Overcoming Tumor Resistance to Gefitinib and Erlotinib in Non-Small Cell Lung Cancer Using Piperlongumine

Terrick Andey, PhD., Shail Modi, PhD.

Massachusetts College of Pharmacy and Health Sciences, School of Pharmacy, Department of Pharmaceutical Sciences, Worcester, MA, USA.

OBJECTIVES

- Gefinitib and erlotinib are approved as first-line therapies for the treatment of metastatic non-small cell lung cancer (NSCLC) harboring certain EGFR mutations.
- However, acquired resistance to these therapies have limited their long-term clinical utility and success of therapeutic outcomes.
- A combinatorial treatment approach with piperlongumine - a natural bioactive from the long pepper fruit (Piper longum) - was pursued to overcome tumor resistance to gefitinib and erlotinib to improve therapeutic outcomes.

METHODS

- Anticancer efficacy assessment of piperlongumine (PPL), gefitinib (GEF), and erlotinib (ERL) were performed in H1299 and H1975 NSCLC cells.
- Two-drug combination regimen comprising sub-cytotoxic concentrations of PPL with GEF, or ERL were investigated in both cell lines in a concentration-dependent manner, and scheduling-specific manner.
- Assay of reactive oxygen species (ROS), apoptosis, and oncogenic protein marker (EGFR, EGFR-L858R, p-EGFR, cleaved-Caspase-3, cleaved-Caspase-7) expression were performed.

CONCLUSION

- Piperlongumine was effective and superior to ERL and GEF in inhibiting the growth of both NSCLC (H1299 and H1975) cells.
- Combination treatment comprising low doses of ERL or GEF with PPL [2.5 µM] resulted in significant anticancer activity in a cell- and schedule-specific manner.
- Concurrent treatment schedule potentiated GEF and ERL effects in H1299.
- Pre-treating with PPL followed by GEF or ERL after 1 h was more effective in H1975 cells.
- The anticancer activities of the combination treatment were associated with apoptosis induction and inhibition of oncogenic protein expression and activation.
- Piperlongumine is a viable drug candidate as adjuvant therapy to gefitinib and erlotinib in NSCLC treatment.

REFERENCES

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DISCLOSURES

Authors have no disclosures to make.

RESULTS

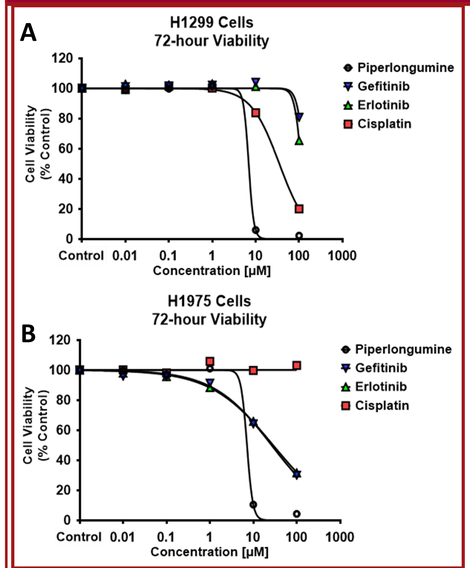

Fig 1. Piperlongumine (PPL) inhibits lung cancer cell viability. (A) H1299 and **(B)** H1975 cells were treated with PPL, ERL, GEF, and CIS for 72 h and analyzed with Alamar Blue (resazurin dye-uptake) assay. Results were presented as line graphs of mean cell viability±SEM and mean IC_{50} values±SEM. [H1299: PPL = 5.84 µM; GEF = 219 µM; ERL = 139 µM; H1975: PPL = 6.05 µM; GEF = 47 µM; ERL = 52 µM].

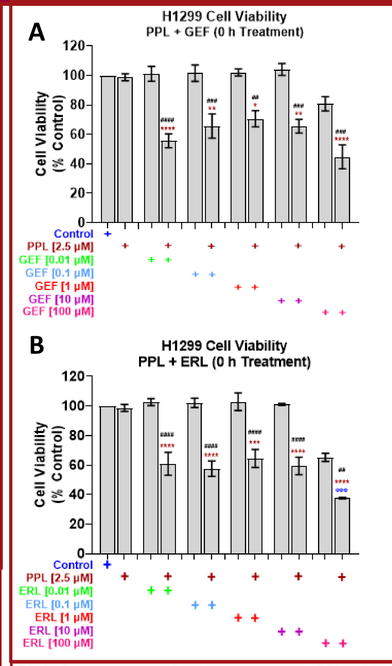

Fig 2. Concurrent combination treatment of PPL with (A) GEF and (B) ERL potentiates anticancer effects in H1299. H1299 cells were treated with PPL [2.5 µM] and varying concentrations of GEF or ERL, concurrently (0 h), for 72 hours and analyzed using Alamar Blue (resazurin dye-uptake) assay. Results were presented as bar graphs of mean cell viability±SEM.

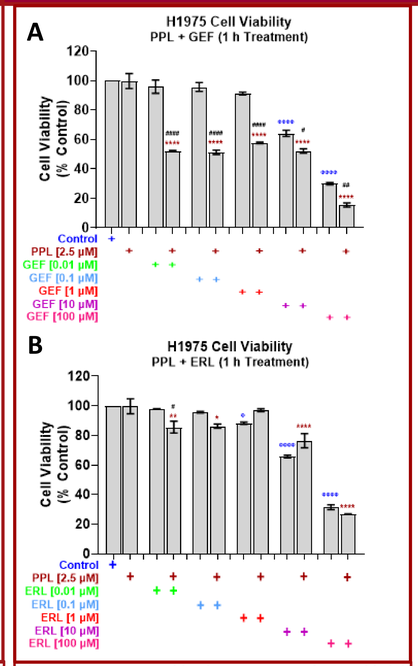

Fig 3. Concurrent combination treatment of PPL with (A) GEF and (B) ERL potentiated anticancer effects in H1975. H1975 cells were treated with PPL [2.5 µM] with varying concentrations of GEF or ERL, sequentially (1 h), for 72 h and analyzed using Alamar Blue (resazurin dye-uptake) assay. Results were presented as bar graphs of mean cell viability±SEM.

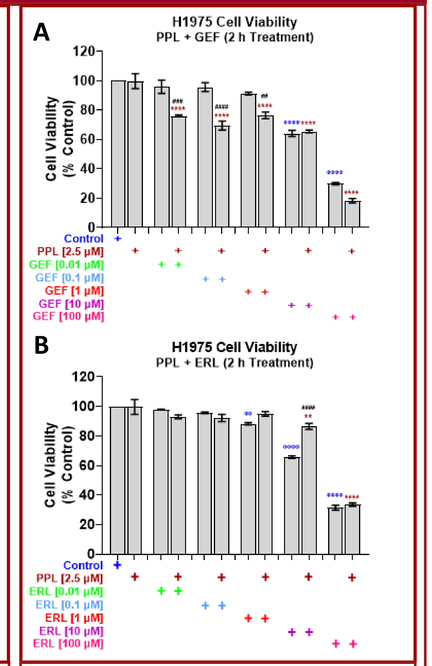

Fig 4. Concurrent combination treatment of PPL with (A) GEF and (B) ERL potentiated anticancer effects in H1975. H1975 cells were treated with PPL [2.5 µM] with varying concentrations of GEF or ERL, sequentially (2 h), for 72 h and analyzed using Alamar Blue (resazurin dye-uptake) assay. Results were presented as bar graphs of mean cell viability±SEM.

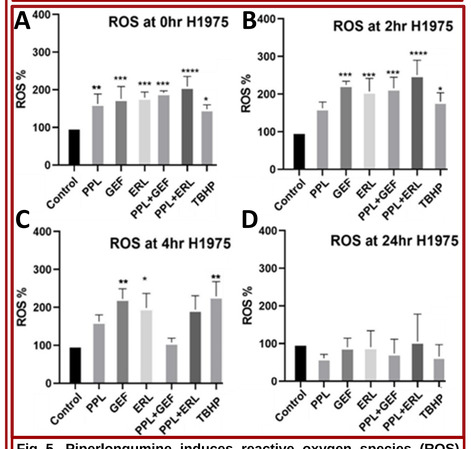

Fig 5. Piperlongumine induces reactive oxygen species (ROS) production in (A-D) H1975 cells, but not H1299. Cells were cultured overnight and assayed for ROS production using DCFDA/H2DCFDA Cellular ROS Assay Kit (Abcam, USA) according to manufacturer's protocol. Cells were exposed to sub-IC_{50} concentrations of PPL [2.5 µM], GEF [25 µM], and ERL [25 µM] alone and in combination, with DMSO and TBHP [100 µM] used as negative and positive controls. Fluorescence readings were taken at and presented as bar graphs.

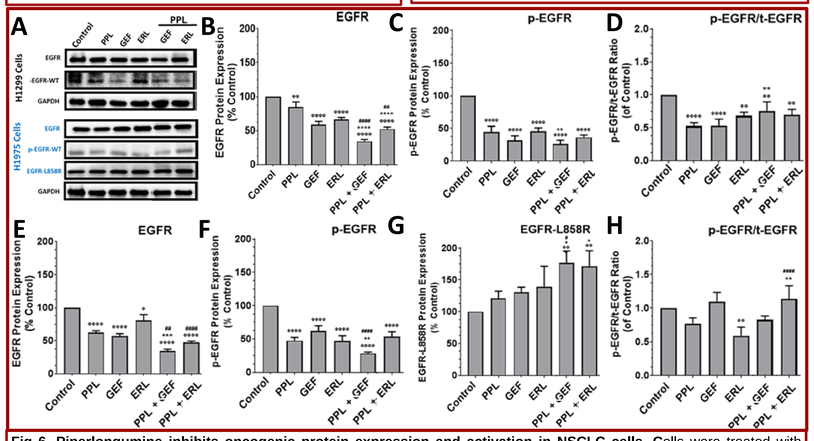

Fig 6. Piperlongumine inhibits oncogenic protein expression and activation in NSCLC cells. Cells were treated with PPL, GEF, and ERL alone and in combination and analyzed for protein expression after 48 h in H1299 **(B-D)** and H1975 **(E-H)**.

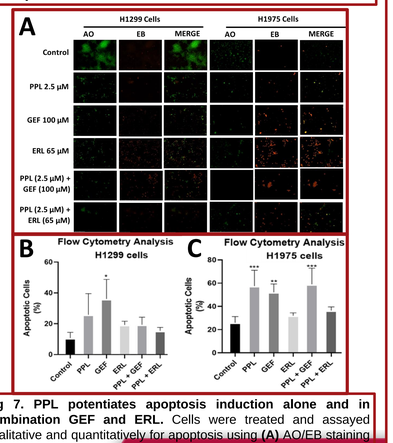

Fig 7. PPL potentiates apoptosis induction alone and in combination GEF and ERL. Cells were treated and assayed qualitative and quantitatively for apoptosis using **(A)** AO/EB staining and **(B-C)** flow cytometry.