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Identification and characterisation of small molecule inhibitors of atypical protein kinase C (aPKC) as anti-cancer agents

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Protein Kinase C (PKC) is a family of at least 12 serine/threonine kinases that have been divided into three distinct enzyme classes – the classic PKCs, novel PKCs, and atypical PKCs (aPKCs). The atypical PKC isoforms (PKCζ and PKCι) are structurally and functionally distinct from the classic (PKCα, PKCβ, PKCγ) and novel (PKCδ, PKCε, PKCη, PKCθ) isoforms, and have been implicated in diverse cellular processes including regulation of polarity, PI3K signalling, and insulin signalling. Meta-analysis of gene expression using expression data from ExpO (International Genomics Consortium) and the Human Body Index (GSE7307) indicated that PKCι is overexpressed in cancers of the lung, ovary, liver and colon. PKCζ is overexpressed in cancers of the small intestine, ovary and cervix. Recent clinical and genetic evidence has suggested that the aPKCs play a key role in tumourigenesis. For instance, PKCι has been identified as an oncogene in non-small cell lung cancer (NSCLC), and its transgenic overexpression in the colon is permissive for carcinogen-induced colon carcinogenesis. PKCζ has been described as a target of Rituximab in follicular lymphoma, and its inhibition has been shown to sensitise cancer cells to a number of commonly used chemotherapeutic agents. Thus inhibition of the aPKC isoenzymes is an attractive target for an anti-tumour therapeutic. The aPKC programme at CRT is in Lead Optimisation and has developed a number of potent ATP-competitive inhibitors of aPKC with distinct chemotypes, e.g. CRT0103391 (IC50PKC*iota; = 8 nM) and CRT0099431 (IC50PKCι = 25 nM). aPKC inhibitors are highly selective for the atypical PKC isoforms against a broad panel of kinases, and show good selectivity within the AGC kinase family including against the classic and novel PKC isoforms. In addition, project compounds display promising early *in vitro* ADME properties. In cell-based assays aPKC inhibitors show direct mode of action biomarker modulation in the sub-400 nM range, exhibiting strong structure-activity relationships, and anchorage-independent tumour cell growth inhibition in the sub-micromolar range. Preliminary pharmacokinetic studies are underway and we now aim to move these compounds into primary efficacy studies.

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A model of synergistic antitumour activity of sorafenib, a multikinase inhibitor of Raf, VEGF and PDGF receptors, with anti-EGFR inhibitors (cetuximab and erlotinib) in a panel of colorectal and lung cancer cell lines

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EGFR and VEGF signalling pathways play a crucial role in tumor cell survival, growth, invasion, proliferation and metastasis. The purpose of this study is targeting both these pathways by using the combination of sorafenib, and cetuximab or erlotinib. This could provide a better anticancer therapeutic strategy.

A panel of human lung (A549, GLC-82, Calu3) and colon (GEO, HCT-15, HCT-116, HT-29, SW480) cancer cells were screened for EGFR and VEGFR expression by Real Time PCR and Western Blot; their ligands have been evaluated by ELISA. These cells lines are characterized for different expression of gene status for p53, K-ras and BRAF (Table 1).

The antiproliferative effects of sorafenib in combination with gefitinib or cetuximab were determined by using a soft agar anchorage-independent growth assay. Combination effects were analyzed by using the isobolographic model according to the Chou and Talalay method. Expression of proteins involved in intracellular cell signaling were assessed by Western Blot. The migration capabilities have been investigated by wound-healing assay.

The EGFR, VEGFR are expressed in almost all cancer cell lines as well as their ligands which were detected in the supernatant. A dose-dependent synergistic effect in growth inhibition was observed by the combined treatment with sorafenib 1 μM and erlotinib 2 μM or with sorafenib and cetuximab 2.5 μM; colorectal cancer cell lines seem to be more sensitive to inhibition. The expression of active phosphorylated EGFR, MAPK and AKT, evaluated after 10, 20, 60 and 120 minutes of treatment, is markedly decreased by both combined drugs especially with sorafenib and erlotinib treatment as well as the downstream pathway of mTOR such as ppS6K and p4EBPI. Moreover the migratory activity was decreased by using sorafenib as single agent and in combination in H1299 NSCLC cell line.

Combination treatment with sorafenib and erlotinib or sorafenib and cetuximab has synergistic antiproliferative properties in human colorectal and lung cancer cell lines providing a rationale for further clinical studies.

Table 1

Human cancer cell lines	K-ras	BRAF	p53
GLC-82	–	–	WT
H1299	WT	Mut	Mut
A549	Mut	WT	WT
Calu3	Mut	WT	Mut
H460	Mut	WT	WT
HT29	WT	Mut	Mut
HCT15	Mut	WT	Mut
HCT116	Mut	WT	WT
SW480	Mut	WT	Mut
GEO	Mut	WT	WT

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Enhancement of antitumour activity of the vascular disrupting agent ABI-011 by nab-paclitaxel and bevacizumab

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Background: The tumor vasculature is an established target for anticancer therapies. Vascular disrupting agents (VDAs) compromise established tumor vasculature and have the potential to destroy tumor masses as well as preventing progression. ABI-011, a novel thiocolchicine dimer, is a potent VDA with antitubulin and topoisomerase 1 inhibitor properties. ABI-011 displayed significant anti-tumor activity in mouse xenograft models of human breast, colon, prostate, and ovarian carcinoma. In this study, the importance of dose, schedule, and sequence for the combination *of* ABI-011 and *nab*-paclitaxel (Abraxane®) or bevacizumab (Avastin®) was evaluated in mice bearing HT29 (colon) xenografts.

Materials and Methods: Potential antagonistic activity of ABI-011 against paclitaxel and docetaxel in vitro was demonstrated in vitro using MX-1 tumor cells. Subcutaneous human colon (HT29) tumors were grown in athymic nude mice and treated intravenously (IV) with sub-optimal dose of ABI-011 alone (10 mg/kg or 20 mg/kg, q4d×3) and in combination with *nab*-paclitaxel (10 mg/kg, q4d×3) and/or bevacizumab (8 mg/kg, 2×wkly). The effect of dose, schedule, and sequence of combination regimen on therapeutic efficacy was tested. ABI-011 was administered 24 h before, concurrent with, or 24 h after *nab*-paclitaxel or bevacizumab treatment.

Results: Antagonistic interaction was observed between paclitaxel, docetaxel and ABI-011 in vitro. There was strong synergy between Avastin and ABI-011, with the combination exhibiting significantly better antitumor activity than either Avastin ($P = 0.028$) or ABI-011 alone ($P = 0.003$). When combined with ABI-011 + Avastin, *nab*-paclitaxel exhibited best additive improvement when administered concurrent with ABI-011 (Concurrent vs 24 hr prior to *nab*-paclitaxel: $P = 0.06$; Concurrent vs 24 hr post *nab*-paclitaxel: $P = 0.01$). The triple combination was superior to the ABI-011 + *nab*-paclitaxel combination ($P < 0.001$) and the ABI-011 + Avastin combination ($P = 0.002$).

Conclusions: ABI-011 alone and in combination with *nab*-paclitaxel demonstrated significant TGI in xenograft models. Possible negative interactions between the two drugs can be avoided by careful scheduling, sequencing and timing of drug administrations. The combination data suggest that effective combination of Avastin, Abraxane, and ABI-011 is feasible. The double or triple drug combination therapy would be expected to be more effective than monotherapy.

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AZD8931, an equipotent, reversible inhibitor of erbB1, erbB2 and erbB3 receptor signalling: characterisation of pharmacological profile

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Background: ErbB family receptors (ErbB1−4) are deregulated in many cancers with their homo- and/or hetero-dimerisation stimulating cell proliferation, invasion, metastasis, angiogenesis and/or survival via numerous