BLU-701 is a highly potent, brain-penetrant and WT-sparing next-generation EGFR TKI for the treatment of sensitizing (ex19del, L858R) and C797S resistance mutations in metastatic NSCLC

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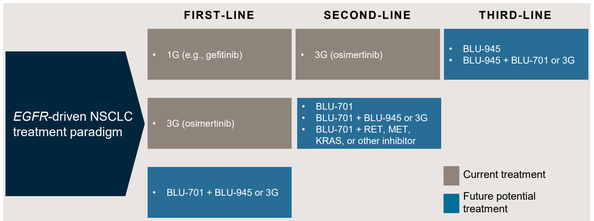
Background

- Lung cancer is the leading cause of cancer death globally.[1] The sensitizing/activating *EGFR* exon 19 deletion (ex19del) and L858R mutations are the genomic drivers in ~17% of patients with lung adenocarcinoma, the most common form of non-small cell lung cancer (NSCLC)[2]
- First- (1G) and third-generation (3G) EGFR inhibitors such as gefitinib and osimertinib, respectively, have improved treatment outcomes for patients with *EGFR*-driven NSCLC, but resistance inevitably emerges, leading to disease progression[3–5] often with central nervous system (CNS) metastases.[6,7] Toxicities driven by inhibition of wild-type (WT) EGFR are frequently reported with 1G inhibitors[3–5]
- The T790M and C797S mutations are the most common on-target resistance mechanism to 1G inhibitors and 3G inhibitors, respectively[3,5]
- There are no approved therapies for patients with disease progression following treatment with a first-line 3G inhibitor or following sequential treatment with first-line 1G and second-line 3G inhibitors[3,5]
- BLU-701 and BLU-945 are fourth-generation (4G) investigational EGFR inhibitors designed for use as monotherapy or combination therapies (together or with other agents) to potently suppress activating and on-target resistance EGFR mutants, and spare WT EGFR, with potential to treat or prevent CNS metastases (Lim SM et al. AACR 2021. Abstract 1467)[8]
- BLU-701 is a highly selective and potent investigational inhibitor of double-mutant EGFR, harboring the ex19del or L858R activating mutations and the C797S resistance mutation
- Here we provide further preclinical data to support the clinical development of BLU-701 in patients with *EGFR*-driven NSCLC

Figure 1: BLU-701 and BLU-945 are optimized for single agent and combination therapy



	1G	3G	4G		Potential combinations	
EGFR mutational coverage[a]	Gefitinib	Osimertinib	BLU-701	BLU-945	BLU-701 + osimertinib	BLU-701 + BLU-945
L858R (LR)						
ex19del						
LR or ex19del / T790M						
LR or ex19del / C797S						
LR or ex19del / T790M / C797S						

[a]Based on biochemical IC_{50}.
1G, first generation; 3G, third generation; 4G, fourth generation; IC_{50}, half-maximal inhibitory concentration.

$IC_{50} \leq 10$ nM
10 nM < $IC_{50} \leq 50$ nM
$IC_{50} > 50$ nM

Methods

- BLU-701 activity was tested in biochemical assays for EGFR mutants and WT EGFR
- Cellular activity was evaluated by a phosphorylation-specific EGFR AlphaLisa assay in WT cell lines and in cell lines expressing *EGFR* mutations
- The *in vivo* antitumor activity of BLU-701 was assessed in a PC9 ex19del cell line-derived xenograft (CDX) tumor model and in tumors grown from Ba/F3 cells expressing EGFRm/C797S

Results

Table 1: BLU-701 is a WT-sparing potent inhibitor of activating mutant EGFRm and double mutant EGFRm/C797S

	EGFR enzyme activity IC_{50} (nM)				
Compound	WT	ex19del	L858R	ex19del/C797S	L858R/C797S
BLU-701	54.4	0.5	2.6	0.5	1.5
Gefitinib	11.5	0.5	1.0	0.5	0.7
Osimertinib[a]	1.6	1.1	1.2	484.4	2354.6

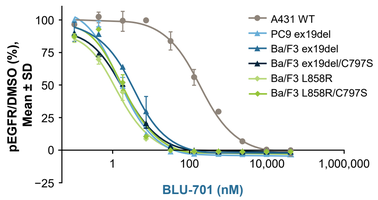
[a]Calculated after pre-incubation with the substrate to account for covalency.

Table 2: BLU-701 potently inhibits EGFR autophosphorylation in EGFRm and EGFRm/C797S-driven cell lines

	Cellular pEGFR inhibition IC_{50} (nM) per cell line and EGFR mutation					
	A431	PC9	Ba/F3			
Compound	WT	ex19del	ex19del	L858R	ex19del/C797S	L858R/C797S
BLU-701	107.3	1.3	3.3	3.3	1.8	3.3
Gefitinib	16.6	2.1	4.6	4.2	6.1	3.8
Osimertinib	113.6	1.8	5.0	10.3	>8000	>7000

pEGFR, phosphorylated EGFR.

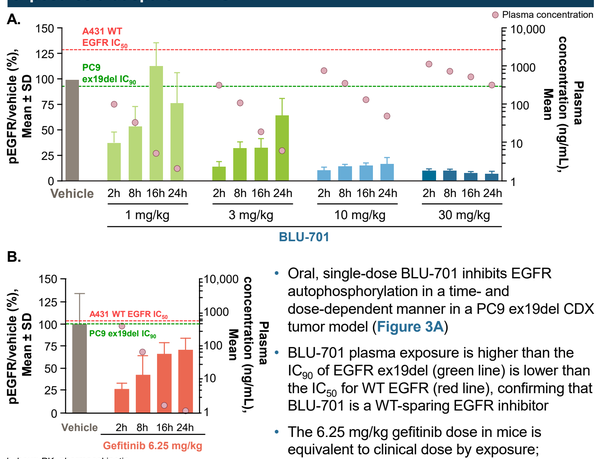
Figure 2: BLU-701 selectively inhibits EGFR autophosphorylation in mutant compared with WT cell lines



- Inhibition of EGFR autophosphorylation was measured by AlphaLISA SureFire in cellular assays after 4 hours of treatment

DMSO, dimethyl sulfoxide; SD, standard deviation.

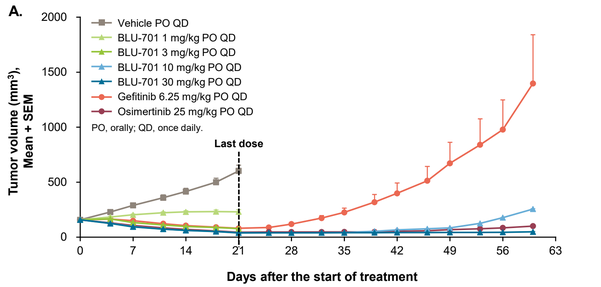
Figure 3: BLU-701 strongly inhibits EGFRm autophosphorylation *in vivo* at exposures that spare WT EGFR



- Oral, single-dose BLU-701 inhibits EGFR autophosphorylation in a time- and dose-dependent manner in a PC9 ex19del CDX tumor model (Figure 3A)
- BLU-701 plasma exposure is higher than the IC_{90} of EGFR ex19del (green line) is lower than the IC_{50} for WT EGFR (red line), confirming that BLU-701 is a WT-sparing EGFR inhibitor
- The 6.25 mg/kg gefitinib dose in mice is equivalent to clinical dose by exposure; plasma exposure was high enough to inhibit both mutant and WT EGFR (Figure 3B)

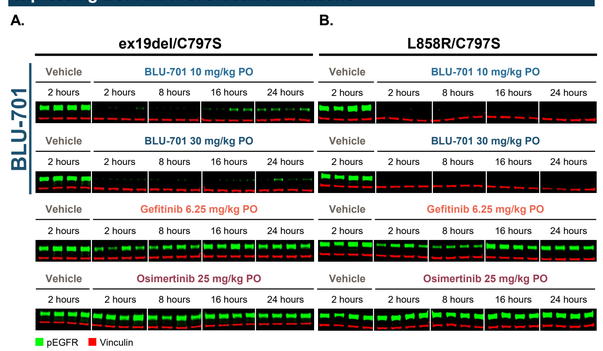
h, hour; PK, pharmacokinetic.

Figure 4: BLU-701 has durable anti-tumor activity in an EGFR ex19del-driven cancer cell line at doses that spare WT EGFR



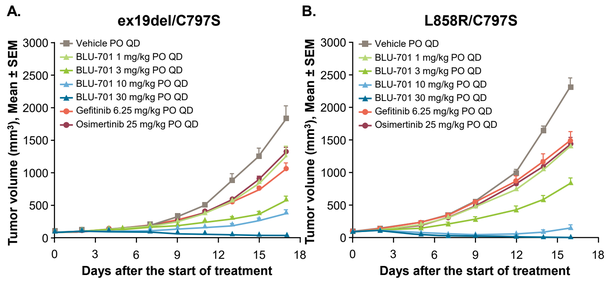
- Oral, once-daily administration of BLU-701 for 21 days resulted in tumor regression in a subcutaneous PC9 ex19del CDX tumor model (Figure 4A) at doses that spared WT EGFR (Figure 3A)
- BLU-701 showed durable tumor responses with 10 mg/kg and 30 mg/kg after the last dose (Day 21)
- The mouse equivalent of the clinical dose of gefitinib, which inhibits mutant and WT EGFR, showed faster regrowth, consistent with limited pharmacodynamic (PD) modulation (after single dose: Figure 3B; after last dose: data not shown)
- PD analysis after the last dose showed sustained and robust inhibition of phospho- and total EGFR (Figure 4B)

Figure 5: BLU-701 inhibits EGFR autophosphorylation in Ba/F3 CDX models expressing EGFRm/C797S double mutations



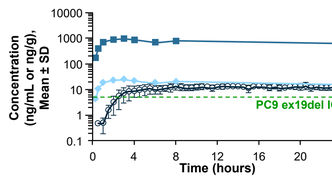
- Oral, single-dose of BLU-701 resulted in sustained inhibition of EGFR autophosphorylation in subcutaneous monoclonal Ba/F3 tumors expressing ex19del/C797S (Figure 5A) and L858R/C797S mutant EGFR (Figure 5B)
- Total EGFR was unchanged after a single oral dose of BLU-701 (data not shown)
- The mouse equivalent clinical doses of osimertinib (25 mg/kg) and gefitinib (6.25 mg/kg) were less potent at inhibiting EGFR autophosphorylation compared with BLU-701 in these tumor models

Figure 6: BLU-701 has dose-dependent anti-tumor activity on Ba/F3 CDX models expressing EGFRm/C797S double mutants



- Oral, once-daily administration of BLU-701 showed tumor inhibition in subcutaneous monoclonal Ba/F3 tumors expressing ex19del/C797S (Figure 6A) and L858R/C797S mutant EGFR (Figure 6B)

Figure 7: BLU-701 plasma concentrations are comparable to brain concentrations, suggesting significant brain penetration



Compound	Microdialysis $Kp_{u,u}$ (AUC)[a]	IV infusion $Kp_{u,u}$ (C_{ss})[a]
BLU-701	0.56	0.98
Gefitinib	–	0.11
Osimertinib	–	0.30

AUC, area under the curve; C_{ss}, steady-state concentration; IV, intravenous.

- Oral, single-dose administration of BLU-701 30 mg/kg to male Sprague-Dawley rats achieved concentrations above pEGFR IC_{90} for over 20 hours in plasma and brain dialysate (Figure 7)
- The calculated $Kp_{u,u}$ in rat was 0.56 ± 0.13 from microdialysis-free AUC and 0.98 from IV-infusion free plasma and brain concentrations measured at steady state (8 hours)

[a]Unbound plasma concentrations were estimated by multiplying total plasma concentrations with unbound fraction; the extent of brain penetration was determined based on unbound brain-to-plasma AUC ratio ($Kp_{u,u}$ brain).

Conclusions

- BLU-701 is a potential best-in-class, selective, potent, fourth-generation EGFR TKI with activity against EGFR ex19del/L858R activating mutations and the EGFRm/C797S double mutant
- BLU-701 shows strong inhibition of EGFR autophosphorylation and inhibition of tumor growth at doses that spare WT EGFR
- BLU-701 indicated CNS penetration with potential to treat and prevent CNS metastases in patients with *EGFR*-driven tumors
- As BLU-701 has activity against the activating EGFR mutants, these pre-clinical data support the clinical development of BLU-701 in *EGFR*-driven NSCLC

References

1. GLOBOCAN World Fact Sheet. November 2020. Available from: https://gco.iarc.fr/today/data/factsheets/populations/900-world-fact-sheets.pdf. Accessed February 26, 2021; 2. Hsu W-H et al. *Ann Oncol.* 2018;29(suppl.1):i3–i9; 3. Leonetti A et al. *Br J Cancer.* 2019;121(9):725–737; 4. Piper-Vallillo AJ, Sequist LV, Piotrowska Z. *J Clin Oncol.* 2020;38(25):2926–2936; 5. Park S et al. *Cancer Res Treat.* 2020;52:1288–1290; 6. Reungwetwattana T et al. *J Clin Oncol.* 2018;36:3290–3297; 7. Aiko N et al. *BMC Cancer.* 2018;18:1012; 8. Lim SM et al. AACR 2021. Abstract 1467.

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