

# 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

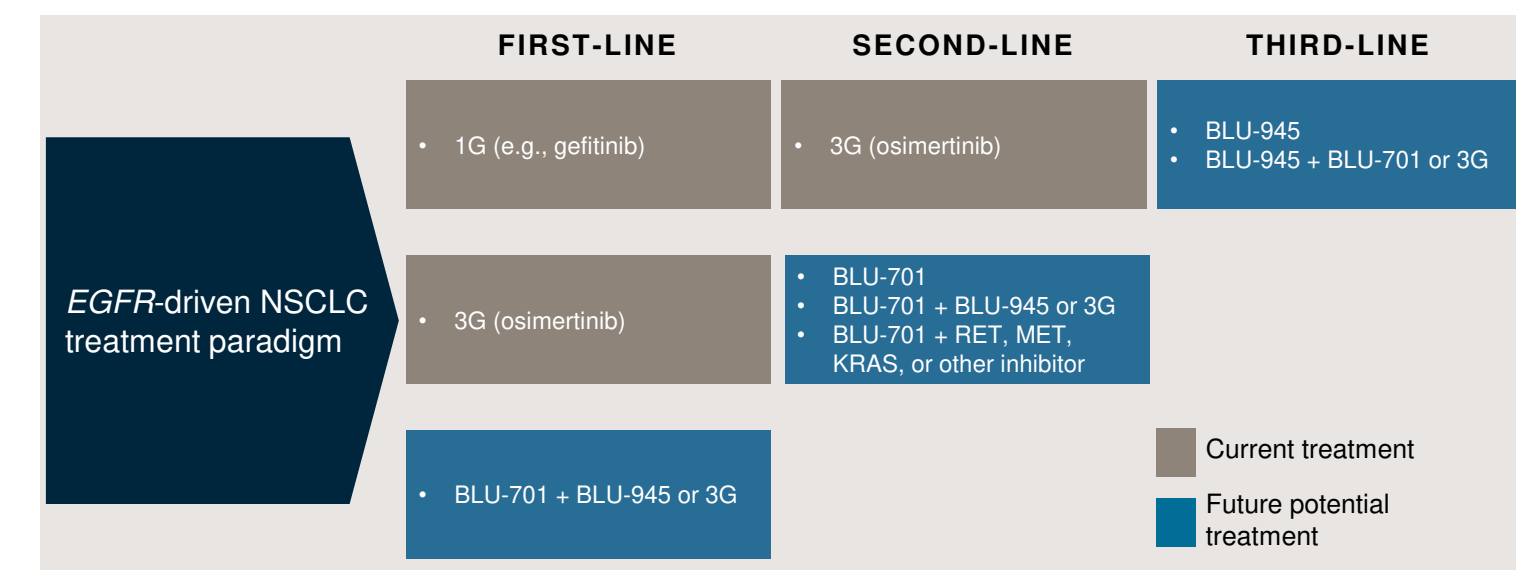
Chiara Conti<sup>1</sup>, John Campbell<sup>1</sup>, Rich Woessner<sup>1</sup>, Jian Guo<sup>1</sup>, Yoav Timsit<sup>1</sup>, Maria Iliou<sup>1</sup>, Scott Wardwell<sup>1</sup>, Alison Davis<sup>1</sup>, Sharon Chicklas<sup>1</sup>, John Hsieh<sup>1</sup>, Meredith Eno<sup>1</sup>, Omar Ahmad<sup>1</sup>, Dilinie Fernando<sup>1</sup>, Kevin Barvian<sup>1</sup>, Joseph Kim<sup>1</sup>, Steven Kazmirski<sup>1</sup>, Emanuele Perola<sup>1</sup>, Tom Dineen<sup>1</sup>, Victoria Brown<sup>1</sup>, Timothy Guzi<sup>1</sup>, Ayşegül Özen<sup>1</sup>, Faith Stevison<sup>1</sup>, Caitlin Utt<sup>1</sup>, Clare Medendorp<sup>1</sup>, Robert Meissner<sup>1</sup>, Marion Dorsch<sup>1</sup>, Klaus Hoeflich<sup>1</sup>

<sup>1</sup>Blueprint Medicines Corporation, Cambridge, Massachusetts, USA

## Background

- Lung cancer is the leading cause of cancer death globally.<sup>1</sup> 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)<sup>2</sup>
- 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<sup>3-5</sup> often with central nervous system (CNS) metastases.<sup>6,7</sup> Toxicities driven by inhibition of wild-type (WT) EGFR are frequently reported with 1G inhibitors<sup>3-5</sup>
- The T790M and C797S mutations are the most common on-target resistance mechanism to 1G inhibitors and 3G inhibitors, respectively<sup>3,5</sup>
- 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<sup>3,5</sup>
- 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 potentially 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)<sup>8</sup>
- 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**



EGFR mutational coverage <sup>a</sup>	1G		3G		4G	
	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						

<sup>a</sup>Based on biochemical IC<sub>50</sub>. 1G, first generation; 3G, third generation; 4G, fourth generation; IC<sub>50</sub>, half-maximal inhibitory concentration.

Potential combinations	IC <sub>50</sub> ≤ 10 nM	10 nM < IC <sub>50</sub> ≤ 50 nM	IC <sub>50</sub> > 50 nM
BLU-701 + osimertinib			
BLU-701 + BLU-945			

## 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**

Compound	EGFR enzyme activity IC <sub>50</sub> (nM)				
	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 <sup>a</sup>	1.6	1.1	1.2	484.4	2354.6

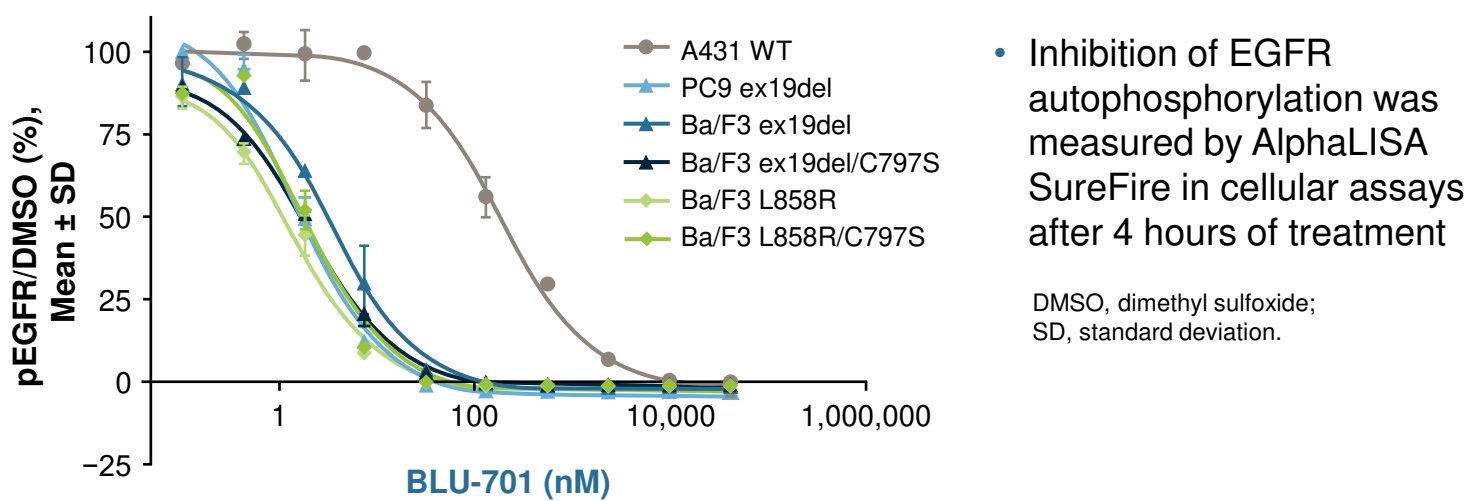
<sup>a</sup>Calculated after pre-incubation with the substrate to account for covalency.

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

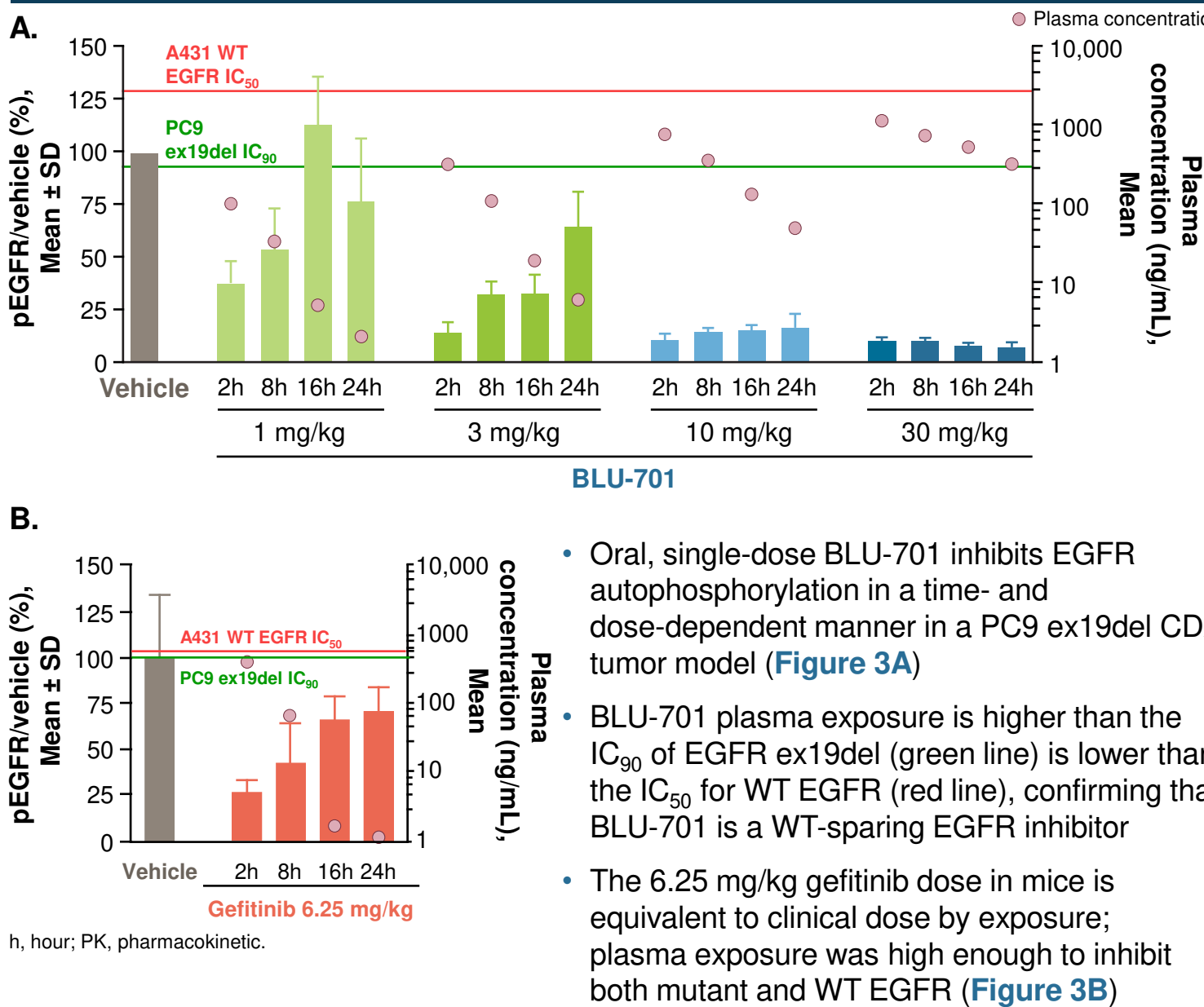
Compound	Cellular pEGFR inhibition IC <sub>50</sub> (nM) per cell line and EGFR mutation					
	A431		PC9		Ba/F3	
	WT	ex19del	ex19del	L858R	ex19del/C797S	L858R/C797S
BLU-701	107.3	1.3	3.3	3.3	1.8	3.3
Gefitinib	16.6	2.1	4.6	4.2	6.1	3.8
Osimertinib	113.6	1.8	5.0	10.3	>8000	>7000

pEGFR, phosphorylated EGFR.

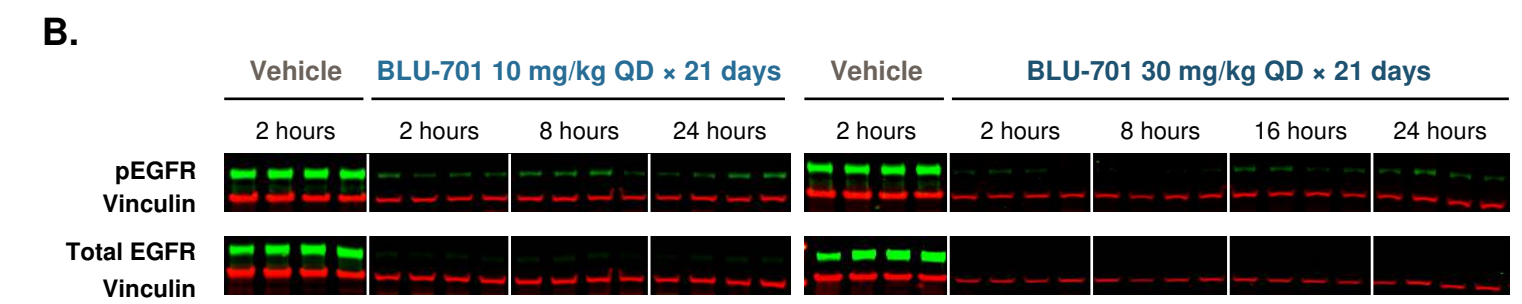
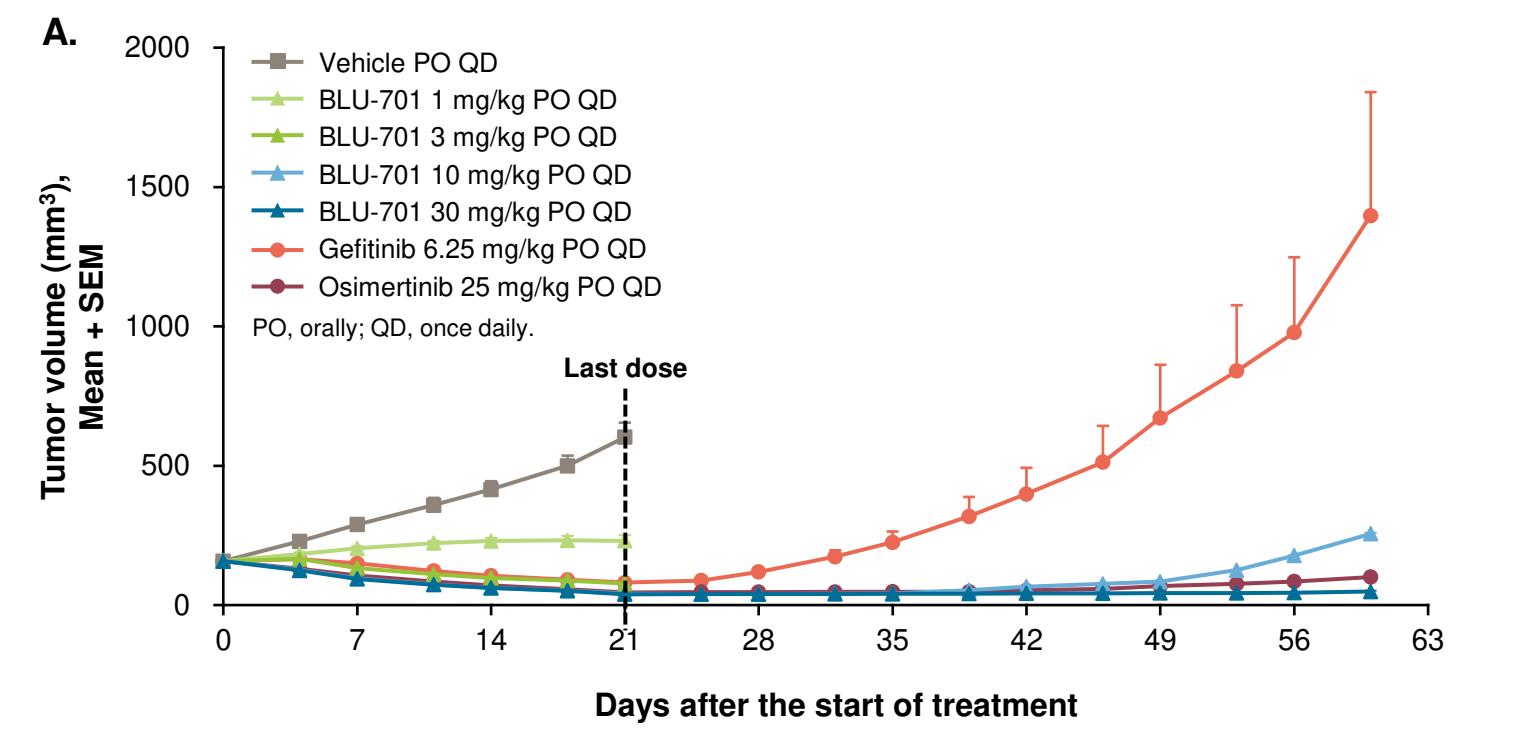
**Figure 2: BLU-701 selectively inhibits EGFR autophosphorylation in mutant compared with WT cell lines**



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

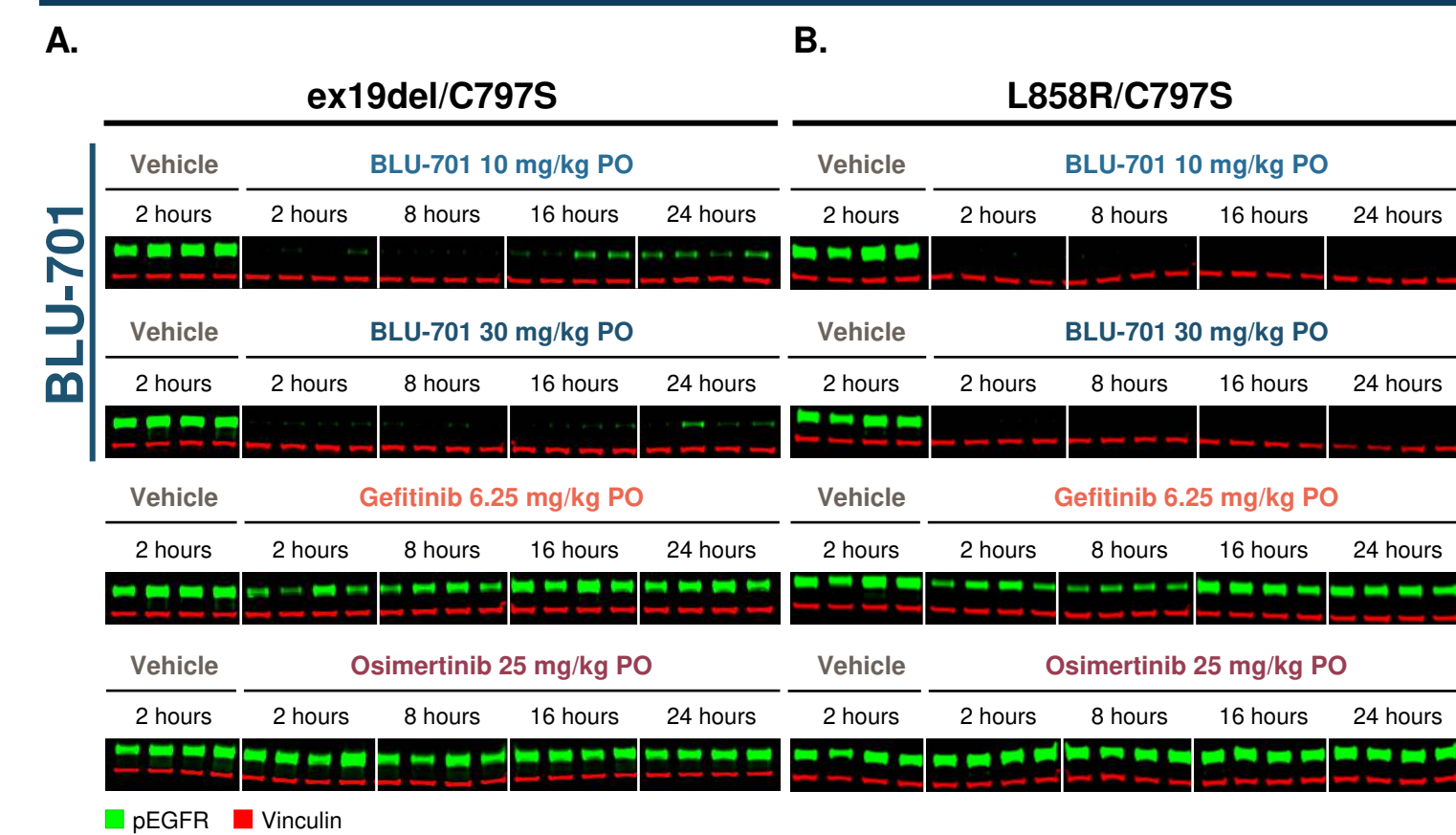


**Figure 4: BLU-701 has durable anti-tumor activity in an EGFR ex19del-driven cancer cell line at doses that spare WT EGFR**



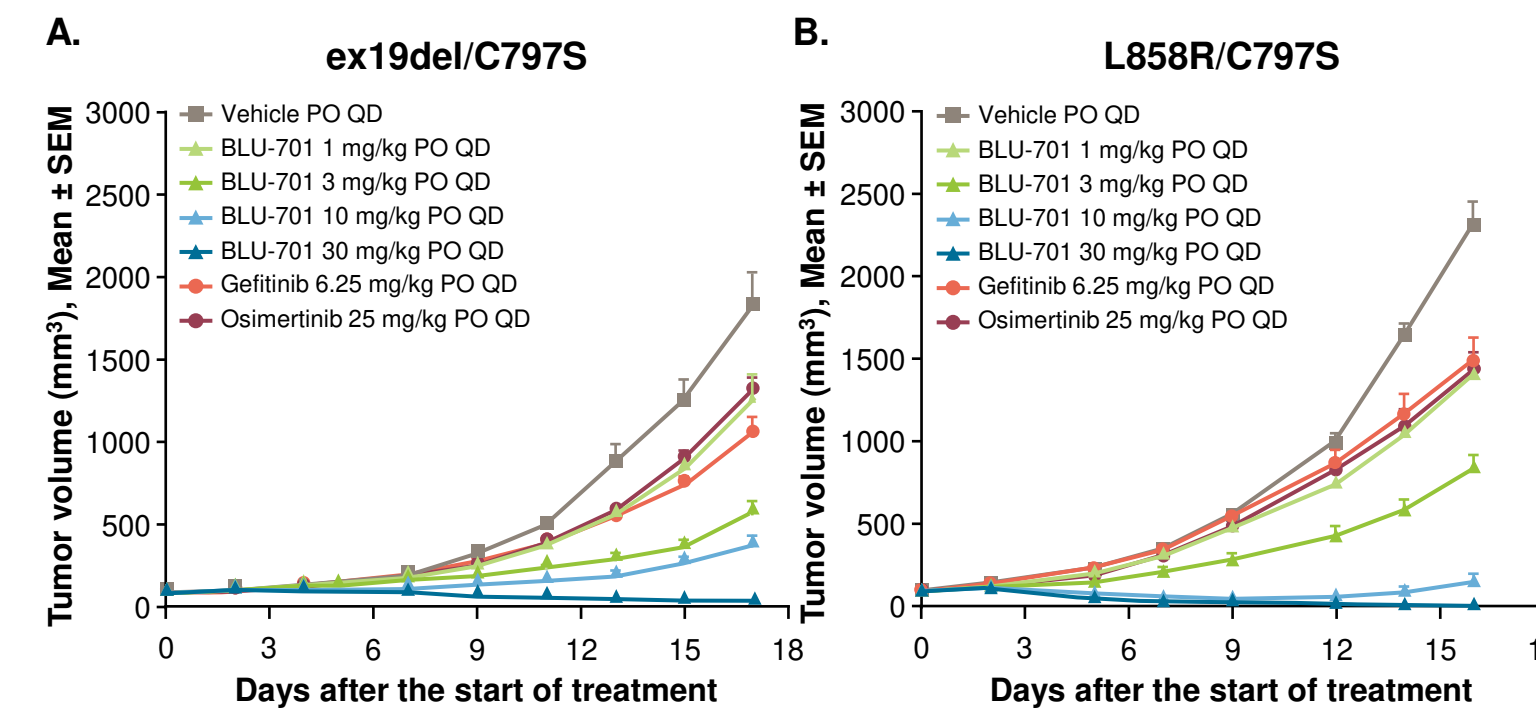
- Oral, once-daily administration of BLU-701 for 21 days resulted in tumor regression in a subcutaneous PC9 ex19del CDX tumor model (Figure 4A) at doses that spared WT EGFR (Figure 3A)
- BLU-701 showed durable tumor responses with 10 mg/kg and 30 mg/kg after the last dose (Day 21)
- The mouse equivalent 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**



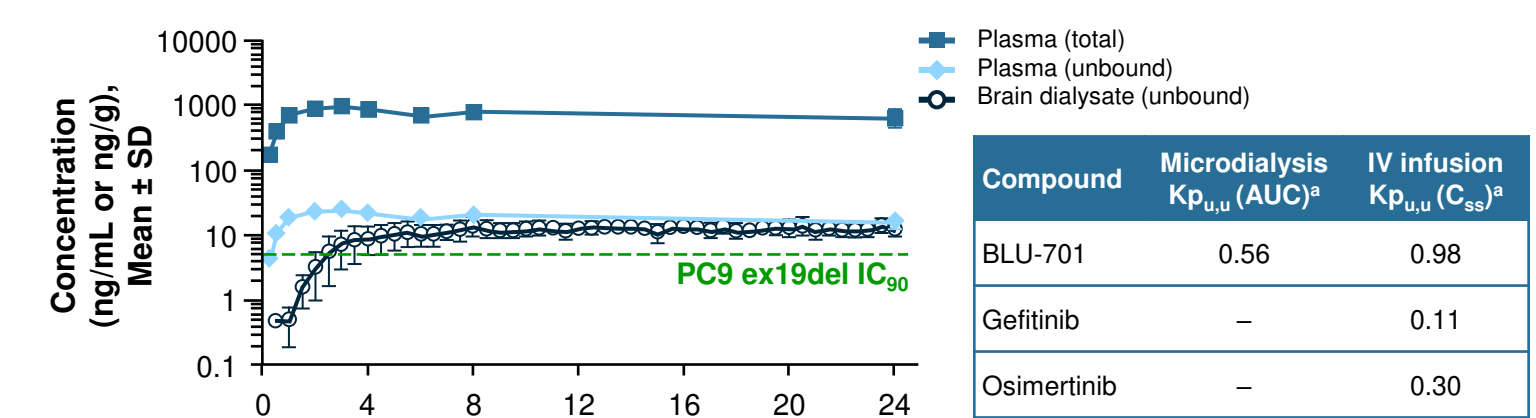
- Oral, single-dose of BLU-701 resulted in sustained inhibition of EGFR autophosphorylation in subcutaneous monoclonal Ba/F3 tumors expressing ex19del/C797S (Figure 5A) and L858R/C797S mutant EGFR (Figure 5B)
- Total EGFR was unchanged after a single oral dose of BLU-701 (data not shown)
- The mouse 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**



- Oral, single-dose administration of BLU-701 30 mg/kg to male Sprague-Dawley rats achieved concentrations above pEGFR IC<sub>90</sub> for over 20 hours in plasma and brain dialysate (Figure 7)
- The calculated K<sub>p,uu</sub> 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)

## 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

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**Acknowledgments**  
Julia Zhu (Blueprint Medicines Corporation) oversaw collaborations with external CROs. Chandra Mudituru (EQRx, Cambridge, MA) and Ebby Job (Blueprint Medicines Corporation, Cambridge, MA) contributed to the BLU-701 program. Pharmaron, Inc. *in vitro* biology team (Beijing, China) and WuXi AppTec oncology and immunology department (Shanghai, China) contributed to the *in vivo* and *in vitro* pharmacology data collection. Medical writing support was provided by Natasha Tracey, PhD, and editorial support was provided by Travis Taylor, BA, all of Paragon, Knutsford, UK, supported by Blueprint Medicines Corporation, Cambridge, MA, according to Good Publication Practice guidelines.

**Disclosures**  
This research was funded by Blueprint Medicines Corporation. Blueprint Medicines Corporation reviewed and provided feedback on the poster. The authors had full editorial control of the poster and provided their final approval of all content. CC, JC, RW, JG, YT, MI, SW, SC, JH, ME, OA, DF, KB, JK, SK, EP, TD, VB, AD, FS, CJ, CM, RM, and MD are employees of and hold equity interest in Blueprint Medicines Corporation. AD, TG, and KH were employees of Blueprint Medicines Corporation at the time of the work.