Effect of additional inhibition of human epidermal growth factor receptor 2 with the bispecific tyrosine kinase inhibitor AEE788 on the resistance to specific EGFR inhibition in glioma cells.

Abstract:
Targeted molecular therapies against the epidermal growth factor receptor (EGFR) are novel, promising and potentially radiosensitising therapeutic approaches in the treatment of glioblastoma, a highly malignant and treatment-refractory brain tumour. Despite a solid rational basis, specific EGFR inhibition has rendered only disappointing clinical results to date. We therefore evaluated the efficacy of additional inhibition of human epidermal growth factor receptor 2 (HER2), the 'non-autonomous amplifier' of EGFR signalling.

Glioblastoma cells (LN-18, LN-229) with different co-expression levels of EGFR and HER2 were treated with specific EGFR and bispecific EGFR/HER2 tyrosine kinase inhibitors (TKIs) (AG1478, AEE788) and experimental radiotherapy, followed by assessment of growth inhibition. Activity of the major downstream signalling pathways Akt and MAPK was determined by immunoblotting. EGFR-overexpressing LN-18 cells (EGFR++++/HER2+) showed resistance and HER2-overexpressing LN-229 cells (EGFR+/HER2++) showed sensitivity to EGFR-specific inhibition. Interestingly, resistance of LN-18 to EGFR inhibition was overcome by AEE788 treatment, supposedly due to its additional HER2 inhibition. Application of AEE788 resulted in blockage of
EGF-dependent EGFR/HER2-heterodimer activation in LN-18 cells, disclosing a possible mediating mechanism for overcoming EGFR-resistance. TKI treatment resulted in significant blockage of both Akt and MAPK signalling pathways, but an incomplete inhibition of PI3K/Akt paralleled the resistance of cells to TKI-induced growth inhibition. Furthermore, the bispecific EGFR/HER2 inhibitor AEE788 showed a radio-sensitising effect in EGFR-overexpressing cells. Taken together, we conclude that inhibition of HER2 in EGFR-overexpressing tumours may harbour the potential to overcome resistance to EGFR-targeted therapy and exert radio-sensitising properties. We suggest that responsiveness to EGFR targeted therapy is mediated through impairment of EGFR/HER2 heterodimer signalling, and thus depends on the ratio of EGFR to HER2 rather than on the amount of individual receptors.