BLU-945, a fourth-generation, potent and highly selective epidermal growth factor receptor tyrosine kinase inhibitor with intracranial activity, demonstrates robust *in vivo* anti-tumor activity in models of osimertinib-resistant non-small cell lung cancer

1467

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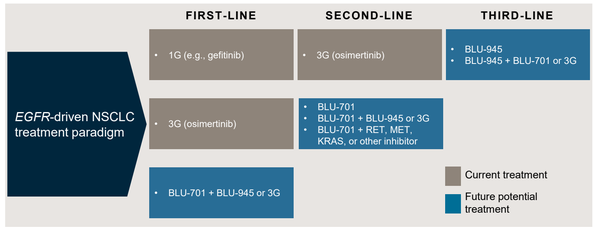
Background

- 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-945 and BLU-701 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 (Conti C et al. AACR 2021. Abstract 1262)[8]
- Previously, we have shown BLU-945 is a selective and potent investigational inhibitor of double-mutant or triple-mutant EGFR (T790M or ex19del/T790M/C797S) and demonstrated robust anti-tumor activity in preclinical models[9]
- Here we provide further preclinical data to support the clinical development of BLU-945 in patients with *EGFR*-driven NSCLC

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



	1G	3G	4G		Potential combinations	
EGFR mutational coverage[a]	Gefitinib	Osimertinib	BLU-701	BLU-945	BLU-945 + osimertinib	BLU-945 + BLU-701
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

- Cellular activity was evaluated by a phosphorylation-specific EGFR AlphaLisa assay in WT cell lines and in cell lines expressing *EGFR* mutants
- The *in vivo* anti-tumor activity and pathway inhibition of BLU-945 was assessed in an engineered triple mutant, osimertinib-resistant cell line-derived xenograft (CDX) and an osimertinib-resistant patient-derived cell xenograft (PDCX) model
- *In vivo* CNS activity was evaluated in an intracranial implantation model of luciferase-expressing YU-1097 patient-derived-cells harboring *EGFR* ex19del/T790M/C797S mutations; tumor burden of intracranial lesions was measured by bioluminescence imaging

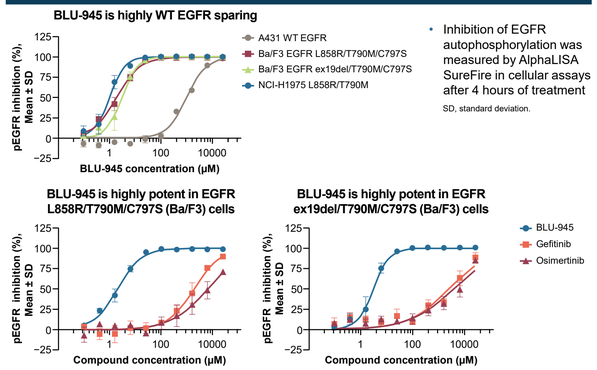
Results

Table 1: BLU-945 is a nanomolar EGFRm/T790M/C797S and EGFRm/T790M inhibitor with >450-fold selectivity over WT EGFR in cellular assays

	Cellular pEGFR inhibition IC_{60} (nM)					
	Cell lines			Engineered Ba/F3 cell lines		
Compound	NCI-H1975 (L858R/T790M)	PC9 (ex19del)	A431 (EGFR WT)	L858R	L858R/ T790M/C797S	ex19del/ T790M/C797S
BLU-945	1.2	129.5	544.4	21.5	2.9	4.4
Erlotinib	>10,000	3.9	140.6	5.9	6655.5	4524.8
Gefitinib	4679.8	1.8	16.5	4.6	6707.7	4864.7
Osimertinib	4.7	2.1	115.9	11.0	7754.6	>10,000

pEGFR, phosphorylated EGFR.

Figure 2: BLU-945 is a highly WT-sparing EGFR T790M/C797S, EGFR ex19del/T790M/C797S and EGFR L858R/T790M mutant inhibitor

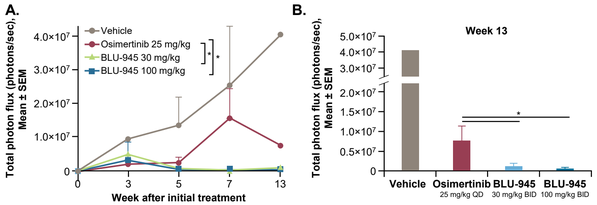


- Inhibition of EGFR autophosphorylation was measured by AlphaLISA SureFire in cellular assays after 4 hours of treatment

SD, standard deviation.

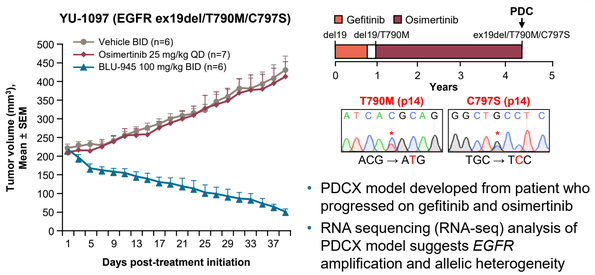
- BLU-945, but not osimertinib or gefitinib inhibit EGFR phosphorylation in the EGFR L858R/T790M/C797S, and EGFR ex19del/T790M/C797S mutant cell lines

Figure 3: BLU-945 intracranial activity in NSCLC PDC-luc (ex19del/T790M/C797S) model, per total photon flux measurements (A) over treatment and (B) at Week 13



n=4–5/group; calculated by SigmaPlot *t*-test, **P*<0.05. BID, twice daily; PDC, patient-derived cell; QD, once daily; SEM, standard error of the mean.

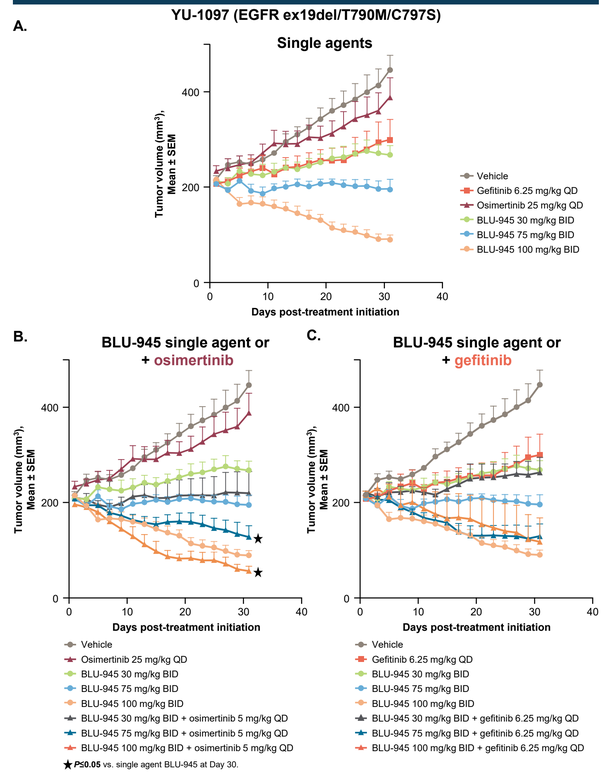
Figure 4: Oral administration of BLU-945 showed significant tumor regression in an osimertinib-resistant EGFR ex19del/T790M/C797S PDCX



- PDCX model developed from patient who progressed on gefitinib and osimertinib
- RNA sequencing (RNA-seq) analysis of PDCX model suggests *EGFR* amplification and allelic heterogeneity

Figure previously presented in reference 9.

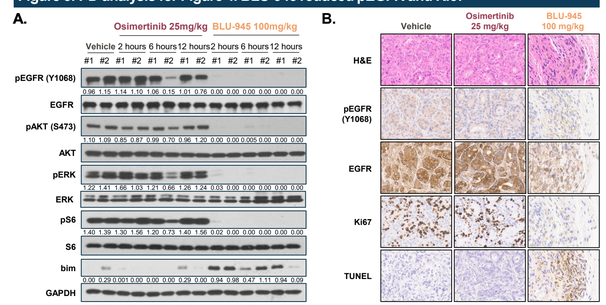
Figure 5: BLU-945 showed significant tumor regression (A) alone or in combination with (B) osimertinib or (C) gefitinib, in an osimertinib-resistant EGFR ex19del/T790M/C797S PDCX



★ *P*≤0.05 vs. single agent BLU-945 at Day 30.

- BLU-945 single agent was sufficient for tumor regression in the YU-1097 model
- Co-dosing BLU-945 with either osimertinib or gefitinib enhanced tumor regression
- Data suggest that BLU-945 can lead to tumor regression as a single agent and that combination with other EGFR TKIs can enhance anti-tumor activity

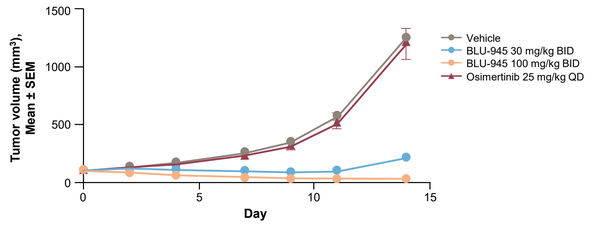
Figure 6: PD analysis for Figure 4. BLU-945 reduced pEGFR and Ki67



H&E, hematoxylin & eosin; PD, pharmacodynamic; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

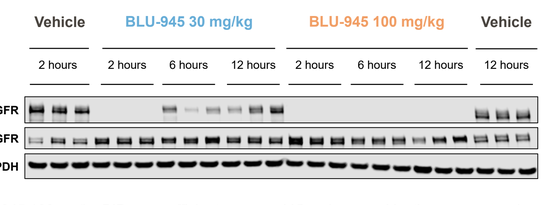
- When treated with BLU-945, tumors demonstrated a marked reduction in pEGFR and Ki67 with a concurrent increase in TUNEL staining (Figure 6B)

Figure 7: Oral administration of BLU-945 showed significant tumor regression in an osimertinib-resistant Ba/F3 CDX (L858R/T790M/C797S) tumor model



- At 30 mg/kg BID and 100 mg/kg BID, BLU-945 led to significant tumor regression
- Osimertinib showed no significant reduction of tumor growth, confirming resistance

Figure 8: PD analysis of BLU-945 single dose in L858R/T790M/C797S Ba/F3 CDX (L858R/T790M/C797S) tumor model



- BLU-945 100 mg/kg BID was sufficient to exceed IC_{90} pharmacokinetic coverage and complete pathway inhibition

IC_{90}, 90%-maximal inhibitory concentration.

Conclusions

- BLU-945 is a potential best-in-class, selective, potent, fourth-generation EGFR TKI with activity against the EGFRm/T790M double and EGFRm/T790M/C797S triple mutants
- BLU-945 demonstrated potent, robust EGFR pathway inhibition and anti-tumor activity in triple-mutant osimertinib-resistant Ba/F3 CDX and PDCX models
- In the same triple-mutant PDCX model, combination of BLU-945 with either gefitinib or osimertinib showed enhanced anti-tumor activity when compared with single-agent treatment
- Clinical development of BLU-945 monotherapy is expected to begin with an international phase 1 dose-escalation trial in patients with *EGFR*-driven NSCLC in the first half of 2021, and future clinical development of BLU-945 in combination with other agents across multiple treatment settings is planned

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