MOLECULAR MECHANISMS UNDERLYING THE SYNERGISTIC INTERACTION OF THE EGFR TYROSINE KINASE INHIBITOR ERLOTINIB WITH THE MULTITARGETED ANTIFOLATE PEMETREXED IN NON-SMALL CELL LUNG CANCER CELLS

Giovannetti Elisa,[1,2] Smid Kees,[1] Mey Valentina,[2] Tekle Christina,[1] Nannizzi Sara,[2] Rodriguez José A,[1] Ricciardi Simona,[2] Del Tacca Mario ,[2] Giaccone Giuseppe,[1]Danesi Romano,[2] Peters Godefridus J[1]

[1]Dept. Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands;
[2]Dept. Internal Medicine, Div. Pharmacology and Chemotherapy, University of Pisa, Italy

Purpose. The EGFR tyrosine-kinase inhibitor erlotinib demonstrated clinical activity in 10% of unselected metastatic non-small-cell-lung cancer (NSCLC) patients, but no advantages in combination with standard chemotherapy. Since the multitargeted antifolate pemetrexed is effective against NSCLC and affects different signaling pathways involved in cellular proliferation and apoptosis induction, this study investigates molecular mechanisms underlying its combination with erlotinib in six NSCLC cell lines.
Methods. Cells were characterized by heterogeneous expression of pemetrexed determinants, such as thymidylate synthase (TS) and dihydrofolate reductase (DHFR), as well as by mutations and polymorphisms potentially affecting chemosensitivity. Pharmacologic interaction was studied using the combination index (CI) method, while effects on cell cycle, apoptosis induction and EGFR, Erk1/2 and Akt phosphorylation were studied with flow cytometry, fluorescence microscopy, and ELISA analyses. Finally, real-time PCR, western blot and activity assays were performed to assess whether erlotinib influenced TS.
Results. MTT assays demonstrated the role of *EGFR* and *k-Ras* mutations, and TS and DHFR expression in erlotinib and pemetrexed sensitivity, respectively. However, synergistic cytotoxicity was detected in all cell lines (CI<1), mostly with the pemetrexed (24h) → erlotinib (72h) sequence, associated with a significant induction of apoptosis. Pemetrexed increased EGFR phosphorylation (up to +80% in H460 cells) and reduced Akt phosphorylation, which was additionally decreased by the combination (-79% in SW1573). Erlotinib significantly reduced DHFR and TS expression and TS activity (from 75 to 14 pmol/h/10^6cells in A549), while the combination additionally reduced TS *in situ* activity, possibly via a decrease of the E2F-1 transcription factor, as detected by western blot.
Conclusions. Erlotinib and pemetrexed synergistically interact against NSCLC cells, regardless of their different genetic signature. Several factors, including induction of apoptosis, modulation of EGFR and Akt phosphorylation, as well as expression of critical genes involved in drug activity, contribute to this synergistic interaction and support its clinical investigation.