1222 POSTER

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.

1223 POSTER

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

1224 POSTER

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.

1225 POSTER

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

signal transduction pathways. We hypothesise that simultaneous, potent inhibition of erbB1 (EGFR), erbB2 (HER2) and erbB3 (HER3) may be more efficacious than first generation of erbB family targeted therapies such as gefitinib and lapatinib, and report here the pharmacological characterisation of AZD8931 in comparison with these agents.

Methods: A broad range of assays modelling erbB family receptor signalling in homo- and hetero-dimers included: *in vitro* evaluation of erbB kinase activity; erbB receptor phosphorylation and proliferation in cells; and *in vivo* testing in a human tumour xenograft panel, with *ex vivo* evaluation of erbB phosphorylation and downstream biomarkers in some cases.

Results: *In vitro,* AZD8931 demonstrated equipotent, reversible inhibition of erbB1, erbB2 and erbB3 phosphorylation in cells (Table). In proliferation assays, AZD8931 was significantly more potent than comparators in some SCCHN and NSCLC cell lines. AZD8931 significantly inhibited xenograft growth *in vivo* in models responsive to erbB1 inhibition alone, or erbB1 and erbB2 together and furthermore had significant effects on erbB1, erbB2 and erbB3 phosphorylation and on downstream signalling pathways, apoptosis and proliferation.

	IC_{50} (μM);95% CIR		
	AZD8931	Gefitinib#	Lapatinib
Isolated kinase			
erbB1	0.012; 1.354	0.018; 1.307	0.302; 1.119
erbB2	0.014; 2.074	0.335; 4.271	0.093; 3.012
***In vitro* phosphorylation** (tumour cell lines)			
erbB1	0.004; 1.377	0.011; 1.601	0.033; 2.913
erbB2*	0.003; 1.817	0.024; 2.827	0.009; 1.952
erbB3	0.004; 1.890	0.095; 2.180	0.013; 2.270

CIR, confidence interval ratio; *ligand dependent; #Selective erbB1 inhibitor

Conclusions: AZD8931 has a distinct pharmacological profile from gefitinib and lapatinib, inhibiting erbB1, erbB2, and erbB3 equipotently and demonstrating greater anti-tumour activity than these more selective agents in some pre-clinical models. A product with this profile may deliver broader clinical activity than more selective erbB family targeted therapies in cancers where simultaneous inhibition of erbB1−3 signaling is likely to be required for optimal clinical benefit (e.g. in combination with anti-hormonal therapy in oestrogen-dependent breast cancer).

Poster presentations (Mon, 21 Sep, 09:00−12:00)
Drug development − Phase I and Phase II

1226 POSTER

Multi-arm Phase IB study of TH-302 in combination with gemcitabine, docetaxel or pemetrexed

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Background: TH-302 is a tumor-selective 2-nitroimidazole prodrug of the DNA alkylator, bromo-isophosphoramide mustard (Br-IPM). In severe hypoxia, TH-302 is reduced and Br-IPM is released. TH-302 was designed to target tumor hypoxic regions that are not well targeted by conventional anti-tumor therapies. In a Phase I study, TH-302 showed activity as a single-agent with a maximum tolerated dose (MTD) of 575 mg/m^2 weekly and mucosal dose limiting toxicity (DLT) with the absence of significant myelosuppression.

Materials and Methods: Eligible patients (pts) for the study (NCT00743379) had ECOG ⩽1, advanced solid tumors, evaluable disease by RECIST, and acceptable hematologic, hepatic and renal function. Pts received TH-302 in combination with standard doses of gemcitabine (G), docetaxel (D) or pemetrexed (P). TH-302 was administered IV on Days 1, 8 and 15 (G only) of a 21 or 28 (G only) day cycle. A standard 3+3 pts design was used. TH-302 starting dose was 240 mg/m^2. CT scans were done every 2 cycles. The objectives of the study were to determine the MTD and DLT of TH-302 and to evaluate the safety, pharmacokinetics (PK) and preliminary efficacy of TH-302 in combination with G, D or P in advanced solid tumors.

Results: 35 pts have been enrolled at TH-302 doses of 240–480 mg/m^2, 11 with G, 11 with D and 13 with P. Median age: 60. ECOG 0/1 in 21/14 pts. Primary tumor: NSCLC (7), ovary (3), colorectal (3), carcinoid (3), other (19). Pts received 1−11+ cycles (median 3+). 17 pts have discontinued for progressive disease (7), clinical deterioration (5), pt decision (2), adverse event (AE; 1), investigator decision (2). One DLT has occurred in each arm (all grade 3): AST elevation at 240 mg/m^2 with G, febrile neutropenia at 240 mg/m^2 with D and oral candidiasis at 480 mg/m^2 with P. Common AEs were skin or mucosal toxicity, nausea, fatigue and vomiting and were mostly grades 1 and 2. Grade 3/4 neutropenia, lymphopenia or thrombocytopenia has occurred in 38, 47 and 18% of pts. TH-302 and Br-IPM PK are not altered by G, D or P. Six of 29 (21%) evaluable pts had a partial response (PR) and 4 are confirmed (1 each with G and D, 2 with P). 24 (83%) pts had a best response of either PR or stable disease (G: 8 of 10, D: 7 of 9, P: 9 of 10). All arms are now dosing at 480 mg/m^2.

Conclusions: TH-302 can be administered safely in combination with full dose G, D or P but may increase the hematologic toxicity of these agents. Encouraging anti-tumor activity was observed.

1227 POSTER

A phase 1, dose-escalation study of BIIB022 (anti-IGF-1R monoclonal antibody) in patients with relapsed or refractory solid tumours

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Background: Antibodies to insulin like growth factor 1 receptor (IGF-1R) have been shown to inhibit tumour cell survival and proliferation pathways as well as tumour cell motility and invasion. BIIB022 is a fully human, non-glycosylated IgG4 monoclonal antibody that binds specifically to IGF-1R thereby blocking the binding of IGF1 and IGF2 ligands.

Material and Methods: This is a Phase I dose-escalation study to evaluate the safety amd toxicity of escalating doses of BIIB022 (1.5−30 mg/kg) administered by IV infusion every 3 weeks (q3 wk) in patients (pts) with advanced solid tumours. Preliminary anti-tumour effects, PK, immunogenicity, FDG-PET imaging, and tissue and blood biomarkers are also being evaluated.

Results: 15 pts (M/F 10/5) have been treated with BIIB022 at doses of 1.5−20 mg/kg.

The median age is 58 yrs (range 40−75) and all had an ECOG performance status ⩽1.

Fourteen pts (93%) experienced at least 1 adverse event (AE). The most common drug-related AEs were headache and gastrointestinal events in 7 (47%) and 6 pts (40%) respectively. Two pts treated at 10 mg/kg experienced Grade 3 events of hypertension and diarrhoea considered possibly related to BIIB022. One pt treated at 20 mg/kg experienced a cardiac dose-limiting toxicity consisting of widespread EKG T wave inversion, and transient apical hypokinesis with secondary grade 3 QTc prolongation consistent with ischaemia/infarction. No Grade 4 AEs were observed. One pt treated at 10 mg/kg had a transient Grade 2 elevation of blood glucose normalising within 7 days. Neither thrombocytopenia nor elevations in fructosamine or hemoglobin A1c values have been seen. The $t_{1/2}$ appears to increase with increasing BIIB022 dose (4.7 days [1.5 mg/kg] – 11.3 days [20 mg/kg]). C_{max} and AUC are dose dependent. IGF1-R downregulation on blood granulocytes occurred within 2 hours of BIIB022 treatment at all doses evaluated with persistent downregulation for the entire 3 week dosing cycle. One pt achieved an FDG PET response, and 6 pts received 3 or more cycles of treatment before progression.

Conclusions: Preliminary results suggest that BIIB022 is tolerated at doses up to 20 mg/kg q3 wk without the metabolic effects noted with other IGF-1R antibodies. Enrollment is continuing with expansion of the 20 mg/kg cohort and a detailed evaluation of safety, anti-tumor activity, PK, and potential biomarkers of activity.