2 Dose (RP2D).
- **Part 2**: The primary objective of Part 2 of this study is to confirm the safety and tolerability of NX-019 at the MTD/RP2D and, for each expansion cohort, assess the preliminary evidence of efficacy as measured by objective response rate (ORR).

Dose Escalation (design and key eligibility criteria)

- 3+3 design
- 28d cycle, 7 dose levels, QD dosing
- Enroll all EGFR mut pts who fail or are ineligible for SoC therapy
- Prior therapy with Osimertinib or Lazertinib required, if indicated
- Measurable disease
- Treated, asymptomatic CNS mets allowed
- Fresh tissue if archival not available
- Blood collection for PK and ctDNA. CSF for PK in CNS

Expansion & Efficacy Confirm. (independent cohorts)

- EGFR Exon 20 ins mutation, **with measurable CNS mets**
- EGFR mutation, non-Exon 20, **with measurable CNS mets**
- EGFR mutation (Exon 20 ins), **no prior Exon 20 ins therapy**
- EGFR rare mutations, **no current targeted therapy**

Key Objectives

- Safety/tolerability
- RP2D and MTD
- PK and biomarkers
- Early efficacy signal
- Anti-tumor activity incl. CNS
- Biomarkers
- Potential for registration based on strength of evidence

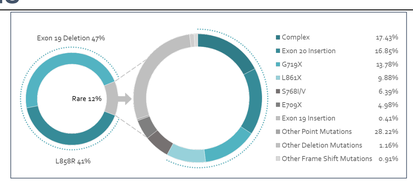
Clinical Sites

- 5 Countries and 26 sites (US, S.Korea, Taiwan, Singapore and Hong Kong)

Currently recruiting patients into Part 1

CONCLUSIONS

NX-019 has activity in these mutations[3]



- NX-019 is a potent and selective inhibitor of mutant EGFR, including classic, resistant and rare mutations such as exon 20 insertions.
- Orally administered NX-019 is highly brain penetrant, active in orthotopic models of CNS metastasis at well tolerated doses, and shows prolonged inhibition of phospho-EGFR in implanted brain tumors.
- NX-019 shows selective inhibition of mutant EGFR whilst sparing WT EGFR function, both *in vitro* and *in vivo*.
- NX-019 is currently undergoing evaluation in a Phase 1 clinical trial.

REFERENCES

1. Riess JW, et al. *J Thorac Oncol,* 2018; 13(10): 1560-1568.
2. Ernani V, et al. *J Oncol Pract, 2019;* 15(11): 563-570.
3. Figure based on: Harrison PT, et al. *Semin Cancer Biol, 2020;* 61:167–179.

Presenter: Keith Wilson (keith@nalotherapeutics.com). Conflicts: KW is an officer and employee of Nalo Therapeutics and holds equity in Nalo Therapeutics.