T790M or C797S confers acquired resistance to tarloxotinib and poziotinib in EGFR exon 20 insertion-driving lung cancer models in vitro

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Introduction

Unmet needs in EGFR mutated lung cancer treatment

- Approximately, 20% of all EGFR mutated lung cancers harbor EGFR exon 20 insertion mutations and represent a unique subset of patients for whom there are currently no effective or approved targeted therapies. First generation (G1) EGFR-TKIs, gefitinib and erlotinib, and second generation (G2) EGFR-TKIs, erlotinib and osimertinib, are not effective in EGFR-20 insertion mutant-driven tumors.

- Recent evidence suggests that the therapeutic potential of the novel hypoxia-activated prodrug, tarloxotinib, a potent pan-EGFR inhibitor, is quite promising. In our previous study, we have shown that tarloxotinib is selectively inhibited in hypoxic tumors, which could drive the clinical development of EGFR TKIs with an improved antitumor activity.

- This secondary mutation preference pattern was the same for tarloxotinib-E and poziotinib.

- Interestingly, secondary mutations showed preference based on the type of the original exon 20 insertion.

- This could be due to the potential for a clinical trial for EGFR and HER2 inhibitors in EGFR-20 insertions.

Materials and Methods

Establishment of Ba/F3 cells expressing mutant forms of EGFR

- The mature pro-drug of Ba/F3 cells (Ba/F3-E) was obtained from RNAi Bio Resource Center (Tsubaki, Osaka, Japan). Ba/F3 cells that express one of the reported EGFR mutations were established.

- Briefly, Ba/F3 cells were cultured in each well of 24-well plates. Those twenty-four hours later, O9MG or T790M at indicated drug concentration were added, and the cells were cultured for additional seven days. After that, a cytotoxicity assay was performed to estimate the growth inhibition of each drug using the Cell Counting Kit 8 (Dojindo Laboratories, Kumamoto, Japan). Each experiment was performed in triplicate.

DNA mutagenesis assay

- DNA acquired resistant cells against tarloxotinib-E or poziotinib were established using ENU (Sigma Aldrich) mutagenesis technique. ENU exposure was performed at the concentration of 0.1 μg/ml for 10 days. Thirty thousand cells were seeded into each well of 96-well plates. Twenty-four hours later, O9MG or T790M at indicated drug concentration were added, and the cells were cultured for additional seven days. After that, a cytotoxicity assay was performed to estimate the growth inhibition of each drug using the Cell Counting Kit 8 (Dojindo Laboratories, Kumamoto, Japan). Each experiment was performed in triplicate.

Results

EGFR exon 20 insertions evaluated in this study

- Using the COSMIC database, we searched for EGFR exon 20 insertion mutations which have been reported in more than 10 lung cancer patients. We selected top 10 mutation subtypes for this study, which covered 72.2% of all reported EGFR exon 20 insertions in lung cancers.

- Esgrin-maturation to tarloxotinib-E plus drug shows synergistic interactions in Ba/F3 models expressing EGFR exon 20 insertion mutations in vitro.

Conclusions

- Tarloxotinib-E plus drug shows synergistic interactions in Ba/F3 cells expressing EGFR exon 20 insertion mutations in vitro.