LNG-451 (BLU-451), a potent inhibitor of EGFR exon 20 insertion mutations with high CNS exposure

Poster Number 3261

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Background

- Epithelial growth factor receptor (*EGFR*) exon 20 insertions (ex20ins) are oncogenic driver mutations that constitutively upregulate EGFR kinase activity, are the third most common type of activating *EGFR* mutation, and are not potently targeted by many inhibitors of common activation mutations such as L858R and exon 19 mutations[1]
- *EGFR* ex20ins are in-frame insertions of 1 to 7 amino acids in the αC helix or following the αC helix[2] with the three most prevalent insertions V769_D770insASV, D770_N771insSVD, and H773_V774insNPH accounting for half of the cases[1]
- Since brain metastases are common in non-small cell lung cancer (NSCLC) with 30% of patients developing them during the course of their disease, brain penetrant EGFR-directed therapies are necessary for better treatment outcomes[3]
- While there are approved therapies such as mobocertinib and amivantamab and others in clinical development, none have demonstrated meaningful central nervous system (CNS) activity, and can be associated with treatment-limiting adverse events, including wild-type (WT) EGFR-mediated toxicities[4,5]
- BLU-451 (formerly known as LNG-451) was designed as a covalent inhibitor to potently inhibit EGFR ex20ins mutations, spare WT EGFR, and be CNS penetrant

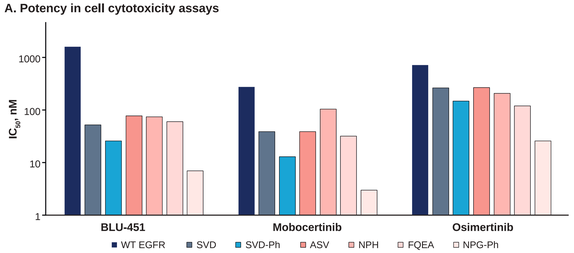
Methods

- BLU-451 activity was tested in tumor cell lines and Ba/F3 engineered cell lines expressing *EGFR* mutations as well as cell lines dependent on WT EGFR
- BLU-451 *in vitro* and *in vivo* characterization was performed in a range of pharmacokinetic (PK) studies to assess brain penetration and to measure efflux ratios in cell lines over-expressing MDR1 and BCRP
- The *in vivo* antitumor and CNS activities of BLU-451 were assessed in a PC9-luc intracranial tumor model

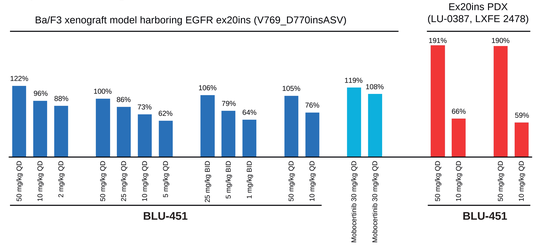
Results

Figure 1: BLU-451 inhibited EGFR ex20ins in cell proliferation models (A) and led to regression in xenograft tumor models (B)

A. Potency in cell cytotoxicity assays



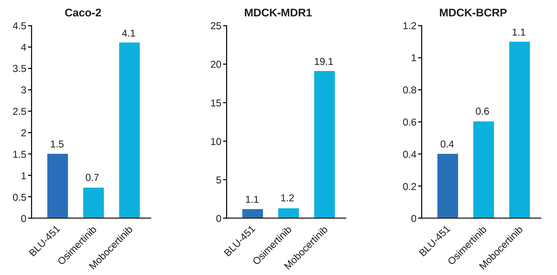
B. Dose dependent tumor growth inhibition, % relative to vehicle



BID, twice daily; IC_{50}, half-maximal inhibitory concentration; PDX, patient-derived xenograft; QD, once daily.

Figure 2: BLU-451 is not a substrate for P-glycoprotein (MDR1) or breast cancer resistance protein (BCRP) and therefore not subject to prominent efflux mechanisms in cells

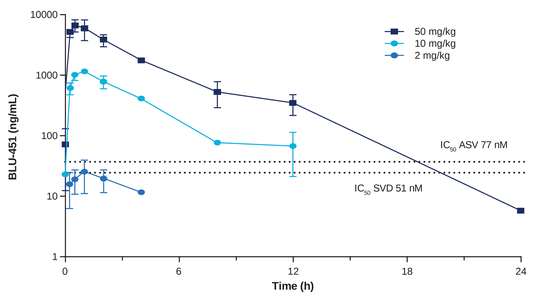
Efflux ratios (B-A / A-B) – *in vitro* assays



- **Figures 3A-B** shows BLU-451 PK in Balb/C mice models at 2 mg/kg, 10 mg/kg (AUC ~4,590 ng-h/mL), and 50 mg/kg
- BLU-451 treatment resulted in suppression of EGFR phospho-Tyr1068 (activation marker) in a Ba/F3 *EGFR* ex20ins V769_D770insASV tumor model (**Figure 3C**)
- Extended BLU-451 pharmacodynamic half-life is expected as EGFR turnover was reported to be 27 hours[6]

Figure 3: BLU-451 was orally bioavailable in mice and its covalent mechanism of action resulted in prolonged suppression of EGFR ex20ins activation in tumors

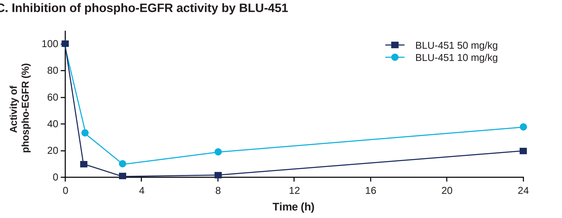
A. BLU-451 PK (oral) in mouse



B. BLU-451 PK parameters in mouse

Dose (mg/kg)	T_{max} (h)	C_{max} (ng/mL)	C_{24h} (ng/mL)	AUC_{0-24} (ng·h/mL)	$AUC_{(0-\infty)}$ (ng·h/mL)	$T_{1/2}$ (h)
2	1.0	25	BLQ	71.6	117	2.68
10	1.0	1,163	BLQ	4,289	4,590	3.06
50	0.5	6,694	5.8	24,340	24,360	2.34

C. Inhibition of phospho-EGFR activity by BLU-451



AUC_{0-24}, area under the curve for 0–24 hours; $AUC_{0-\infty}$, area under the curve extrapolated to infinity; BLQ, below limit of quantitation; C_{24h}, concentration at 24 hours; C_{max}, maximum concentration; $T_{1/2}$, half-life; T_{max}, time to maximum concentration.

- Brain-to-plasma ratios were determined in mice and rats following oral doses of BLU-451. Absolute values (**Figure 4A**) are shown for brain (ng/g) and plasma (ng/mL)
- Rat brain-to-plasma ratios were determined after BLU-451 30 mg/kg oral dose (AUC_{0-8h}) (**Figure 4B**)
- BLU-451 was evaluated in rat CNS steady state intravenous infusion models to derive the following PK parameters
  - High steady-state brain and plasma levels (874 ng/g, 431 ng/mL)
  - Brain to plasma ratio = 0.62
  - Cerebrospinal fluid (CSF) levels: 26 ng/mL (suggesting 2.96% free in brain given the lack of transporter activity[7]),
  - Unbound brain (CSF) /unbound plasma concentration ratio = 0.66 (2.77% free in rat plasma) in a rat CNS steady state intravenous infusion model

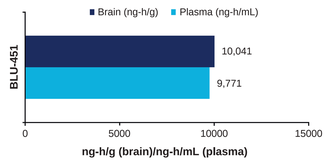
Figure 4: BLU-451 demonstrated CNS exposure

A. Brain and plasma ratios (and concentrations) of BLU-451

Study	BLU-451 50 mg/kg
Mouse (Balb/C – male) PO, @ 1 h	**0.72** 2450/3399
Mouse xenograft PO, 10 days, 1 h	**0.59** 4326/7375
Rat PO @ 1 h	**1.10** 2835/2571

PO, oral.

B. Brain and plasma $AUC_{(0-8h)}$ of BLU-451 30 mg/kg oral dose



- Anesthetized animals were incised along the skin over the midline to expose coronal and sagittal suture junctions and luciferase-expressing PC-9-luc tumor cells (2×10^5) were injected into the right lateral ventricle
  - PC9 cells carry *EGFR* exon 19 deletion mutations
  - Half-maximal inhibitory concentration (IC_{50}) for BLU-451 in PC9-luc *in vitro* cell growth inhibition model was 13 nM
- BLU-451 treatment resulted in tumor regression in a PC9-luc human lung cancer intracranial murine tumor model (**Figure 5A**)
- Whole animal luciferase bioluminescence imaging (BLI) demonstrated that BLU-451 treatment resulted in brain tumor regression and suppression of metastatic dissemination (**Figure 5B**)
- *Ex vivo* analysis showed that BLU-451 reduced luminescence in the brain and spinal cords consistent with activity in the CNS compartment (**Figure 5C**)

Figure 5: BLU-451 is a CNS penetrant, mutant EGFR inhibitor with activity demonstrated in a PC9-luc human lung cancer intracranial murine tumor model

A. BLU-451 resulted in tumor regression in PC9-luc human lung cancer intracranial murine tumor model

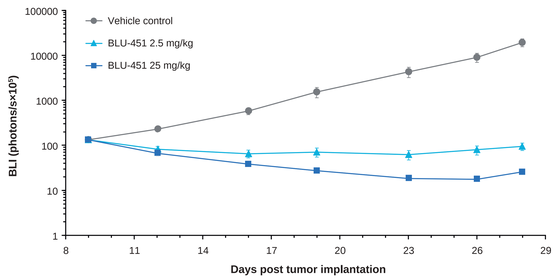
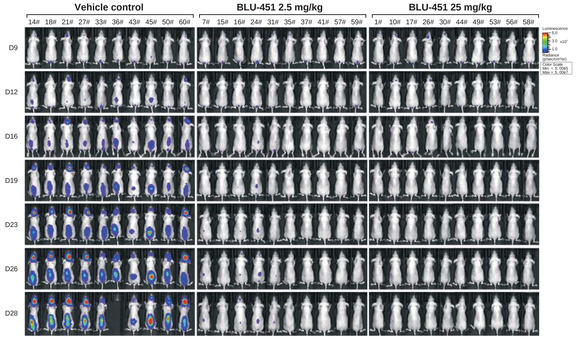
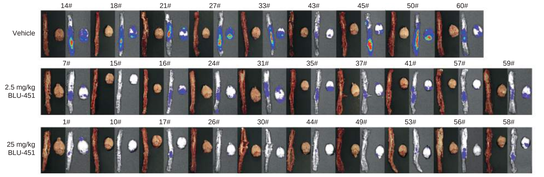


Figure 5: (continued)

B. Whole animal luciferase bioluminescence imaging



C. *Ex vivo* brain and spinal cord luminescence analysis



Conclusions

- BLU-451 is a WT EGFR sparing, selective, CNS-penetrant investigational EGFR ex20ins covalent inhibitor
  - BLU-451 was not a substrate for P-gp (MDR1) or BCRP in *in vitro* assays which is consistent with the potential for CNS activity
  - BLU-451 was orally bioavailable in mouse and rat
  - BLU-451 showed an extended pharmacodynamic half-life for inhibition of EGFR phosphorylation in tumors, as expected given its covalent mechanism of action
- In a murine intracranial tumor model, BLU-451 treatment resulted in measurable tumor regression
- These *in vitro* and *in vivo* PK and pharmacodynamic results strongly support a first-in-human phase 1/2 clinical trial of BLU-451 in patients with advanced or metastatic solid tumors harboring *EGFR* ex20ins mutations (NCT05241873)[8]

References

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Disclosures

PGP, AP, BR, TS, DJE, HJ, and BWM were employees of Lengo Therapeutics, formerly of San Diego, California, when this study was conducted. RT, HA, GH, and IA are employees of Jubilant Biosys Limited, Bengaluru, India. Data in this poster were generated by Lengo Therapeutics and its collaborators.

Poster available for download at:

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