

# Antiproliferative activity of PEP005, a novel agent that activates PKC $\delta$ and inhibits PKC $\alpha$ , alone and in combination with cytotoxic agents in human solid tumor cancer cell lines

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**Background.** PEP005 (3-Ingenyl angelate) is a novel agent extracted from *Euphorbia peplus* that activates PKC $\delta$  and inhibits PKC $\alpha$  (A Ghoul *et al.*, NCI-AACR-EORTC 2005), resulting in cytotoxic (Can Res 2004, 64:2833) and antiproliferative effects (Blood 2005, Epub). Currently, PEP005 is being developed as a topical treatment of actinic keratoses and basal cell skin cancer and as a systemic treatment for leukemia by an Australian company, Peplin Ltd. This study aimed to evaluate the antiproliferative effects of PEP005 alone and in combination with several cytotoxic agents in a panel of human cancer cell lines deriving from solid tumors.

**Methods.** Antiproliferative effects of PEP005 were first evaluated in a panel of colon (HT29, HCT116, COLO205 and HCC2998), breast (MCF7, MDA-MB-435), lung (HOP62, HOP92), and ovarian (IGROV1, OVCAR3) cancer cell lines characterized for the expression of various PKC isoforms (NCI screen). The cytotoxic effects of PEP005 were compared to that of staurosporine, cisplatin, oxaliplatin, doxorubicin, gemcitabine, 5FU, vinorelbine, and docetaxel using the MTT assay.

**Results.** Antiproliferative effects of PEP005 were shown to be concentration- and time-related (duration of exposure  $\geq 24$ h, being the most effective). In our panel, the cell lines that were most sensitive to PEP005 were COLO205, MDA-MB-435 and HCC2998 (IC<sub>50</sub>: 0.01, 2.6, 30  $\mu$ M, respectively). Exposure of COLO205 to PEP005 induced accumulation in the G<sub>0</sub>/G<sub>1</sub> phase of the cell cycle (with inhibition of the S phase) as measured by flow cytometry. In this cell line, apoptosis induction (Annexin V/Propidium iodide) was observed at concentrations of 0.03-0.3  $\mu$ M followed by cellular secondary necrosis. In our cellular panel, PEP005 was shown to display a unique spectrum of activity, suggesting unique mechanisms of sensitivity/resistance to this agent as compared to other anticancer agents. In COLO205 cells, sequential and concomitant combinations of PEP005 with several cytotoxic agents, using the median effect analysis described by Chou and Talalay, were evaluated using simultaneous (24 hours) and sequential exposure modalities (24 hour exposure for each drug with/without a 24h drug free washout period between the 2 drug exposures). Readouts were performed 48 and 72 hours after growth in a drug free medium. Additive and/or antagonistic effects were reported with cisplatin. Concomitant and sequential combinations of oxaliplatin with PEP005 were either antagonistic or additive. Interestingly, synergistic effects were observed when a 24h washout period was incorporated between PEP005 and oxaliplatin exposure. Similar results were observed with gemcitabine for which optimal cytotoxicity was obtained when PEP005 exposure was followed by a 24h washout period prior to gemcitabine exposure. Combinations of PEP005 and 5FU or topoisomerase II lead to various degrees of synergistic activity when these drugs were administered either before or after PEP005. Finally, combinations of tubuline interactive agents (vinorelbine or docetaxel) followed by PEP005 appeared mostly synergistic.

**Conclusion.** PEP005 a novel agent that modulates PKCs, displays antiproliferative effects, apoptosis induction, and a unique spectrum of activity in human solid tumor cell lines. Combination with several classical cytotoxic agents showed schedule-dependent additive and/or synergistic activity. Based on these data, single agent PEP005 and PEP005-based combinations should be further explored in clinical trials.