C7-03 Tumor & Cell Biology, Wed, 10:30 - 12:15

Efficacy of BIBF 1120, a potent triple angiokinase inhibitor, in models of human non-small cell lung cancer is augmented by chemotherapy

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Background: VEGF is a prime driver of endothelial cell survival, proliferation and migration in the tumor vasculature. In addition, preclinical evidence points to a role of FGF and PDGF in tumour angiogenesis, mediated by endothelial and smooth muscle cells and pericytes. The indolinone kinase inhibitor, BIBF 1120, concomitantly blocks VEGFR, FGFR and PDGFR signaling at low nM concentrations in vitro and affects tumor vasculature in xenograft models, as shown by DCE-MRI. BIBF 1120, with its unique triple angiokinase profile, has demonstrated encouraging results in phase I clinical trials in cancer patients and is now in phase II combination studies.

Methods: The human NSCLC models H460 and Calu-6 were used to evaluate the antitumor effects of BIBF 1120 in combination with taxanes and pemetrexed. Cell proliferation, apoptosis, and survival were analysed in cultured tumor cells and endothelial cells (HUVEC). In vivo xenografts growing s.c. in nude mice were treated with suboptimal doses of either BIBF 1120 (25 mg/kg daily p.o.), taxotere (7.5 mg/kg once weekly i.v.) or pemetrexed (100 or 150 mg/kg qdx5 i.p.), alone or in combination. TUNEL staining of frozen tumor sections was used to determine the fraction of apoptotic tumor cells.

Results: In vitro proliferation of VEGF-stimulated HUVEC was inhibited by BIBF 1120 (24% inhibition at 10 nM) and paclitaxel alone (17% at 1 nM), with additive effects (92%) seen with the combination. In parallel, the fraction of apoptotic cells increased from ~25% in cultures treated with BIBF 1120 or paclitaxel alone to ~50% in cultures treated with the combination. The proliferation of H460 NSCLC cells in vitro was poorly inhibited even at high concentrations of BIBF 1120 alone (10% at 1 μM), compared to 10 nM paclitaxel alone (42%) or the respective combination (94% inhibition). In vivo studies with the NSCLC models revealed that combination of BIBF 1120 with docetaxel in H460 xenografts has clear antitumor efficacy with a T/C ratio of 27% at dose levels where the single-agent treatments had no or little efficacy (T/C 75% and 66%, resp.). Similarly, BIBF 1120 in combination with pemetrexed in Calu-6 xenografts resulted in additive antitumor activity (T/C 23%) compared with single-agent treatment (T/C 37% and 42%, resp.). The enhanced in vivo efficacy was accompanied by an increase in tumor cell apoptosis detected by immunohistochemistry.

Conclusions: Combining BIBF 1120 with paclitaxel has a marked impact on the proliferation and survival of tumor and endothelial cells in vitro. Combinations of BIBF 1120 with docetaxel or pemetrexed show improved antitumor activity in vivo compared to single-agent treatment. These results lend support to further clinical studies of BIBF 1120 in combination with established chemotherapeutic agents.

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Efficacy of BIBW 2992, a potent irreversible inhibitor of EGFR and HER2 in human NSCLC xenografts and in a transgenic mouse lung-cancer model

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Background: Gefitinib (Iressa) and erlotinib (Tarceva) are both selective and reversible inhibitors of the epidermal growth factor receptor (EGFR) tyrosine kinase. In clinical trials, both compounds showed efficacy specifically in NSCLC patients. Several EGFR mutations associated with clinical response to Iressa or Tarceva have been identified in patients with NSCLC. These include complex in-frame deletions in exon 19 mainly covering the ELREA amino acid sequence or point mutations within exon 21 (e.g. L858R). However, many responding patients develop clinical resistance and relapse. In the majority of cases, the molecular basis for resistance seems to be the acquisition of a secondary T790M point mutation in the kinase domain of EGFR. This bulky amino acid substitution most likely weakens the binding of both reversible inhibitors. By contrast, BIBW 2992, currently in clinical development, is an irreversible, dual EGFR/HER2 inhibitor. BIBW 2992 has demonstrated encouraging results in phase I with 3 objective responses in NSCLC patients, 2 harboring EGFR-del-19 mutations. The present study investigates BIBW 2992 in tumor models with the EGFR L858R/T790M double mutation.

Methods: To assess the profile of BIBW 2992 on various mutants of the human EGFR we generated stable clones of Ba/F3 cells expressing the EGFR-L858R mutant, or various exon 19 deletion mutants, alone or in combination with the T790M resistance mutation. Drug sensitivity was assessed in standard MTT assays. The in vivo efficacy of BIBW 2992 on NSCLC tumors carrying the EGFR-L858R/T790M mutation was evaluated in nude mice bearing human NSCLC xenografts as well as in a transgenic mouse model. Mice were treated orally once daily with BIBW 2992 at 15 or 20 mg/kg. Tumor growth and body weights were monitored 3 times a week.

Results: BIBW 2992 displayed potent inhibitory activity in all cell lines carrying EGFR mutants, including those with the T790M resistance mutation. As expected, erlotinib and gefitinib were active in all exon 19 deletion mutants but failed to inhibit the proliferation of Ba/F3 cells carrying the T790M mutation. In vivo, BIBW 2992 induced long-lasting growth suppression in the H1975 xenograft model which carries the EGFR-L858R/T790M mutation. The T/C values (treated/control) at the end of the experiment were 12% and 18 % at 15 and 20 mg/kg/d respectively. Reversible inhibitors such as lapatinib or gefitinib did not display any anti-tumor activity in this model suggesting that the irreversible binding of BIBW 2992 is key to its activity in these mutant cells. Furthermore, the efficacy of BIBW 2992 was confirmed in a transgenic mouse model based on tetracycline-inducible expression of EGFR-L858R/T790M in pneumocytes. Treatment of mice with established tumor burden with BIBW 2992 resulted in tumor regressions. Treatment was well tolerated by the animals during all experiments.

Conclusion: The orally bioavailable, irreversible and dual EGFR/HER2 inhibitor BIBW 2992 shows efficacy in multiple human NSCLC models. Our data support further clinical investigation of BIBW 2992 in NSCLC patients harboring sensitizing as well as resistance mutations.