



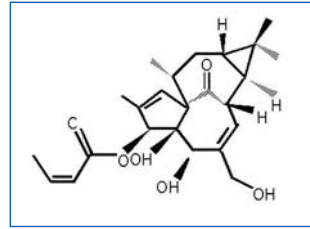
ANTIPROLIFERATIVE ACTIVITY OF PEP005, A NOVEL AGENT THAT ACTIVATES PKC δ AND INHIBITS PKC α , ALONE AND IN COMBINATION WITH CYTOTOXIC AGENT IN HUMAN SOLID TUMOR CANCER CELL LINES

Karim A. Benhadji¹, Maria Serova², Aïda Ghoul¹, Esteban Cvitkovic³, Steven M. Ogbourne⁴, Peter Welburn⁴, Sandrine Faivre⁵, François Lokiec¹, Fabien Calvo², Eric Raymond⁵

¹Rene Huguenin Cancer Center, Saint-Cloud, France, ²U716 Saint-Louis Hospital, Paris, France, ³CAC Oncology, Le Kremlin Bicêtre, France, ⁴Peplin Ltd, Newstead, Australia, ⁵Department of Medical Oncology, Hopital Beaujon, Clichy, France.

INTRODUCTION

- PEP005 is an ingenol angelate extracted and purified from Euphorbia peplus.
- Extracts of Euphorbia peplus have been used in traditional medicine for treating a number of diseases, including warts, corns, waxy growths, skin cancer, asthma, and catarrh.
- PEP005 is a PKC modulator, it activates PKC δ and inhibits PKC α .
- In vitro and In vivo, PEP005 demonstrated anti-proliferative and antitumor effects in a range of solid tumors (melanoma, colon cancer, lung cancer) and leukemia.
- PEP005 is currently under investigation for topical treatment of basal cell carcinoma.
- In this study we explored the antitumor potential of PEP005 as a single agent and in combination with cytotoxic agents in a panel of solid tumor cell lines.



STUDY OBJECTIVES

- To evaluate the anti-proliferative effects of PEP005 as single agent in a panel of selected human cancer cell lines.
- To compare the anti-proliferative effects of PEP005 with that of other cytotoxic agents.
- To evaluate the anti-proliferative and cell cycle effects of combinations using PEP005 with other anticancer agents.

MATERIAL & METHODS

Panel of cancer cell lines tested in present study

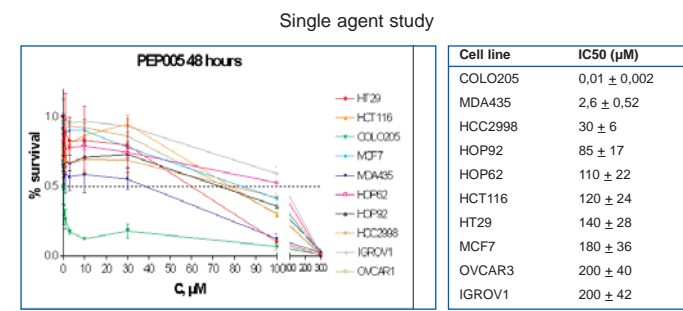
Cell line	NCI screen characteristics						
	p53	MDR	MMR	HER2/neu	EGFR	PKC δ	PKC α
Colon HT29 HCT116 COLO205 HCC2998	mut mut mut mut	Low Low Low Low	High Low High Low	Low Low High High	High High High High		High Low
Breast MCF7 MDA-MB-435	wt mut	Low Low	Low Low	Low High	Low Low	Low High	Low
Ovarian OVCAR3 IGROV1	mut wt?	Low Low	High Low	High High	High High	Low Low	High
Lung Hop62 Hop92	mut mut	High Low	High Low	Low High	Low High	Low High	

* MTT assay: cells seeded at 2*10³/well in 96-well plates and treated with different concentrations of drugs for various times, incubated with 0.4 mg/ml MTT for 4 hours at 37°C. After incubation, the supernatant was discarded, the cell pellet was resuspended in 0.1 ml of DMSO and the absorbance was measured at 560 nm. Growth inhibition curves were plotted as a percentage of untreated control cells.

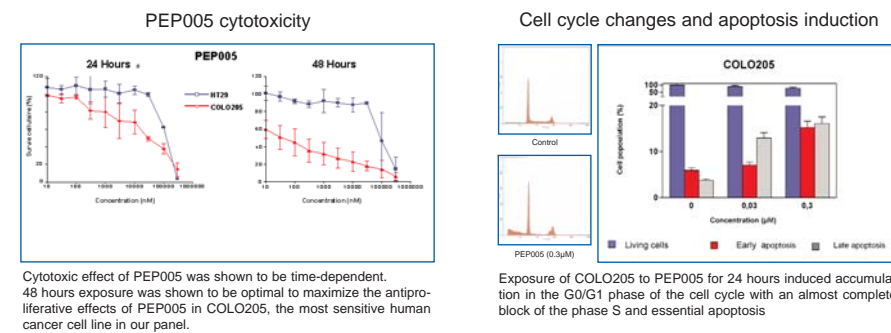
** Effects of drug combinations were evaluated using the Chou and Talalay method which is based on the median-effect principle. Combination index (CI) values of < 0.8 indicate synergy, the value between 0.8 and 1 indicates additive effects, and values > 1 indicates antagonism.

*** Cell cycle and apoptosis were studied by FACS analysis

RESULTS : SINGLE AGENT STUDY



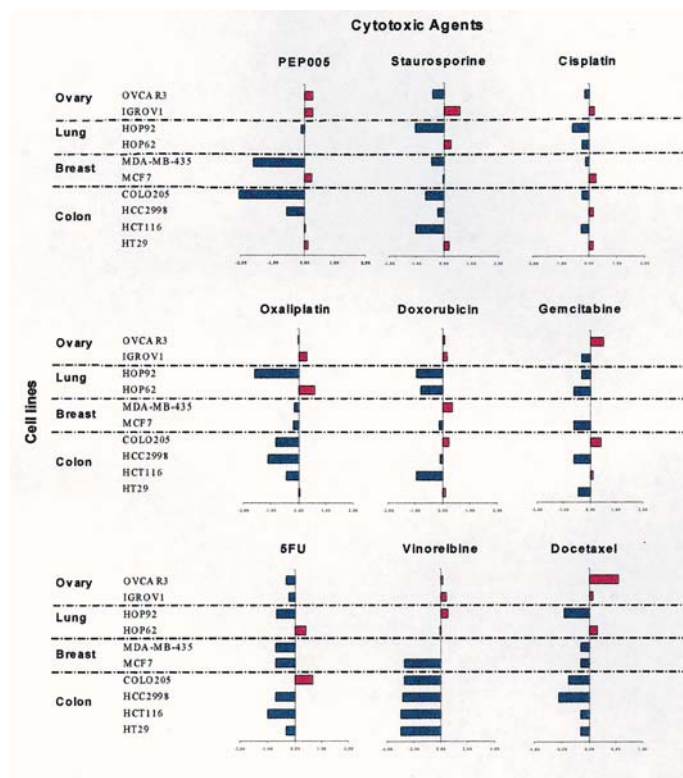
In our panel, most sensitive cell lines to PEP005 were COLO205, MDA-MB-435 and HCC2998



Cytotoxic effect of PEP005 was shown to be time-dependent. 48 hours exposure was shown to be optimal to maximize the anti-proliferative effects of PEP005 in COLO205, the most sensitive human cancer cell line in our panel.

Exposure of COLO205 to PEP005 for 24 hours induced accumulation in the G0/G1 phase of the cell cycle with an almost complete block of the phase S and essential apoptosis

Comparative study of PEP005 cytotoxicity with other anticancer drugs



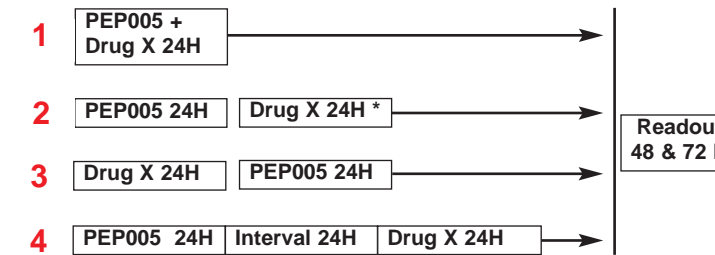
PEP005 displays a unique toxicity profile as compared to several other anticancer agents (including stauroporine another PKC modulator).

RESULTS : COMBINATION STUDY

Drug combinations schedules

Effects of drug combinations were evaluated using the Chou and Talalay method which is based on the median-effect principle. Combination index (CI) values of < 0.8 indicate synergy, the value between 0.8 and 1 indicates additive effects, and values > 1 indicate antagonism.

Schedules 1, 2, 3 and 4 evaluating the effects of PEP005 based combination. 1, 2, 4 - sequential exposure, 3 - simultaneous exposure

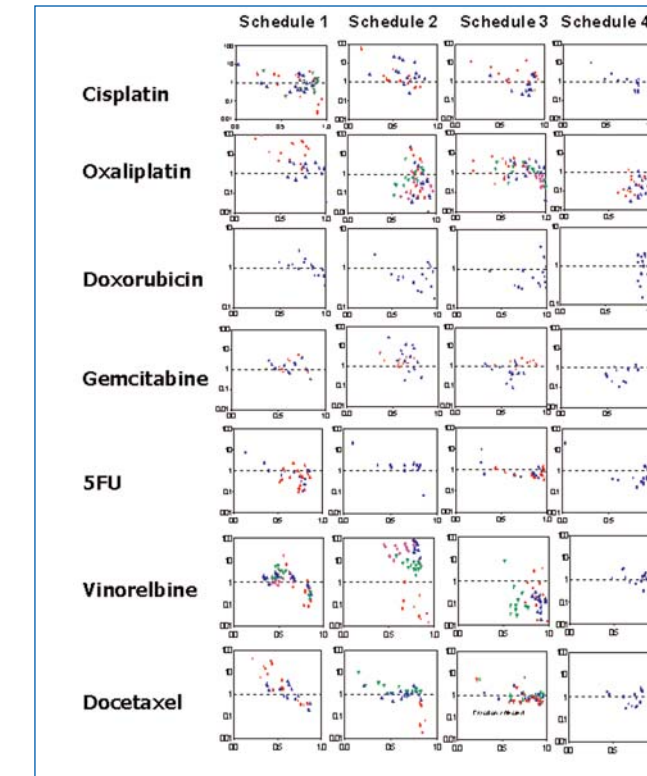


Summary of antiproliferative effects of PEP005-based combination chemotherapy

Combination	Schedule			
	1	2	3	4
PEP/Cisplatin	Additive	Antagonism	Additive	Additive/ Synergy at high concentrations
PEP/Oxaliplatin	Antagonism	Additive/ Synergy	Antagonism/ Additive	Synergy
PEP/Doxorubicin	Additive/ Synergy at high concentrations	Synergy	Synergy	Additive/ Synergy
PEP/Gemcitabine	Antagonism	Antagonism	Additive/ Synergy	Synergy
PEP/5FU	Antagonism	Synergy	Additive	Synergy
PEP/Vinorelbine	Additive	Antagonism	Synergy	Additive/ Synergy
PEP/Docetaxel	Antagonism/ Synergy at high concentrations	Additive	Synergy	Additive/ Synergy

Combination of PEP005 with different classical cytotoxic agents in COLO205 cells showed schedule-dependent additive and/or synergistic activity.

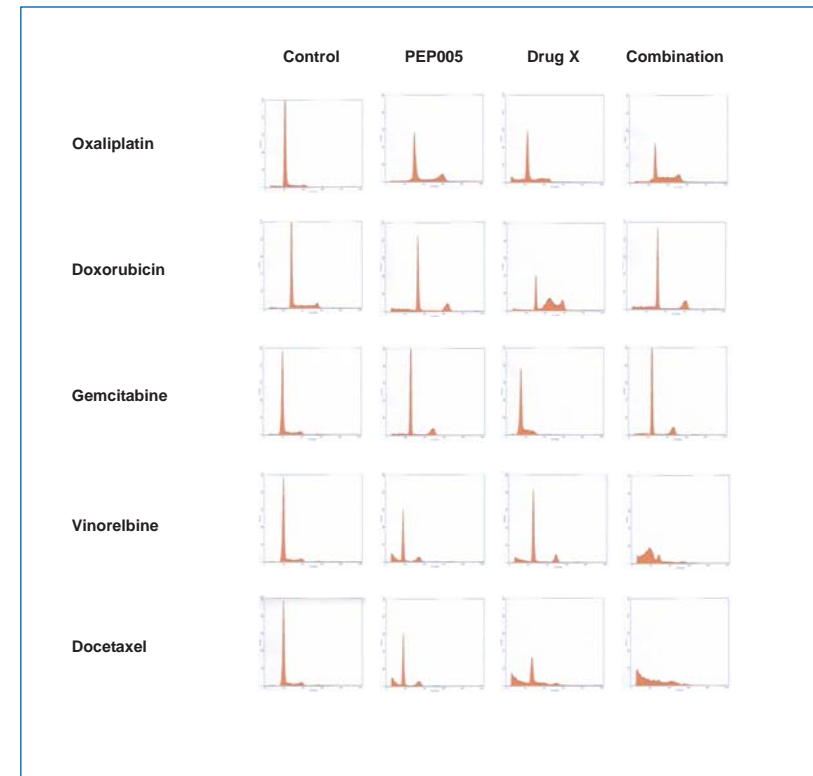
Cytotoxicity of PEP005 combinations in COLO205 cell line



Combination Study

Combination index (CI) < 0.8 indicates synergy, > 1.2 antagonism while a CI between 0.8 and 1.2 corresponds to an additive effect. Present data are the values from independent experiments.

Cell cycle effects of combination studies in COLO205 cell line



Drugs were used at concentrations corresponded IC50s.

Summary of cell cycle effects of PEP005-based combination chemotherapy

Schedule studied	Drug effects	Combination effect
PEP005 - Washout - Oxaliplatin	Apoptosis / phase S accumulation	Phase S accumulation
PEP005 - Doxorubicin	G2/M accumulation	Apoptosis / S phase inhibition
PEP005 - Washout - Gemcitabine	S phase inhibition	Apoptosis / S phase inhibition
Vinorelbine - PEP005	Apoptosis / S phase inhibition	Apoptosis
Docetaxel - PEP005	Apoptosis	Apoptosis

CONCLUSION

- PEP005 given as a single agent, displayed anti-proliferative effects in a range of human solid tumors. These effects are concentration and time dependent, related to the cell cycle arrest and apoptosis induction.
- PEP005 increases the anti-proliferative effects of several chemotherapeutic agents including oxaliplatin, doxorubicin, 5FU, vinorelbine and docetaxel being strongly sequence-dependent for several combinations.
- The data supports the further development of PEP005 in combination with other agents.