298 POSTER

Oxaliplatin induces the expression of genes involved in capecitabine activation: preliminary results of a pharmacodynamic study in esophageal cancer

L. Pendyala[1], G. Wilding[2], P. Smith[3], R. Iyer[4], H. Nava[5], C. Nwogu[6], M. Javle[7]. *[1]Roswell Park Cancer Institute, Medicine, Buffalo, USA; [2]Roswell Park Cancer Institute, Biostatistics, Buffalo, USA; [3]Roswell Park Cancer Institute, Pharmacology, Buffalo, USA; [4]Roswell Park Cancer Institute, Medicine, Buffalo, USA; [5]Roswell Park Cancer Institute, Surgery, Buffalo, USA; [6]Roswell Park Cancer Institute, Surgery, Buffalo, USA; [7]Roswell Park Cancer Institute, Medicine, Buffalo, USA*

Background: Capecitabine (CAP) is activated to 5-fluorouracil (5FU) in 3 enzymatic steps involving carboxylesterase (CE), cytidine deaminase (CDA) and thymidine phosphorylase (TP). Our *in vitro* studies indicate that Oxaliplatin (OXP) induces CE gene expression. 5FU, OXP and radiation (XRT) is an effective neoadjuvant regimen for esophageal cancer (EC) (J Clin Oncol 20: 2844, 2002). Study aims: Explore if OXP induces expression of genes related to CAP activation in tumor biopsies (TB) and peripheral blood mononuclear cells (PMN) and correlate these changes with OXP pharmacokinetics (PK) and response in a phase I neoadjuvant study of OXP, CAP and XRT for EC.
Materials and Methods: OXP (85 mg/m^2) administered on days (D) 1, 15 and 29. CAP dose levels (DL) I: 1000, II: 1250 or III: 1500 mg/m^2 in 2 divided doses, Mon-Fri, weekly with XRT (1.8 Gy daily $\times$28). CAP + XRT started on D3. Expression of CE, CDA and TP genes in TB and PMN was evaluated before treatment and on D2 (24 hrs post-OXP) using real time QRT-PCR with comparative C_T method. After chemoradiation, patients (pts) underwent esophagectomy. Platinum in plasma ultrafiltrate was measured using Atomic Absorption Spectrophotometry and PK parameters were derived using WINNONLIN. Statistical techniques included were the signed rank test and the Spearman rank correlation.
Results: 16 pts were treated (3 at DLI, 6 at DLII and 7 at DLIII). 8 underwent esophagectomy: 3 complete responses, 2 microscopic residual disease (<1 cm), 3 downstaged. 3 pts progressed. TB and PMN gene expression data were available for 15 pts. CDA and TP had similar level of expression in TB and PMN; CE had lower expression in PMN ($p < 0.01$). No correlation noted between TB and PMN gene expression. Induction of one or more of the 3 genes was noted in TB in several pts. The changes in gene expression did not correlate with the PK of oxaliplatin. PMN did not show the same trend in expression changes as the TB. CE expression in D2 TB and the fold increase in its expression from the pretreatment showed significant correlation to response ($p \leqslant 0.03$). TP expression in D2 TB was also related to response ($p = 0.03$). Induction of one or more genes by OXP appears to be associated with response and lack of induction with PD.
Conclusions: OXP induces the expression of genes involved in CAP activation to 5FU in EC and this induction may be related to response. PMN do not serve as a surrogate tissue for studying expression changes for these genes.

299 POSTER

Randomized phase II trial of irofulven/prednisone, irofulven/capecitabine/prednisone, or mitoxantrone/prednisone in hormone refractory prostate cancer (HRPC) patients failing first-line docetaxel: preliminary results

L. Hart[1], J. Hainsworth[2], T. Ciuleanu[3], S. Oudard[4], E. Berger[5], J. Alexandre[6], K. Chi[7], D. Ruether[8], C. Kahatt[9], J. MacDonald[10]. *[1]Florida Cancer Specialists, Florida, USA; [2]Sarah Cannon Cancer Center, Nashville, USA; [3]Ion Chiricuta Institute of Oncology, Cluj, Romania; [4]Hôpital Européen Georges Pompidou, Paris, France; [5]North Shore Hematology/Oncology, East Setauket, USA; [6]Hôpital Cochin, Paris, France; [7]British Columbia Cancer Agency, Vancouver, Canada; [8]Tom Baker Cancer Centre, Calgary, Canada; [9]CAC Oncology, Le Kremlin-Bicêtre, France; [10]MGI PHARMA, Inc, Bloomington, USA*

Background: First-line docetaxel-based chemotherapy shows a survival benefit for HRPC patients (pts), although little data exist regarding post-docetaxel second-line chemotherapy. Based on evidence of irofulven (IROF) activity in HRPC observed in prior Phase I/II studies, a randomized Phase II study in docetaxel-refractory HRPC pts was initiated using IROF/prednisone (P) or IROF/capecitabine (Cap)/P versus mitoxantrone (M)/P.
Methods: Between Jun-04 and Jan-06, pts with histologically-proven metastatic HRPC who progressed by RECIST or PSAWGR criteria during or within 3 months of completing prior docetaxel treatment were randomized to 1 of 3 treatments in a 2:2:1 ratio: *Arm A*: IROF (0.45 mg/kg, Day [D]1, 8 every [q] 3 weeks [w]) and P (10 mg po daily); *Arm B*: IROF (0.4 mg/kg D1, 15), Cap (2000 mg/m^2 D1−15 q4w) and P; *Arm C*: M (12 mg/m^2 q3w) and P. Pts had adequate hematologic and organ function, Karnofsky performance status (KPS) $\geqslant$70%, and were stratified by baseline pain status. Primary endpoint was time to progression (TTP); secondary endpoints included survival, PSA response, pain response and safety assessment.
Results: As of May-06, 134 pts from 34 centers in 9 countries were randomized and treated (Arm A/B/C: 53/54/27). Median age for each group was 64/67/63 years, KPS $\geqslant$80% for each group was 79%/94%/59% of pts, median baseline PSA (ng/mL) 144/136/243, disease-related pain at baseline 61%/58%/63%; other characteristics, including metastatic site distribution, were comparable among arms. *Efficacy*: With a median follow-up of 9.7 months (range 1.7–22.6), progression was reported in 84% of pts and 49% have died across all treatment groups.
Safety: 129 pts were evaluated. Median cycles/pt (A/B/C) 3/2/3; most common Gr 3–4 toxicities (% pts): asthenia (6%/10%/0%), vomiting (2%/12%/0%) and diarrhea (4%/6%/0%). The most common laboratory Gr 3–4 abnormalities were neutropenia (17%/10%/44%) and thrombocytopenia (15%/14%/4%).

	Arm A, IROF/P N = 53	Arm B, IROF/Cap/P N = 54	Arm C, M/P N = 27
PSA decrease >50% or PR (RECIST)	5 (9%)	11 (20%)	1 (4%)
Stable disease >12 w	11 (21%)	7 (13%)	4 (15%)
TTP (months), Median (95% CI)	1.9 (1.4–2.4)	2.1 (1.8–2.4)	1.0 (0.5–1.6)
Overall survival (months), Median (95% CI)	10.7 (7.6−13.8)	10.2 (6.3−14.1)	7.2 (2.3−12.2)

Conclusion: Preliminary results suggest a longer survival and TTP, a higher PSA response, and an acceptable safety profile for IROF/P and IROF/Cap/P compared to M/P. Based on these data, IROF may have a role in treating docetaxel-resistant HRPC pts.

300 POSTER

Phase I study of an oral isotype-selective histone deacetylase (HDAC) inhibitor in patients (pts) with advanced solid tumors

L.L. Siu[1], M. Carducci[2], E.X. Chen[1], M. Maclean[1], R. Pili[2], S. King[2], T. Patterson[3], Z. Li[3], A. Kalita[3], G.K. Reid[3]. *[1]Princess Margaret Hospital, University Health Network, Medical Oncology and Hematology, Toronto, Canada; [2]Sidney Kimmel Cancer Center at Johns Hopkins, Medical Oncology, Baltimore, USA; [3]MethylGene Inc., Montreal, Canada*

Background: MGCD0103 is a unique non-hydroxamate, orally available inhibitor of human HDACs. Deregulation of HDAC activity is associated with malignant diseases in humans and small molecule inhibitors of HDACs have emerged as a novel therapeutic class of molecules with anticancer potential. Their proposed mechanism of anticancer activity is via regulation of aberrant gene expression at the transcriptional level, leading to inhibition of proliferation, induction of apoptosis and/or cell differentiation in cancer cells.
Methods: A phase I trial of MGCD0103 given intermittently as an oral dose 3$\times$/weekly, 2 weeks out of 3 is being conducted in pts with advanced solid tumors. Objectives included: safety, tolerability, pharmacodynamic (PD) and pharmacokinetic (PK) evaluation of HDAC activity.
Results: Six dose levels have been evaluated: 12.5, 20, 27, 36, 45 and 56 mg/m^2. 34 pts with the following demographics have received MGCD0103: M:F = 22:12, median age (range) = 59 (29−75). From 28 pts the following are known: ECOG 0:1:2=10:17:1; primary tumor sites: colorectal (8), renal (5), lung (4), others (11); prior chemotherapy, radiotherapy and immunotherapy were given to 22, 12 and 2 pts respectively. A total of 65 cycles have been administered (N = 28) mean = 2.3 and range = 1–11. Most common adverse events in pts (N = 27) are: Grade 1–3 fatigue 18 pts (67%), nausea 13 pts (48%); Grade 1–2 vomiting, 8 pts (30%) and anorexia, 6 pts (22%). MTD has been reached. PD evaluations include inhibition of HDAC activity and induction of H3 histone acetylation. The portion of pts with >20% inhibition of HDAC activity increased with dose as compared to baseline levels and induction of histone acetylation exceeded 50% in a majority of pts (3 out of 5) at 45 mg/m^2. Terminal $t_{1/2}$ in plasma was found to be approximately 9 hrs and duration of the PD effects persisted for 72 hrs after dosing, supporting this intermittent dosing regimen. Stable disease beyond 2 cycles has been achieved in 4 pts to date. Serum IL-6 levels may correlate with fatigue encountered on study.
Conclusion: Evaluations indicate MGCD0103 can be safely administered on a 3$\times$/weekly 2 weeks out of 3 schedule to pts with solid tumors.

Preliminary PD data suggest that MGCD0103 can inhibit HDAC activity in a dose-dependent manner, and induce histone acetylation in peripheral blood mononuclear cells. Both these effects have been shown to persist for 72 hours following dosing.

301 POSTER

The design, synthesis and biological evaluation of a set of C2-aryl substituted pyrrolo[2,1-c][1,4]benzodiazepine dimers

P.W. Howard[1], Z. Chen[1], S.J. Gregson[1], M. Stephenson[1], S. Klee[1], J.M. Hartley[2], G. Subur[2,3], J.A. Hartley[1], D.E. Thurston[1]. *[1]Spirogen Ltd, London, United Kingdom; [2]University College London, Department of Oncology, London, United Kingdom; [3]The School of Pharmacy, Dept of Pharmaceutical & Biological Chemistry, London, United Kingdom*

The pyrrolobenzodiazepines (PBDs) are a family of naturally occurring antitumour antibiotics. Many of the most potent family members contain an *endo-exo* unsaturated motif associated with the pyrrolo C-ring of the PBD.

We have previously reported the synthesis and potent *in vitro* and *in vivo* antitumour activity of SG2285, a PBD dimer which retains the *endo-exo* unsaturation motif in the form of a C2-aryl substituent conjugated to a 2,3 double bond. We now report the synthesis and biological evaluation of a set of 10 analogs with diverse C2-aryl substituents. These C2-aryl substituents were selected on the basis of the biological evaluation of approximately 80 C2-aryl PBD monomers.

The novel dimers were prepared from a key enol triflate PBD intermediate by Pd(0) catalysed coupling with the appropriate aryl boronic acids. Removal of the N10 Troc protecting group afforded the free PBD imines, which were converted to their bisulphite adducts in order to improve water solubility and modulate the DNA-reactivity of the PBD unit.

The C2-aryl PBD dimers were evaluated in the K562 human leukaemia cell line (96 hrs continuous exposure) with IC_{50} values in the range 0.02−43 nM. The 3-methoxyphenyl analog (SG2965) and the 3,4-benzodioxole analogs (SG2962 and 2965) were particularly potent with IC_{50} values of 20, 80 and 50 pM, respectively. These molecules were also found to be efficient DNA interstrand cross-linking agents in both plasmid and cellular DNA. In the case of the bisulphite adducts, the cross-links were found to form more slowly in cells reaching a maximum at approximately 24 hrs.

These studies confirm the potent activity of C2-aryl PBD dimers of this type, and SG2285 is currently undergoing preclinical development.

302 POSTER

Processing of 1-nitroacridine-induced DNA-DNA cross-links by topoisomerase I is associated with enhanced cellular survival: a possible role of topoisomerase I in the removal of DNA cross-links

K. Lemke[1], A.K. Larsen[2], A. Skladanowski[1]. *[1]Gdansk University of Technology, Pharmaceutical Technology adn Biochemistry, Gdansk, Poland; [2]Hôpital Saint-Antoine, INSERM U673 and Université Pierre et Marie Curie, Paris, France*

1-nitroacridines like nitracrine (Ledakrin) are potent antitumor agents with clinical activity toward ovary, lung and breast cancers. These compounds are activated by bioreduction and bind covalently to DNA. This is accompanied by formation of DNA cross-links and covalent DNA-protein complexes. Earlier studies showed that 1-nitroacridines could be activated by thiols *in vitro* to reactive species which bind DNA and proteins. On the other hand, DNA topoisomerases have been indicated in the processing of DNA adducts. We here show that modification of plasmid DNA with nitracrine and, to a lesser degree, the nitracrine derivative C-857 inhibited the catalytic activity of purified topoisomerase I in a dose-dependent manner. The inhibition is associated with the induction of DNA single strand breaks and the formation of covalent DNA-topoisomerase I complexes. In contrast, no detectable effects were observed for purified human topoisomerase IIα. Further studies revealed that both nitracrine and C-857 form unusual DNA cross-links between different DNA molecules. Unexpectedly, the high molecular weight cross-linked DNA formed in the presence of nitracrine, but not by C-857, was completely resolved after further incubation with topoisomerase I. Accordingly, the In Vivo link assay revealed the formation of covalent DNA-topoisomerase I complexes in LNCaP cells after treatment with nitracrine but not with C-857. DNA cross-linking was accompanied by the formation of double stranded DNA breaks that were particularly pronounced for cells treated by C-857 suggesting that the topoisomerase I-mediated processing of the cross-linked DNA may play a role in DNA repair. In agreement, cells with decreased topoisomerase I levels showed increased sensitivity to nitracrine but unchanged sensitivity to C-857. In conclusion, our results suggest a novel role for topoisomerase I in the removal of DNA-DNA cross-links which is accompanied by increased cellular survival.

303 POSTER

Additive and synergistic effects of irofulven and capecitabine in human prostate cancer cells

M. Serova[1], A. Ghoul[2], S. Faivre[3], E.S. Van Laar[4], S.A. Wadman[4], S.J. Waters[4], F. Lokiec[2], E. Cvitkovic[5], F. Calvo[1], E. Raymond[3]. *[1]U716 Laboratory of pharmacology, IGM Saint-Louis Hospital, Paris, France; [2]Department of Clinical Pharmacology, Rene Huguenin Cancer Center, Saint-Cloud, France; [3]Department of Medical Oncology, Beaujon Hospital, Clichy, France; [4]MGI Pharma, Bloomington, MN, USA; [5]CAC Oncology, Le Kremlin-Bicêtre, France*

Background: Irofulven (6-hydroxymethylacylfulvene, MGI 114) is a novel DNA-interacting anticancer drug derived from the mushroom natural product, illudin S. Irofulven displays a broad range of activity against human tumors in vitro and in vivo and is currently under study in clinical trials as a single agent and in combination with several other anticancer drugs. To optimize the clinical use of irofulven, the present study examined the cytotoxic effects of combining irofulven with 5′-DFUR or 5-FU, the active metabolite of capecitabine, in human prostate cancer cells.

Materials and Methods: Antiproliferative effects of irofulven, 5′-DFUR and 5-FU were evaluated in two prostate cancer cell lines, PC3 and DU145, with different expression levels of thymidine phosphorylase (TP), a key enzyme for capecitabine metabolism. Drug interaction studies were performed using isobolograms according to the method of Chou & Talalay.

Results: Single agent irofulven produced cytotoxic effects against human PC3 and DU145 prostate cancer cells with IC50s of 4.2+0.9 μM and 1.4+0.6 μM, respectively. Sensitivity to 5′-DFUR was directly correlated with TP expression level. PC3 cells expressed less TP and were less sensitive to 5′-DFUR than DU145 (IC50s of 62 and 33 μM, respectively). Combination of irofulven with 5′-DFUR produced additive/synergistic activity over a broad range of concentrations in both PC3 and DU145 cells, and similar effects were observed when irofulven was combined with 5-FU. While there was no clear schedule dependency for the tested combinations, both cell lines showed a trend favoring 5-FU/5′-DFUR exposure prior to irofulven. Cell cycle analysis showed consistent outcomes for 5′-DFUR and 5-FU with accumulation of cells in the S-phase of cell cycle, while combinations with irofulven were associated with cell cycle blockage in G1/S. Irofulven induced cellular apoptosis as a single agent, however, in combination with either 5′-DFUR or 5-FU the observed apoptosis was markedly increased.

Conclusion: Irofulven displays additive/synergistic anti-proliferative effects when combined with 5′-DFUR and 5-FU over a broad range of concentrations in human prostate cancer cells. Cell cycle arrest in S-phase and apoptosis appear as primary mechanisms of cytotoxicity of irofulven-based combinations. Based on these data, the irofulven-capecitabine combination should be further explored using a schedule that preferably gives capecitabine prior to irofulven.

304 POSTER

Combination treatment of new molecular-targeted therapies and the DNA minor groove binder brostallicin

C. Geroni, E. Pesenti, D. Ballinari, M. Ciomei, V. Croci, A. Marsiglio. *Nerviano Medical Sciences, Oncology, Nerviano, Italy*

Brostallicin is a DNA minor groove binder, currently in Phase I/II trials as single agent or in combination. It has antitumor effect and strong pro-apoptotic activity in experimental tumors. The mechanism of DNA interaction is novel, since it binds covalently to DNA only in the presence of glutathione and glutathione S-transferase (GSH/GST). As a consequence, brostallicin is more effective on tumors expressing relatively high levels of GSH/GST. Multiple combinations of brostallicin with “classical” antitumor drugs have been previously studied; synergy was observed with cisplatin, gemcitabine and irinotecan. Based on the role of combination chemotherapy in cancer treatment, and its importance for the efficacy of newer therapies, we evaluated whether brostallicin could synergize with novel molecular-targeted dugs. In this study we examined the effects of combining brostallicin and different molecular-targeted agents (such as erlotinib or gefitinib or imatinib) on different experimental tumors. In vitro and in vivo studies were performed on tumors cells sensitive to brostallicin; for each combination, tumor cells sensitive to the kinase inhibitor were selected. In vitro cells were treated with increasing doses of brostallicin and/or the kinase inhibitor for 72 h. At the end of treatment, cell proliferation of treated and control cells was determined by a cellular ATP monitoring system. In vivo, DU145 human prostate carcinoma, A549 human lung carcinoma, HCT-116 human colon carcinoma and K562 human AML models, transplanted in nude mice were used to determine the effect of brostallicin/molecular targeted agents combinations. Drugs were administered at their best schedule and route and the simultaneous treatment was used for all the tested combinations. The effect of the antitumor treatment was determined as the delay, in days, in the onset