Table 1

Breast cancer cell line	Treatment[a]			
	E	E+B	E+F	E+F+B
MCF-7 *(ERa+, ERb+)*	100.0±2.9[b]	19.8±1.0	87.6±1.7	20.1±0.2
BT-474 *(ERa+)*	100.0±16.3	36.9±0.6	82.9±6.2	29.2±0.2
MDA-MB-231 *(ERb+)*	100.0±7.7	106.2±2.4	105.1±2.8	98.5±3.6

[a] 44E = Estradiol 100 nM, B = Bevacizumab 200 ug/mL, F = Fulvestrant 100 nM, T = Tamoxifen 100 nM [b] Proliferation 48 hours (%) ±SD.

Furthermore, the combination of the antiangiogenic with an antiestrogen enhanced this antiproliferative effect, that was also related to the reduction in the levels of VEGF-A in the culture medium and to diminished ER alpha phosphorylation. The combined treatment also altered the phosphorylation of Akt and Erk1/2 signaling kinases.

Conclusions: Our results suggest that in estrogen dependent breast cancer cells the anti-proliferative effect of bevacizumab depends on estradiol concentration, that in turn affects VEGF production levels, using a different mechanism to apoptosis. The combination of bevacizumab with antiestrogens enhances this antitumoral effect, altering intracellular signaling pathways of proliferation and survival.

71 POSTER

Radiosensitization by B-Raf inhibitor PLX4720 correlates with genotype status in anaplastic and differentiated thyroid carcinomas

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Background: Undifferentiated or anaplastic thyroid cancers (ATC) comprise only 5% of all thyroid carcinomas in the US per year but account for one third of annual thyroid cancer-related mortality. Treatment is surgical resection with post-operative radiation and chemotherapy, or palliation for patients with unresectable tumors. These modalities prolong survival by a matter of months. B-Raf and its downstream effector MEK are critical to the molecular pathogenesis of ATCs and 24% of ATCs express the B-Raf mutation V600E. The aim of this study was to determine whether the specific B-Raf V600E inhibitor, PLX4720, is a radiosensitizer in ATCs *in vitro*.

Materials and Methods: Cell lines ARO 81−1 (ATC), WRO 82−1 (follicular TC), NPA 87 (papillary TC) and SW 579 (poorly differentiated TC) were sequenced for the B-Raf V600E mutation. IC50 values of PLX4720 with and without varying doses of radiation were determined using luminescent cell viability and clonogenic assays. PLX4720 concentration-dependent expression of MEK and phospho-MEK were determined by western blotting of total cell lysates.

Results: ARO and WRO were heterozygous for B-Raf V600E; NPA 87 was homozygous for B-Raf V600E; and SW 579 was homozygous for wild type (wt) B-Raf genotype. The IC50 of PLX4720 in SW (wt B-Raf) was significantly higher than for the cell lines encoding B-Raf V600E mutations. In the ARO and NPA cell lines, 8 Gy radiation combined with PLX4720 led to a significant decrease in cell viability, an effect not observed in SW or WRO cells. Preliminary clonogenic studies corroborate these luminescent cell viability data. In ARO, WRO and NPA, a concentration-dependent decrease in phospho-MEK expression was observed, though total levels of MEK remained unchanged. In wt B-Raf cell line SW, there was no drug-dependent decrease in phospho-MEK expression.

Conclusions: Inhibitory effects of the B-Raf specific inhibitor PLX4720 in ATC *in vitro* are enhanced by radiation. Inhibitory effects of PLX4720 correlate with B-Raf V600E genotype of various TC cell lines. Concentration-dependent inhibition of MEK phosphorylation by PLX4720 in TC is limited only to cells encoding B-Raf V600E.

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Semi-rational design of β-catenin targeting peptides for the inhibition of Wnt-dependent signaling

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Wnt-dependent signaling is a conserved signal transduction pathway involved in embryonic development and tissue self-renewal. Deregulation of this pathway is associated with several forms of cancer such as colon cancer, hair follicle tumors and several leukemias.[1] The carcinogenic activation of Wnt-signaling is commonly triggered by the reduced ability of a cell to degrade β catenin, a key component of the signal transduction cascade. Restoring this ability therapeutically would impact cancers that depend on deregulated Wnt-signaling to grow. The manipulation of requisite protein-protein interactions is one approach to interfere with the pathway. Due to large interaction areas however, the precise manipulation is complicated. In principle, an isolated peptide of a protein could be used to inhibit protein-protein interactions. However, such small peptides usually exhibit little or no secondary structure when excised from the stabilizing protein context. The “stapled peptide” strategy in which an all-hydrocarbon cross-link is generated by olefin metathesis of nonproteogenic amino acids is an efficient approach to increase the helical character of peptides to target α-helical binding motifs.[2 4] Unlike their unstapled analogues these hydrocarbon-stapled peptides have shown to be helical, protease resistant, and cell permeable.

Here we report the semi-rational design of stapled α-helical peptides targeting β-catenin. The peptide sequences are derived from α-helical segments of multiple proteins binding to β-catenin. The usage of phage display-based sequence optimization and the incorporation of an all-hydrocarbon cross-link facilitated an increase in affinity to β-catenin by more than two orders of magnitude. In addition, it was possible to render the peptides cell permeable, therefore omitting the use of transfection agents. These investigations were carried out with fluorescein-labeled peptides. In an established Wnt-driven reporter system active peptides have shown robust inhibition of Wnt-mediated transcription.

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Design of a novel covalent EGFR mutant-selective inhibitor

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Background: Erlotinib and gefitinib are effective first-line therapeutics for NSCLC patients who harbor activating mutations in Egfr. Unfortunately, most patients relapse due to the emergence and/or acquisition of secondary mutations or amplification of potential escape pathways. A T790M mutation in Egfr is detected in approximately 50% of patients with drug-resistant tumors, which renders erlotinib/gefitinib ineffective. Although irreversible inhibitors in current clinical studies, such as PF299804, BIBW2992 and HKI-272 demonstrate anti- $Egfr^{T790M}$ activity *in vitro*, they have higher affinity for wild-type (WT) Egfr, resulting in dose-limiting toxicity such as diarrhea and skin rash. We have developed a novel covalent irreversible inhibitor that selectively and potently inhibits both $Egfr^{T790M}$ and the initial activating Egfr mutations but, importantly, is WT-sparing. Such a drug has the potential to effectively treat first- and second-line NSCLC patients with Egfr mutations. Further, covalent inhibitors provide many advantages including improvements in potency, selectivity, prolonged duration of action and translational biomarker opportunities.

Material and Methods: Cell lines expressing $Egfr^{WT}$, $Egfr^{D746-750}$ and $Egfr^{T790M/L858R}$ were used to evaluate the activity of Egfr inhibitors in cell proliferation assays and in pEgfr signaling. An Egfr-specific biotinylated irreversible covalent probe was used to determine the correlation between target-site occupancy and inhibition of pEgfr signaling. Washout experiments were performed to assess prolonged duration of action in cells by the covalent inhibitor.

Results: In cell proliferation assays CNX-419 showed a >50-fold selectivity for the $Egfr^{T790M/L858R}$ and activating Egfr mutations over WT and a >10-fold selectivity in pEgfr signaling. The biotinylated covalent probe confirmed a direct correlation between target-site occupancy by the small molecule and inhibition of pEgfr signaling. Washout experiments demonstrated that pEgfr activity continued to be inhibited after compound removal, confirming the compound’s prolonged duration of action.

Conclusions: CNX-419 is a potent irreversible small molecule that is selective for the activating and drug-resistant mutants of Egfr while sparing of WT-Egfr. Development of such a drug should increase the therapeutic window for NSCLC therapy, allowing adequate dosing to inhibit mutant Egfr while avoiding side effects of current therapies caused by WT-Egfr inhibition.