156 POSTER

DNA damage induced by camptothecins is stabilized by G-quadruplex ligands

C. Leonetti[1], M. Porru[1], E. Salvati[1], C. D'Angelo[1], A. Orlandi[2], M. Franceschin[3], F. Zunino[4], M.F.G. Stevens[5], A. Biroccio[1]. [1]*Regina Elena Cancer Institute, Experimental Chemotherapy Laboratory, Rome, Italy;* [2]*Tor Vergata University, Anatomic Pathology Institute, Rome, Italy;* [3]*Sapienza Università, Dipartimento di Chimica, Rome, Italy;* [4]*Fondazione IRCCS Istituto Nazionale Tumori, Department of Experimental Oncology and Molecular Medicine, Milan, Italy;* [5]*the University of Nottingham, School of Pharmacy, Nottingham, United Kingdom*

Background: We previously reported that the combination of the G-quadruplex (G4) ligand RHPS4 with standard camptothecins has a strong synergistic interaction *in vitro* and produced a marked antitumor activity on xenografts. The present study aims at investigating the mechanisms involved in the specific interaction between G4 ligands and camptothecins by using well-established G4 ligands and novel camptothecins.
Material and Methods: Chromatin immunoprecipitation (ChIP) assay was performed to evaluate the presence at the telomeres of the different topisomerase isoforms after treatment with G4 ligand compounds. Combination index test based on clonogenic assay was used to study synergism, additivity or antagonism of the different G-quadruplex/camptothecin combination. Co-immunofluorescence experiments were performed to measure the formation of damaged foci and FACS analysis to evaluate cell cycle perturbation/apoptosis. *In vivo* experiments were carried-out to evaluate the therapeutic efficacy of novel camptothecins/RHPS4 combination.
Results: We found that the G4 ligand RHPS4, Coron and Pip-piper increased Topo I at telomeres, while no change of TopoIIα was observed in treated compared to untreated cells. We therefore studied the effect of G4 compounds in combination with either the TopoI inhibitor SN-38 or the topoisomerase II poison doxorubicin. A marked reduction of clonogenic ability and a strong synergism effect was observed in the G4/camptothecin combination, while the combination with doxorubicin showed only a slight decrease of cell survival. Of note, the sequence of administrating the two drugs is critical in determining the chemo-sensitizer activity of the G4 ligands to SN-38, since the opposite sequence of treatment did not produce an increase in camptothecin cytotoxicity. Analysis of damage foci revealed that SN-38 produced a strong phosphorylation of γH2AX both at the telomeric (TIFs) and non telomeric regions but cells recovered the damage as it is evident by the decrease of γH2AX foci at 48 hrs after the treatment. Interestingly, DNA damage was highly increased in cells treated with SN-38/RHPS4 combination and the percentage of γH2AX-positive cells and the number of TIFs were stabilized by the G4 ligands. In addition, the synergistic effect of SN-38/G4 ligands was confirmed with the novel ST1481, IDN5174 and ST1968 camptothecins in combination with RHPS4. Finally, treatment of mice with ST1481 and RHPS4 was able to inhibit tumor weight, delay tumor regrowth and increase survival of mice bearing colon cancer xenografts.
Conclusions: Our data demonstrate the high therapeutic efficacy of TopoI inhibitors/G4 ligands combination and suggest that the stabilization of DNA damage by G4 ligands can account for the antitumor effect of this therapeutic strategy both *in vitro* and in xenografts.

157 POSTER

The mTOR kinase inhibitor AZD8055 induces cell death in Her2+ tumours partially or intrinsically resistant to ErbB2 inhibitors

S.V. Holt[1], G. Marshall[1], A. Logie[1], Z. Howard[1], S. Fenton[1], J. Dry[1], D. James[1], E. Brown[1], R.W. Wilkinson[1], S.M. Guichard[1]. [1]*AstraZeneca, Cancer Bioscience, Alderley Edge, United Kingdom*

The PI3K-Akt-mTOR pathway is one of the most frequently activated pathways in human tumours. Oncogenic drives such as PI3K or KRas mutations, PTEN loss of function or ErbB2 amplification produce high dependency on the PI3K/mTOR pathway for growth and survival. mTOR is part of 2 complexes mTORC1 and mTORC2. mTORC1 activates S6K and 4EBP1, both involved in cap-dependent translation. mTORC2 directly activates AKT on Ser 473. Clinically, mTOR is the only validated target in the PI3K pathway. However, allosteric inhibitors of mTOR such as rapamycin inhibit mTORC1 only, leading to AKT hyperactivation, limiting their clinical activity.
AZD8055 is a mTOR kinase inhibitor, targeting mTORC1 and mTORC2 inducing greater growth inhibition and cell death than rapamycin. The aim of this study was to establish the activity and biomarkers profile of AZD8055 in Her2-overexpressing models both as single agent and in combination *in vitro* and *in vivo*.
A gene expression and copy number analysis selected a series of Her2+ cell lines with differential PI3K activation. AZD8055 induced significant growth inhibition and cell death in this cell line panel. A subset of cell lines were established as xenografts and tested *in vivo*. AZD8055 induced growth inhibition or regression in BT474, MDA-MB-453 and H1954 xenograft models at a dose of 20 mg/kg daily. In BT474 cells, AZD8055 increased the growth inhibitory effect of herceptin in vitro. In vivo, BT474 xenografts were hypersensitive to herceptin and the added beneficial effect of AZD8055 was limited. In MDA-MB-453 moderately sensitive to lapatinib *in vitro*, the combination of AZD8055 and lapatinib induced cell death while only growth inhibition was observed with either agent. Finally, AZD8055 induced regression in a primary explants model of Her2-amplified breast cancer xenografts resistant to herceptin. In MDA-MB-453, the combination effect was associated with greater inhibition of mTORC1 and mTORC2 markers, induction of apoptosis as well as abrogation of pHer3 feedback activation. Taken together this study demonstrates that AZD8055 enhances the efficacy of Her2 targeted agents in sensitive models. AZD8055 is also active in tumour models partially or intrinsically resistant to ErbB2 inhibitors, suggesting that it may have broad activity in ErbB2+ tumours.

158 POSTER

3D tumour models for the assessment of tumour micro-environment targeted therapies

D. Onion[1], R. Argent[1], R. Kumari[1], S. Watson[1], A. Axel[2], B. Hall[2]. [1]*University of Nottingham, Ex vivo Cancer Pharmacology Centre of Excellence, Nottingham, United Kingdom;* [2]*OrthoBiotech, Oncology Research & Development, Beerse, Belgium*

Background: It is becoming increasingly apparent that the interaction between the tumour and the surrounding stromal cells is critical to tumour growth, invasion and metastasis. As a consequence, tumour-microenvironment targeted therapies and therapies which disrupt the paracrine signalling pathways that support tumour growth are under development. Current pre-clinical *in vitro* and *in vivo* tumour models lack human stromal cells and so are inadequate for the assessment of novel tumour microenvironment targeted therapies.
Methods: We have developed a novel 3D matrix reconstitution assay, where the paracrine interaction between tumour derived fibroblasts and epithelial cells are restored, allowing the real-time assessment of tumour-microenvironment driven tumour growth in the presence of targeted therapies.
Results: Primary tumour associated fibroblasts and mesenchymal stem cells drive growth and proliferation of non-small cell lung cancer cell lines of adenocarcinoma (A549, NCI-H358, NCI-H460) and squamous subtypes (SK-MES1) as well as early stage disease (NCI-H322M). The paracrine interaction promotes 3D structure formation and invasion into laminin rich basement membrane extract. Using fluorescent cell labelling and real-time viability assays the 3D matrix reconstitution assay allows the rapid and scalable assessment of targeted therapies in comparison to platinum doublet standards of care in the context or tumour micro-environment driven growth.
Conclusion: The 3D matrix reconstitution assay provides a more accurate model for pre-clinical testing of therapies which target the tumour microenvironment or the downstream pathways of their paracrine interaction with tumour cells.

159 POSTER

TGF-β1 as a therapeutic target in high risk endometrial carcinomas

L. Muinelo-Romay[1], E. Colas[2], A. Gil-Moreno[2], J. Barbazán[1], L. Alonso-Alconada[1], T. Curiel[1], J. Cuevas[1], J. Reventós[2], R. López-López[1], M. Abal[1]. [1]*Complexo Hospitalario Universitario de Santiago de Compostela, Translational Oncology Laboratory/Medical Oncology Department/SERGAS, Santiago de Compostela, Spain;* [2]*Research Institute Vall d'Hebron University Hospital, Biomedical Research Unit, Barcelona, Spain*

Background: Endometrial cancer is among the three most common cancers in females in industrialized countries. In the majority of cases the tumor is confined to the uterus at time of diagnosis and presents a good prognosis. However, after primary surgery, from 15% to 20% of these tumors recur and have limited response to systemic therapy. We thus aimed to perform gene expression profiling associated with high risk of recurrence in endometrial carcinomas, and evaluate new therapeutic approaches targeting the molecular pathways involved in the acquisition of an aggressive tumor phenotype.
Material and Methods: We performed cDNA microarray analysis in 60 human endometrial carcinomas and compared the gene expression profiles associated with low (IA and IB endometrioid tumors) and high (II, III, IV and IC endometrioid, serous papillary and clear cell carcinomas) risk of

recurrence. The main molecular alterations defining high risk tumors were identified by Ingenuity Pathways Analyses software. We further validated the experimental approach and characterized the effects of TGF-β1 and the TGF-β1 receptor kinase inhibitor SB-431542 in an *in vitro* invasion assay with Hec1A endometrial cell line.
Results: Gene expression profiling identified a number of molecular pathways associated with high risk of recurrence in endometrial cancer, and designated a prominent role to TGF-β signaling in the acquisition of an aggressive phenotype. We showed that TGF-β1 promoted morphologic and molecular alterations consistent with an epithelial to mesenchymal transition in Hec1A cells. Moreover, TGF-β1 was able to promote Hec1A cells invasion and SB-431542 reversed these effects. We further demonstrated in a 3D inverted invasion assay that the TGF-β pathway represents a key molecular event in the initial steps of carcinoma invasion.
Conclusion: Our study indicates that the acquisition of a high risk of recurrence phenotype in endometrial carcinomas strongly relies on TGF-β1. The results highlight the promising utility of TGF-β pathway inhibitors for the development of targeted therapies in endometrial cancer.

160 POSTER

Cancer-associated IDH1 and IDH2 mutations: therapeutic opportunities

S. Jin[1], L. Dang[2], S. Gross[2], E. Driggers[3], K. Yen[4], M. Bittinger[5], C. Thompson[6], L. Cantley[7], M. Su[8], V. Fantin[5]. *[1]Agios Pharmaceuticals Inc, Cell Biology, Cambridge, USA; [2]Agios Pharmaceuticals Inc, Biochemistry, Cambridge, USA; [3]Agios Pharmaceuticals Inc, Cell Metabolomics, Cambridge, USA; [4]Agios Pharmaceuticals Inc, In Vivo Pharmacology, Cambridge, USA; [5]Agios Pharmaceuticals Inc, Molecular Oncology, Cambridge, USA; [6]University of Pennsylvania, Abramson Cancer Center, Philadelphia, USA; [7]Harvard Med Sch, Dept of Medicine, Boston, USA; [8]Agios Pharmaceuticals Inc, R&D, Cambridge, USA*

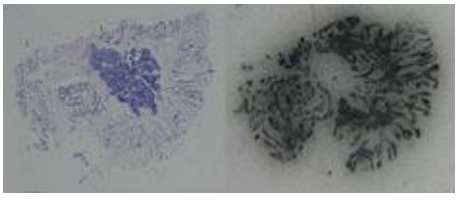
Somatic mutations in the enzymes isocitrate dehydrogenase IDH1 and IDH2 are a common feature of more than 70% of grade II−III gliomas and secondary glioblastomas, 20−30% cytogenetically normal acute myeloid leukemia, and a variety of other malignancies at lower frequencies. These mutations are all heterozygous and occur at amino acid residues of the IDH substrate binding site, resulting in loss of the enzymes' ability to catalyze conversion of isocitrate to ±-ketoglutarate, and gain of function of a neoactivity to catalyze the NADPH-dependent reduction of ±-ketoglutarate to R(−)-2-hydroxyglutarate (2-HG). Elevated levels of R(−)-2-HG are found in tumors of malignant gliomas, and in malignant cells and serum of AML patients, that harbor IDH mutations. In addition, patients with a rare inherited neurometabolic disorder, 2-hydroxyglutaric aciduria, exhibit elevated levels of 2-HG in their CNS and are predisposed to the development of gliomas. Altogether these findings suggest the hypothesis that 2HG functions as an oncometabolite, and that the excess 2HG which accumulates in vivo may contribute to the formation and progression of cancers. We also demonstrate here that 2-HG metabolite is a tractable metabolic biomarker of mutant IDH enzyme activity in clinical samples. It is possible that small molecule inhibitors of mutant IDH enzymes may have therapeutic applicability in multiple cancers harboring IDH mutations.

161 POSTER

Localisation and characterisation of ET-1 binding to human colorectal cancers and evaluation of the orally active ETA receptor antagonist zibotentan (ZD4054)

S. Haque[1], M. Loizidou[1], X. Shiwen[2], D. Abraham[2], N. Farooqui[1], H. Welch[1], O. Ogunbiyi[1], J. Growcott[3], M. Dashwood[4]. *[1]University College London, Department of Surgery, London, United Kingdom; [2]University College London, Centre for Rheumatology and Connective Tissue Disease, London, United Kingdom; [3]AstraZeneca, Cancer & Infection Discovery Medicine, Manchester, United Kingdom; [4]University College London, Department of Clinical Biochemistry, London, United Kingdom*

Background: Endothelin-1 (ET-1) acts via two endothelin receptors, ET_A receptor and ET_B receptor. ET-1 and ET_A receptor, which promote cancer growth and progression, are overexpressed in colorectal cancer tissues. We investigated the distribution of ET_A receptor and ET_B receptor in patient tissue sections. The affinity (K_d) and receptor density (B_{max}) of ET-1 was determined in whole tissue homogenates and colorectal fibroblasts. In addition the effect of the orally active ET_A receptor specific antagonist zibotentan (ZD4054) on ET-1 receptor binding (IC_{50}) was evaluated against subtype selective laboratory compounds.
Material and Methods: ET-1 receptor distribution and binding characteristics (K_d; B_{max}) were determined using *in vitro* autoradiography on patient sections, whole tissue homogenates and primary fibroblasts isolated from human colon tissues. Immunohistochemistry (IHC) was used to identify fibroblasts, endothelial cells and surrounding collagen type XI.
Results: ET-1 binding to cancer and normal colon tissue had similar characteristics. However there was greater ET_A receptor than ET_B receptor binding in colorectal cancer sections. Within both cancer and normal tissues, the strongest binding was to stromal cells, in particular fibroblasts, confirmed by immunohistochemistry. Further characterisation performed on primary fibroblasts revealed high density and affinity ET-1 binding in these cells (Bmax 3.03 ng/mg and K_d 213.6). Inhibition studies showed ET_A receptor antagonists (BQ123; zibotentan) were more effective at reducing ET-1 binding (IC_{50} values 0.1 μM, 10 μM respectively) than the ET_B receptor antagonist BQ788 (IC_{50}; 1 mM).
Conclusions: ET-1 binds strongly to receptors within colon cancer stroma structures, such as cancer-associated fibroblasts and endothelial cells, and is consistent with ET-1 signalling contributing to colorectal cancer growth, desmoplasia and neovascularisation. Furthermore, we have demonstrated that the orally active ET_A receptor antagonist zibotentan reduces ET-1 binding to colorectal cancer tissues. This study provides further evidence for the potential therapeutic use of the specific ET_A receptor antagonist zibotentan as an adjuvant treatment for colorectal cancer.



Tumour sections: Haematoxylin & eosin staining (left); ET-1 binding to colorectal cancer using autoradiography (right).

162 POSTER

MEK162 (ARRY-162), a novel MEK 1/2 inhibitor, inhibits tumor growth regardless of KRas/Raf pathway mutations

S. Winski[1], D. Anderson[1], K. Bouhana[1], R. Impastato[1], R. Woessner[1], J. Zuzack[1], B. Tunquist[1], J. Garrus[1], T. Pheneger[1], P. Lee[1]. *[1]Array BioPharma Inc., Pharmacology, Boulder CO, USA*

MEK1/2, a dual specific kinase, is downstream of both Ras and Raf and required for the activation of ERK1/2. Mutated, oncogenic forms of Ras and Raf are commonly found in cancer and are implicated in uncontrolled cell proliferation. Tumors which harbor these mutated forms are reported to be highly sensitive to MEK inhibition. Interestingly, in a survey of 15 xenograft models, the greatest efficacy of MEK162 was observed in models which did not harbor these activating mutations. For example, treatment of BxPC-3 pancreatic carcinoma, CRC13B2 colon carcinoma, HT1080 fibrosarcoma and NCI-H1975 NSCL carcinoma (EGFR T790M) resulted in varying degrees of anti-tumor activity, including partial and complete regressions, in response to daily administration of 30–100 mg/kg MEK162. These models were chosen for further study into the underlying mechanisms of MEK antitumor activity in the absence of Ras/Raf mutation.
MEK inhibitors are reported to affect angiogenesis, through direct effects on endothelial cell proliferation, and tumor cell apoptosis, through increasing the pro-apoptotic protein BIM. The anti-angiogenic effect of MEK162 was first examined in an *in vivo* vascular endothelial cell growth factor (VEGF)- and basic fibroblast growth factor (bFGF)-induced matrigel invasion assay. MEK162 was a highly potent inhibitor of neoangiogenesis (100% inhibition, 10 mg/kg daily administration) and was equally efficacious as other known angiogenesis inhibitors (sunitinib, axitinib). Investigation of angiogenesis endpoints in xenografts, however, did not support a role for angiogenesis in the activity of MEK162. Established tumors from the above models were treated with 100 mg/kg MEK162 (5 days) and examined for VEGF and microvessel density (CD31 staining). No significant decreases were observed in these markers. Western blot analysis of tumor lysates confirmed that MEK was potently inhibited, as evidenced by profound pERK inhibition, and that BIM levels were increased. Taken together, these data suggest that while MEK162 is a potent inhibitor of neoangiogenesis, effects on established vascular systems are more complex and that stimulation of pro-apoptotic pathways may be the major contributor to the potent anti-tumor activity observed *in vivo*. These data further support the preclinical investigation of the effects of MEK inhibitors on apoptotic protein expression and the clinical investigation of MEK inhibitors in tumors with both wild-type and mutated Ras/Raf pathways.