# Potent *in vitro* Activity of Tarloxotinib for EGFR and HER2 Mutations Refractory to Current EGFR Tyrosine Kinase Inhibitors

2200

Kenichi Suda[1], Masaya Nishino[1], Takamasa Koga[1], Toshio Fujino[1], Yoshihisa Kobayashi[1], Avanish Vellanki[2], Vijaya G. Tirunagaru[2], Robert C. Doebele[3] and Tetsuya Mitsudomi[1]

[1]Division of Thoracic Surgery, Department of Surgery, Kindai University Faculty of Medicine, Osaka-Sayama JAPAN, [2]Rain Therapeutics, Inc., Newark, CA, [3]University of Colorado, Division of Medical Oncology, Aurora, CO.

## Introduction

**Unmet needs in oncogene-driven lung cancer**

- Lung cancer patients harboring EGFR or HER2 exon 20 mutant tumors represent a unique subset of patients for whom there are currently no effective or approved targeted therapies
- Approximately 10–12% of EGFR-mutant NSCLC tumors have an in-frame insertion within exon 20 of EGFR and are generally resistant to EGFR TKIs[1] with gefitinib, erlotinib and afatinib showing low response rates of 8-11% [2,3]
- Approximately 3% of patients with NSCLC harbor HER2 mutations, and 90% of these are exon 20 mutated[4]
- Osimertinib is currently the standard of care for EGFR-mutated NSCLCs with T790M mutation following therapy with earlier generation EGFR TKIs. Several EGFR resistance mutations, including L718Q, G724S, L792X, and C797X have been reported to osimertinib therapy in the EGFR T790M+ setting [5,6]
- Osimertinib is also approved as first line therapy for patients with sensitizing EGFR mutations. C797X, L718Q and S768I have been detected in ctDNA following osimertinib treatment in the FLAURA trial[7]. New therapies are needed for the osimertinib resistance mutations as there are currently no approved drugs for patients with osimertinib resistance

**Tarloxotinib: a hypoxia-activated irreversible pan-ErbB inhibitor**

- Tarloxotinib is a hypoxia-activated prodrug (HAP) that releases a potent irreversible pan-ErbB TKI (tarloxotinib-E) under pathophysiological hypoxia present in solid tumors
- Tarloxotinib was designed to increase therapeutic ratio over conventional EGFR-TKI therapy, thus inhibiting mutant forms of EGFR and HER2 with increased dose-intensity in hypoxic tumors
- Tumor selective release of tarloxotinib-E increases dose intensity and significantly enhances the tolerability due to reduced WT EGFR-mediated side effects compared to approved EGFR TKIs


Step 1: Tarloxotinib (prodrug) receives an electron from a reductase (STEAP4)
Step 2: Absent oxygen, radical anion intermediate fragments to form Tarloxotinib-E (active drug)
When oxygen present, $O_2$ scavenges electron, and prodrug is retained

**Figure 1.** Tarloxotinib conversion to its irreversible pan ErbB inhibitor. Addition of a hypoxia trigger (blue) to tarloxotinib-E significantly reduces the potency of the prodrug, allowing for administration of a higher relative dose

## Methods

**Establishment of Ba/F3 cells expressing mutant forms of EGFR or HER2.** The murine pro-B cell line Ba/F3 (RCB0805) was obtained from RIKEN Bio Resource Center (Tsukuba, Japan). Ba/F3 cells that express a human *EGFR* activating mutation (either E746_A750 del (exon 19 deletion), L858R mutation, or one of exon 20 insertions*) with/without a secondary *EGFR* mutation were established as previously described[9]. Several Ba/F3 lines which were established in the previous study were also used in this study. All Ba/F3 cells which express a *ERBB2* (HER2) exon 20 insertion mutation** were established in our previous study[10]. Ba/F3 cells with the secondary HER2 C805S mutation, which confers resistance to poziotinib, were also established in a previous study[10].

* A763insFQEA, V769insASV, D770insSVD, H773insNPH, or H773insNPH
** A775_G776insYVMA, G776delinsVC, P780_Y781insGSP

**Cell growth inhibition assay.** Cell growth inhibition assay for one of the following TKIs, tarloxotinib-E (activated form), tarloxotinib (pro-drug before activation), afatinib, poziotinib, and osimertinib, were preformed as previously described[9]. Briefly, 2000 cells were seeded in each well of 96-well plates. Twenty-four hours later, DMSO or a TKI at indicated drug concentration were added, and the cells were cultured for additional 72 hours. We used a colorimetric assay to estimate the growth inhibition of each drug using the Cell Counting Kit-8 reagent (Dojindo Laboratories, Kumamoto, Japan). Each experiment was performed in triplicate.

## Tarloxotinib-E is more potent than tarloxotinib

- Among 26 Ba/F3 cell lines used, 6 cell lines had tarloxotinib (prodrug) $IC_{50}$ > 1000 nM (highest drug concentration tested), whereas, 8 cell lines had tarloxotinib-E (activated form) $IC_{50}$ < 0.457 nM (lowest concentration tested)
- Excluding these 14 cell lines, we compared $IC_{50}$ values in each cell line between tarloxotinib and tarloxotinib-E. Tarloxotinib had 80.3 times higher $IC_{50}$ value (range: 25.1 – 170.3) compared to tarloxotinib-E, demonstrating the potential to generate a wide therapeutic window with this novel prodrug strategy

## Biochemical activity of tarloxotinib-E on various EGFR and HER2 mutations

- Tarloxotinib-E was tested using radiometric *in vitro* kinase assays at Reaction Biology Corporation at ATP $K_m$
- Tarloxotinib-E was potent across EGFR and HER2 mutations tested. Modest loss of potency was observed for C797S triple mutations (del exon 19 or L858R/T790M/C797S) but maintained for del exon 19 or L858R/C797X

A

| Kinase | $IC_{50}$ (nM) |
|---|---|
| EGFR Exon 20 insertions | |
| EGFR | <0.38 |
| EGFR (A763_Y764insFHEA) | <0.38 |
| EGFR (D770_N771insNPG) | <0.38 |
| EGFR (D770GY) | <0.38 |
| EGFR del 19 | |
| EGFR (d746-750) | <0.38 |
| EGFR (d746-750/C797S) | <0.38 |
| EGFR (d746-750/C797A) | <0.38 |
| EGFR (d746-750/T790M/C797S) | 3.24 |
| EGFR L858R | |
| EGFR (L858R) | <0.38 |
| EGFR (L858R, T790M) | <0.38 |
| EGFR (L858R/C797S) | <0.38 |
| EGFR (L858R/C797S/T790M) | 5.4 |
| EGFR Atypical | |
| EGFR (G719C) | <0.38 |
| EGFR (G719S) | <0.38 |
| EGFR (L747S) | <0.38 |
| EGFR (L861Q) | <0.38 |

B

| Kinase | $IC_{50}$ (nM) |
|---|---|
| ERBB2 Exon 20 insertion | |
| ERBB2 (V777_G778insCG) | <0.38 |
| ERBB2 mutations | |
| ERBB2 (D769H) | <0.38 |
| ERBB2 (D769Y) | <0.38 |
| ERBB2 (V777L) | <0.38 |
| ERBB2 (R896C) | <0.38 |
| ERBB WT | |
| ERBB2/HER2 | <0.38 |
| ERBB4/HER4 | <0.38 |

**Table 1.** Biochemical $IC_{50}$ values of tarloxotinib-E for various EGFR. (**A**) and HER2/ERBB2 (**B**) mutant kinases in radiometric kinase assays at respective ATP Km.

## Tarloxotinib-E potently inhibits EGFR exon 20 insertion mutations *in vitro*

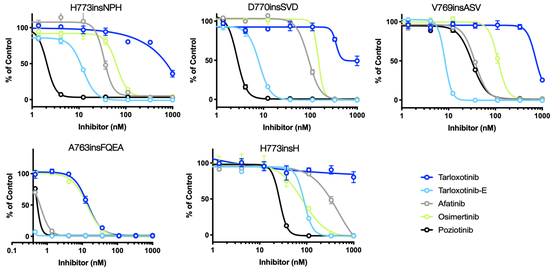


**Figure 2**. Growth inhibition of Ba/F3 cell lines expressing various EGFR exon 20 insertion mutations.

| $IC_{50}$ (nM) | Afatinib | Poziotinib | Osimertinib | Tarloxotinib | Tarloxotinib-E |
|---|---|---|---|---|---|
| A763insFQEA | 0.7 | 0.7 | 14.6 | 15.2 | <0.5 |
| V769insASV | 35.5 | 28 | 118.4 | 675.9 | 7.6 |
| D770insSVD | 86.0 | 27.9 | 184.7 | 990.1 | 7.3 |
| H773insNPH | 35.8 | 2.2 | 61.9 | 714.0 | 9.9 |
| H773insH | 325 | 22.8 | 77.7 | >1000 | 73.1 |

**Table 2.** Cellular potency in Ba/F3 cells expressing various EGFR exon 20 insertion mutations.

## Tarloxotinib-E potently inhibits HER2 exon 20 insertion mutations *in vitro*

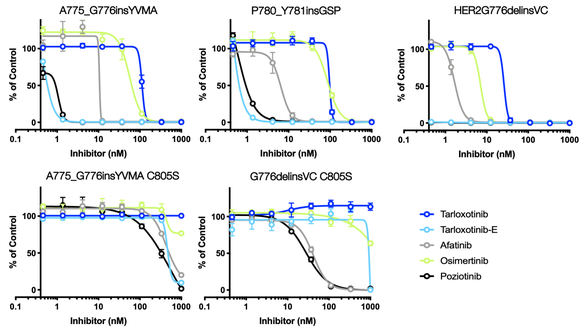


**Figure 3.** Growth inhibition of Ba/F3 cell lines expressing HER2 exon 20 insertion mutations with or without C805S secondary mutations.

| $IC_{50}$ (nM) | Afatinib | Poziotinib | Osimertinib | Tarloxotinib | Tarloxotinib-E |
|---|---|---|---|---|---|
| A775_G776insYVMA | 8.08 | 0.64 | 68.29 | 122.93 | 0.72 |
| G776delinsVC | 2.05 | 0.02 | 7.23 | 22.21 | <0.5 |
| P780_Y781insGSP | 6.50 | 1.03 | 88.45 | 73.53 | 0.87 |
| YVMA+C805S (YVMACS) | 544.9 | 315.8 | >1000 | >1000 | 592.0 |
| VC+C805S (VCCS) | 41.3 | 27.9 | >1000 | >1000 | 608.9 |

**Table 3.** Cellular potency in Ba/F3 cells expressing HER2 exon 20 insertion mutations.

## Tarloxotinib-E activity in EGFR C797S mutations *in vitro*

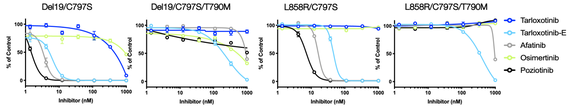


**Figure 4.** Growth inhibition of Ba/F3 cell lines expressing EGFR C797S double and triple mutations with del19 and L585R ± T790M.

| $IC_{50}$ (nM) | Afatinib | Poziotinib | Osimertinib | Tarloxotinib | Tarloxotinib-E |
|---|---|---|---|---|---|
| Del 19 | <0.457 | <0.457 | 0.6 | 16.2 | <0.5 |
| Del 19 + C797S | 2.8 | 1.6 | 791.4 | 408.2 | 5.1 |
| Del 19 + T790M + C797S | 821.7 | >1000 | 464.2 | >1000 | 197.9 |
| L858R | <0.457 | <0.457 | 2.6 | 27.4 | <0.5 |
| L858R + C797S | 19.0 | 7.1 | >1000 | >1000 | 54.2 |
| L858R + T790M + C797S | 845.0 | >1000 | >1000 | >1000 | 348.9 |

**Table 4.** Cellular potency in Ba/F3 cells expressing EGFR C797S double and triple mutations with del19 and L585R ± T790M.

## Efficacy of tarloxotinib for first-line osimertinib resistance mutations

- Osimertinib is currently a standard of care for EGFR-mutated NSCLCs with acquired resistance to first or second generation EGFR-TKIs due to the T790M mutation[5]. Various EGFR tertiary mutations that confer resistance to osimertinib (following 1st and 2nd gen EGFR-TKIs) are reported in the literature[5,8]
- EGFR C797S, L718Q+C797S, L718Q+exon 20 insertions and S7681 were reported as secondary mutations to frontline osimertinib treatment[9]
- Tarloxotinib and tarloxotinib-E were evaluated for activity against these mutations with del19/L858R to mimic osimertinib resistance in the first-line setting (without T790M)

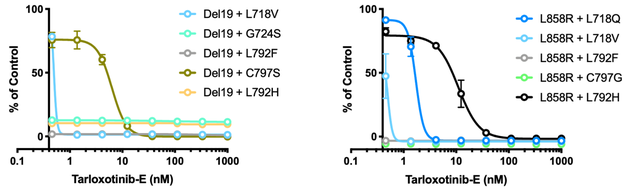


**Figure 5.** Growth inhibition of Ba/F3 cell lines expressing various secondary osimertinib resistance mutations.

| $IC_{50}$ (nM) | Afatinib[8] | Osimertinib[8] | Tarloxotinib | Tarloxotinib-E |
|---|---|---|---|---|
| Del19 + L718V | 0.5 | 10.6 | 46.7 | 0.68 |
| Del19 + G724S | 0.03 | 3.79 | 1.1 | <0.5 |
| Del19 + L792F | 0.3 | 4.01 | 16.9 | <0.5 |
| Del19 + L792H | 0.6 | 11.9 | 0.6 | <0.5 |
| Del19 + C797S | 2.8 | 791.4 | 408.2 | 5.1 |
| L858R + L718Q | 3.37 | 533.0 | 190.1 | 1.9 |
| L858R + L718V | 0.7 | 167.9 | 41.3 | 0.4 |
| L858R + L792F | 0.97 | 29.5 | 189.9 | <0.5 |
| L858R + L792H | 1.25 | 44.7 | 6.5 | 7.8 |
| L858R + C797G | 1.38 | 561.2 | 465.3 | <0.5 |
| L858R + C797S | 6.68 | 918.0 | >1000 | 54.2 |

**Table 5.** Cellular potency in Ba/F3 cells expressing various secondary osimertinib resistance mutations.

## Conclusions

- Tarloxotinib-E (active drug) demonstrates potent *in vitro* activity across a variety of EGFR and HER2 mutations using biochemical kinase assays
- Tarloxotinib-E potently inhibits proliferation of EGFR and HER2 exon 20 insertion mutations in Ba/F3 cell lines
- Tarloxotinib-E potently inhibits proliferation of secondary osimertinib resistance mutations *in cis* with del19 and L858R
- Tarloxotinib-E lost potency in Ba/F3 cell lines with EGFR C797S double and triple mutations as well as HER2 exon 20 insertion mutations when C805S was introduced
- Tarloxotinib-E is consistently more potent than tarloxotinib in Ba/F3 cell lines with various EGFR and HER2 mutations
- EGFR /HER2 exon 20 insertions and osimertinib resistance mutations represent an attractive opportunity for tarloxotinib

## References

1. Arcila *et al.* EGFR exon 20 insertion mutations in lung adenocarcinomas: prevalence, molecular heterogeneity, and clinicopathologic characteristics. Mol. Cancer Ther. 12, 220–229 (2013).
2. Naidoo *et al.* Epidermal growth factor receptor exon 20 insertions in advanced lung adenocarcinomas: Clinical outcomes and response to erlotinib. Cancer 2015;121:3212–20.
3. Yasa *et al. Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations: A combined post- hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6. Lancet Oncol 2015;16:830–8.*
4. Arcila *et al.* Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. Clin. Cancer Res. 18, 4910–4918 (2012).
5. Thress *et al.*, Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M, Nat. Med. 21 (6) (2015) 560–562.
6. Le *et al.*, Landscape of EGFR-Dependent and -Independent Resistance Mechanisms to Osimertinib and Continuation Therapy Beyond Progression in EGFR-Mutant NSCLC. Clin Cancer Res. 2018 Dec 15;24(24):6309-6319.
7. Ramalingam *et al.* Osimertinib As First-Line Treatment of EGFR Mutation-Positive Advanced Non-Small-Cell Lung Cancer. J Clin Oncol 2018;36:841–9.
8. Nishino *et al.* Effects of secondary EGFR mutations on resistance against upfront osimertinib in cells with EGFR-activating mutations in vitro. Lung Cancer. 2018 Dec;126:149-155.
9. Ramalingam *et al.* Mechanisms of acquired resistance to first-line osimertinib: preliminary data from the phase III FLAURA study. In: Proceedings from the 2018 ESMO Congress; October 19-23, 2018; Munich, Germany. Abstract LBA52.
10. Koga *et al.*, Activity of a novel HER2 inhibitor, poziotinib, for HER2 exon 20 mutations in lung cancer and mechanism of acquired resistance: An in vitro study. Lung Cancer. 2018 Dec;126:72-79

Poster presented at the AACR Annual meeting 2019, March 29 - Apr 3, 2019, Atlanta