P.201 SUSCEPTIBILITY OF MANTLE CELL LYMPHOMA (MCL) AND NON-SMALL CELL LUNG CANCER CELL LINES TO TARGETING BCL-2 USING THE SMALL MOLECULAR INHIBITOR, GX15-070

Introduction: Mantle cell lymphoma (MCL) consistently over expresses bcl-2 despite not carrying the t(11;14) while 40-60% of non-small cell lung cancer (NSCLC) tumors also over-express the protein. The attenuation of apoptosis by bcl-2 is thought to contribute to the malignant process and increase resistance to some cytotoxic agents. GX15-070 is a small molecular inhibitor of the Bcl3 binding groove of bcl-2. We set out to assess the effect of this agent on the viability of a series of MCL and non small cell lung cancer cell lines.

Methods: NSCLC (H460 and H1299) and MCL (Granta 519, NCI-H1, Z138C, H112, VM2) cell lines previously characterized in detail were used. Bcl-2 expression was assessed at both protein and mRNA levels. Cells were grown in standard media and exposed to a range of concentrations of GX15-070. The dose-response curve was assessed by measuring viability at 24 and 48 hours using the MTT assay for the adherent NSCLC cell lines and WST-1 assay for the non-adherent MCL cell lines.

Results: All five MCL and both NSCLC cell lines over-expressed bcl-2 by western blot. In two MCL cell lines bcl-2 is over-expressed due to bcl-2 gene amplification. All five MCL cell lines showed sensitivity to GX15-070 at a range of concentrations. On the other hand the two NSCLC cell lines at 48 hours exhibited inhibited resistance to this agent at all concentrations tested with only a minor reduction in viability at the highest concentration.

Discussion: Our results demonstrate that using GX15-070 to target bcl-2 is a very effective anti neoplastic approach in MCL cell lines but less effective in the NSCLC cell lines. This suggests that the MCL phenotype is more dependent on bcl-2 expression and function than the NSCLC phenotype. The specific mechanisms of bcl-2 over-expression do not seem to determine sensitivity to GX15-070. The profound cytotoxicity of the MCL cell lines to GX15-070 warrants further investigation in vivo in animal models and clinical trial based settings. Work assessing the utility of combining this agent with other cytotoxic agents is currently ongoing and will be presented.

P.202 CHARACTERIZATION OF SMALL MOLECULAR WEIGHT INHIBITORS OF POLO-LIKE KINASE-1

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Polo-like kinase 1 (Plk-1) is a serine/threonine protein kinase involved in the entry into, progression through and exit from mitosis with roles in centrosome maturation, bi-polar spindle formation, chromosome separation and cytokinesis. Plk-1 belongs to the Plk family, comprising the structurally related Plk-1,-2, -3 and -4 proteins, that are characterized by having an n-terminal kinase domain and a c-terminal ‘polo-box’ domain involved in protein-protein interactions. Plk-1 is ubiquitously expressed in normal tissues and is over-expressed in a variety of human tumours including lung, colon, stomach, breast, ovary, head and neck, and melanomas and its over-expression often correlates with poor prognosis. Depletion of Plk-1 by siRNA in tumour cells leads to, amongst other things, the inhibition of centrosome maturation resulting in a mitotic block and eventually apoptosis. Further, interference with Plk-1 expression in mouse xenograph tumour models by injection of Plk-1 anti-sense oligonucleotide into the tail vein leads to tumour regression, with no observable animal toxicity. Taken together, these observations indicate that Plk-1 is potentially a target for cancer therapy. The strategy of developing ATP mimetic inhibitors as therapeutics for the inhibition of protein kinases in the treatment of cancer has recently had success with the approval of Gleevec for the treatment of AML. Further, ATP mimetic inhibitors directed towards several other protein kinases, including, Aurora, Cdk1/2, IKKgb, Raf, VEGFR/2 I/2, JAK, PDGFR/Kit, PI3 and PKCs are currently undergoing clinical evaluation in the cancer setting. In order to identify ATP-mimetic inhibitors of Plk-1, we screened a kinase-directed chemical library against recombinant Plk-1 and identified several chemical classes with good potency. The characterization of these compounds will be presented. FACS analysis of potent and selective PIK-1 inhibitors shows that tumour cells arrest with an S phase DNA content and apoptosis after a long mitotic block. Consistent with Plk-1 ablation data, cells appear arrested in prometaphase/metaphase as judged by western blot analysis of mitotic markers, time-lapse photography and immunocytochemistry. Further, these compounds have potent antiproliferative effects on a variety of tumoral cell lines. These preliminary data indicate that the inhibition of Plk-1 could be a useful approach in the treatment of a wide range of human cancers.

Kinesins are eukaryotic microtubule-associated motor proteins. There are over 40 known kinesins, approximately 15 of which are closely associated with mitosis. Kinesin spindle protein (KSP) is a mitotic kinesin that is an early player in mitosis (prophase / prometaphase). It plays a key role in spindle pole separation and production of the bipolar spindle, as well as centrosome separation and maturation. Moreover, KSP is expressed predominately in proliferating cells and is absent from postmitotic neurons, and thus presents the opportunity for development of a novel anti-mitotic therapy potentially alleviating the hallmark side effects of traditional ‘microtubule disruptors’. (taxanes and vinca alkaloids), which are limited clinically by peripheral neuropathy. To this end, reported studies with small molecule KSP inhibitors have demonstrated tumor growth inhibition without peripheral neuropathy. We report here the in vitro and in vivo characterization of a potent KSP inhibitor: ARRY-649. ARRY-649 is a member of a series of KSP inhibitors discovered and optimized by structure-based design. ARRY-649 is exceptionally potent with cellular IC50’s as low as 300 nM in mechanistic assays and 200 pM in functional assays, respectively. Enzymatic kinetic analysis demonstrates ARRY-649 is uncompetitive with respect to ATP and noncompetitive with respect to tubulin. Cellular imaging studies show that the normal mitotic spindle configuration can be disrupted, with formation of monopolar spindles, with as little as a single digit nanomolar concentrations of ARRY-649. Additionally, ARRY-649 has shown the ability to inhibit histone (H3) phosphorylation in tumor xenograft studies, and has demonstrated excellent efficacy in xenograft studies. Notable, ARRY-649, at its maximally tolerated dose of 5 mg/kg, i.p. on a Q4x3 schedule caused dramatic tumor regression and complete responses in established HT-29 xenografts. In this HT-29 xenograft model, ARRY-649 was also able to shrink very large tumors (> 800 mg). This work clearly establishes that ARRY-649 is one example of a novel series of KSP inhibitors with excellent therapeutic potential.

P.204 ANTI-TUMOUR EFFECT OF CELECOXIB IN GlioBLASTOMA CELLS: INDUCTION OF DNA DAMAGE LEADING TO G1 CELL CYCLE ARREST DEPENDENT ON P53 ACTIVATION

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Aims: Cyclooxygenase (COX) inhibitors have shown efficacy against a range of tumours. We sought to define the underlying antineoplastic mechanisms of celecoxib (a selective COX-2 inhibitor) in human glioblastomas, by determining whether celecoxib induces DNA damage, leading to cell cycle arrest and/or apoptosis, and whether these effects are dependent on p53 activation.

Methods: We tested the anti-tumour effect of celecoxib on cell viability of U87 (contains wild-type p53) and U251 (contains mutant p53) glioblastoma cells by [3H]-thymidine incorporation assay, respectively. Activation of p53 upon DNA damage by celecoxib was verified by analysing total p53 and phospho-p53 Ser15 (which is sensitive to DNA damage) protein expression. The consequence of DNA damage by celecoxib on cell cycle progression dependent on functional p53 was analysed by flow cytometry (FACS) analysis on propidium iodide-stained U87, U251 and E6 (which degrades p53)-transfected U87 cells. Induction of G1 cell cycle arrest by celecoxib was verified by measurement of p21 mRNA expression. The effect of celecoxib on cell apoptosis dependent on p53 was analysed by FACS analysis on Annexin V-FITC-stained U87 and U251 cells.

Results: The EC50 of celecoxib was 130.6, 101.7, 85.6 uM (in U87 cells) and 164.8, 90.2, 79.9 uM (in U251 cells), at 24, 48 and 72 hours, respectively. 5 and 24 hours post treatment significantly arrested U87 cells at G1 phase (66.2 ± 2.6% cell population versus 56.1 ± 1.3% in non-treated controls), which corresponded with increased p21 mRNA expression. Cell cycle progression was not affected by celecoxib in U251 and E6-transfected U87 cells, which contained low amount of functional p53. Celecoxib (30 μM, 48 hours) significantly increased cell apoptosis in U251 cells (36.3 ± 1.1% cell population versus 27.8 ± 1.0% in controls), but not in U87 cells.

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Conclusion: Detailed understanding of celcoxib anti-tumour mechanisms holds promise for novel therapeutic approaches to glioblastoma multiforme. Our study showed that celcoxib reduced cell viability by induction of DNA damage and inhibition of DNA synthesis in human glioblastomas, leading to G1 cell cycle arrest dependent on p53 activation.

P.205 SCHEDULE-DEPENDENT ANTITUMOR ACTIVITY OF YM155, A NOVEL SMALL MOLECULE SURVIVIN SUPPRESSANT, IN EXPERIMENTAL HUMAN HORMONE REFRACtORY PROSTATE CARCINOMA XENOGRAFT MODELS


YM155 is a small molecule survivin suppressant which induces the downregulation of survivin and demonstrates potent antitumor activities in experimental human hormone refractory prostate cancer (HRPC) models (Proceedings AACR, NC-I-BORTC, 2005, 2803). Survivin, a new member of the inhibitor of apoptosis (IAP) gene family, is a protein that inhibits apoptosis and regulates cell division. Survivin expression in clinical tumors has been shown to correlate with poor prognosis and low survival of patients. Therefore, survivin represents a promising new target for cancer therapy. In this study, schedule dependence of YM155 measured antitumor activity was investigated in an experimental animal model. YM155 was administered as a fixed total dose to the PC-3 xenografted mice once-daily i.v. bolus 3 times a week or intra-tumoural injection, to compare the antitumor activities among the schedules. YM155 administered as a once-daily i.v. bolus showed significant inhibition of tumor growth. When the same total dose was divided and administered as a 3 times daily i.v. bolus or as an i.m. injection, the antitumor activity of YM155 was significantly more potent than that of the once-daily bolus injection. The antitumor activities of YM155, paclitaxel, cisplatin and doxorubicin in the same animal model were also evaluated. YM155 was administered as 6-day and 3-day infusions once for 2 weeks, or once or i.v. bolus for 5 times for 2 weeks. Paclitaxel and cisplatin were administered daily by i.v. bolus 5 times weekly for 2 weeks. Doxorubicin was administered by i.v. bolus once weekly for 2 weeks. YM155 administered as an i.v. bolus showed maximally 64% tumor growth inhibition. With the YM155 infusion, the activity was enhanced and massive tumor regressions (>100% tumor growth inhibition) were observed. The 6-day infusion and 3-day infusion once for 2 weeks were found to be optimal in showing maximum time-dependent efficacy. When compared with other conventional chemotherapeutic agents, YM155 infusion showed significant and dose-dependent antitumor activity below its maximum tolerated dose (MTD), while cisplatin and doxorubicin were only active at the MTD. Paclitaxel was almost as efficacious as YM155 at the MTD, but this efficacy was accompanied by a significant body weight decrease. In conclusion, YM155 shows time-dependent antitumor activities, and the continuous infusion of YM155 is associated with higher efficacy but no increase in toxicity. Further investigations of YM155 in pre-clinical models of various tumor types would be worthwhile for the development of a novel survivin-targeted therapeutic approach. A phase II, open-label study of YM155 with 7-day continuous i.v. infusion in HRPC patients is currently being conducted.

P.206 ANTISENSE-MEDIATED ML-IAP DOWN REGULATION SENSITIZES MELANOMA CELLS TO CISPLAtIN

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Malignant melanoma is an aggressive form of skin cancer which is highly resistant to conventional therapies. The melanoma inhibitor of apoptosis protein (ML-IAP) is a potent inhibitor of apoptosis and strongly upregulated in melanoma cells but undetectable in most normal tissues including melanocytes. We designed 20-mer antisense oligonucleotides that efficiently downregulated ML-IAP expression and sensitized melanoma cells to cisplatin, which may have therapeutic potential in treatment of chemoresistant melanoma.

P.207 DEVELOPMENT OF SRNA-BASED METHOD FOR ENHANCING OF TUMOUR CELL GROWTH

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Cancers often have upregulated or inappropriately expressed genes that lead to uncontrolled cell growth. These include e.g. genes coding for proteins involved in cell cycle control, signalling pathways, adhesion events or apoptosis. Such genes may serve as possible targets for anticancer therapies orientated specifically towards tumour cells.

siRNAs (small-interfering RNAs) are 21–23 nucleotide, double-stranded RNA that mediate RNA interference. The term of RNA interference refers to post-transcriptional gene silencing based on degradation or translation arrest of target RNA. Gene-silencing activity mediated by siRNAs has been demonstrated in mammalian cells, where siRNAs have shown great specificity and efficacy in silencing of target mRNAs through a base-paring-dependent mechanism.

The aim of our studies was to examine the influence of a number of siRNAs directed against specific molecular targets on the proliferation rate of human prostate cancer cells LNCaP. These included e.g. wnt1, TR3, bcl-2, HGF, checkpoint, akt1, TIF2 or MRP-1 genes. We evaluated their efficacy depending on concentration and incubation time. The proliferation of LNCaP cells was slightly inhibited after 24 hours of incubation with single siRNAs (the percentage of inhibition reached no more than 13%). However, the observed effects were significantly enhanced after additional 48 hours of incubation. The inhibition of proliferation ranged from 4 to 38 percent. We also found that the antiproliferative activity of siRNAs varied depending on concentration used, however the correlation was not linear. We continued our evaluation for the most efficient concentration of each siRNA.

In contrast to our promising results obtained for siRNAs used individually, we did not observe any additive effects in case of the combination of two different siRNAs. Surprisingly, we even found that the combination of two siRNAs showed lower antiproliferative activity as compared to the activities of siRNAs applied individually. However much work remains to optimise efficacy, our results confirm the possible therapeutic advantages of siRNAs.

P.208 EFFECTS OF RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN-3 (RHIGFBP-3) ON HUMAN MALIGNANT PLEURAL MESOTHELIOMA CELL GROWTH AND APOPTOSIS

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Malignant pleural mesothelioma (MPP) has a reserved prognosis, despite aggressive treatment with chemotherapy and prolonged radiotherapy, thus continuing to present a therapeutic challenge. We and others have shown that the insulin-like growth factor system (IGFs), play an important role in the regulation of solid tumour cell growth and apoptosis. Aim: The aim of the present study is to evaluate the effects of rhIGFBP-3 treatment in regulating MMP cell growth and apoptosis in vitro. Methods: We used MMP cells (NCI-H28 and NCI-H2052) and normal human mesothelial cells (LP-9). Western ligand and immunoblot analysis, PCR and real time quantitative RT-PCR methods were used to characterize the various components of the IGF system. Cell proliferation was determined by a colorimetric MTS assay, anchorage-independent growth was examined in a methylcellulose-based clonogenic assay and TUNEL was used to assess apoptosis. Cells were treated with rhIGF-1 (50–100 ng/ml) and rhIGFBP-3 (0.5, 1 and 5 µg/ml), des-IGF-I (110 ng/ml) and TGF-beta 1 (1 ng/ml) for 6, 12 and 24 hrs, alone or in combination. Results: NCI-H28, NCI-H2052 and LP-9 cells express IGF-2, -3, -5, IGF-1 and GH receptor IGF-3 and IGBP-2 in conditioned medium. rhIGF-1 and des-IGF-I treatment increased MMP cell proliferation by 64% and 49%, respectively compared to serum free media (SFM, p < 0.05). In contrast, rhIGFBP-3 treatment significantly decreased proliferation in LP-9 (19%) and NCI-H2052 cells (12%) rhIGFBP-3 treatment decreased proliferation in a dose dependant manner in all cell lines of about 60%. The effect of endogenous IGFBP-3 was examined by inducing its synthesis with TGF-beta 1 which significantly increased gene expression after 12 and 24 hr in LP-9 cells by 2.3 fold, p < 0.05 and in NCI-H2052 cells by 5.8 fold, p < 0.01. Approximately 80% of TGF-beta 1 treated cells were apoptotic. One of the best indicators of tumorigenicity in vitro is the anchorage independence of tumour cells, which was examined in this study using the cloning assay. Treatment of cells with rhIGFBP-3 and TGF-beta 1 markedly decreased colony formation in both MMP cell lines. In conclusion, exogenous and endogenous IGFBP-3 induced apoptosis in both normal mesothelial and malignant mesothelioma cells in vitro. TGF-beta 1 also increased apoptosis and induced proliferation and tumorigenicity, effects that may be mediated by increasing endogenous IGFBP-3 production. These results suggest that treatment with rhIGFBP-3 may have a role as adjuvant therapy in malignant pleural mesothelioma. This study is supported by a grant from Mesothelioma Applied Research Foundation to Dr C Camacho-Hübner.
P.210 MAINTENANCE OF EGFR PHOSPHORYLATION BY THE IGF-1R IN THE PRESENCE OF GEFITINIB IN LUNG CANCER CELLS: CO-TARGETING THE EGFR AND IGF-1R MAXIMIZES ANTI-TUMOUR EFFECTS

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Resistance to anti-epidermal growth factor receptor (EGFR) strategies is an emerging clinical problem and elucidation of these resistance mechanisms and their co-targeting is critical in order to improve drug efficacy or delay/prevent the acquisition of resistance. This pre-clinical study demonstrates that the insulin-like growth factor-1 receptor (IGF-1R) plays a key role in mediating the partial intrinsic resistance to the EGFR tyrosine kinase inhibitor (TKI) gefitinib (IressaTM) developed within 2 and 6 months respectively. Each agent alone resulted in inhibition of cell proliferation after 48h in a dose dependent manner except for trastuzumab, which did not alter cell proliferation of DLD-1 cells. The inhibitory effect of ZD1839, GW572016 and trastuzumab on cell proliferation and apoptosis differs for the two colon cancer cell lines. Moreover, the relationship between inhibition of EGFR and HER-2 and their expression is complicated in IGF-1R signalling, impinging on EGFRpY1173 activity forms a resistance mechanism hindering the efficacy of gefitinib. Consistent with this, the subsequent co-targeting of the IGF-1R and the EGFR with ABDP and gefitinib respectively showed that the combination treatment resulted in a small but additive effect on growth in comparison with either gefitinib or ABDP as single agents, however, chronic exposure of the combination resulted in total cell loss whereas acquired resistance to gefitinib and ABDP monotherapies developed within 2 and 6 months respectively. IRESA is a trademark of the AstraZeneca group of companies.

P.211 THE IN VITRO EFFECT OF TWO TYROSINE KINASE INHIBITORS AND A MONOCLONAL ANTIBODY IN TWO COLON CANCER CELL LINES

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Background: Cancer chemotherapy has been one of the major medical advances in the last few decades. However, the drugs used for this therapy have several toxic side effects. In contrast, targeted therapy is directed against cancer-specific molecules and signaling pathways and is therefore less likely to have more specific and less toxic side effects. There are multiple types of targeted therapies available, including monoclonal antibodies, inhibitors of tyrosine kinases and antiangiogenic treatments. In the present study, we studied the effect of monoclonal antibody trastuzumab, raised against HER-2, the epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 and EGFR/HER-2 tyrosine kinase inhibitor GW572016 in the proliferation of two human colon cancer cell lines.

Materials and methods: Caco-2 and DLD-1 colon cancer cell lines were treated with two tyrosine kinase inhibitors ZD1839 and GW572016 and trastuzumab. Cell proliferation was estimated using the colorimetric MTT assay. Apoptosis was assessed by evaluating DNA fragmentation and by an Annexin V binding assay. The expression of HER-2 and EGFR was studied using real time RT-PCR.

Results: Each agent alone resulted in inhibition of cell proliferation after 48h in a dose dependent manner except for trastuzumab, which did not alter cell proliferation of DLD-1 cells. Further, ZD1839 combined with GW572016 resulted in an additional inhibitory effect on Caco-2 cells but not on DLD-1 cells. In contrast, trastuzumab did not enhance the inhibitory effect of ZD1839 and GW572016 on Caco-2 cell line. In addition to the inhibition of D1-1 proliferation by ZD1839, cell apoptosis increased 24 h after treatment. None of the tested agents altered apoptosis in DLD-1 cells. EGFR and HER-2 mRNA levels decreased in both DLD-1 cells with ZD1839 or the combination of ZD1839 and GW572016. However GW572016 alone increased HER-2 mRNA levels. In Caco-2 cells, HER-2 mRNA decreased with ZD1839, GW572016, trastuzumab or their combinations. In contrast, EGFR mRNA levels increased with any of the agents tested and their combinations.

Conclusion: The inhibitory effect of ZD1839, GW572016 and trastuzumab on cell proliferation and apoptosis differs for the two colon cancer cell lines. Moreover, the relationship between inhibition of EGFR and HER-2 and their expression is complicated and cell line- and agent- dependent. Further studies are necessary for elucidating the activated pathways.

P.212 EXPLORATION OF THE ANTIANGIOGENIC POTENTIAL OF SATRAPLATIN AND ITS ACTIVE METABOLITE JM118

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Background: Satraplatin is a novel oral platinum analog with potent cytotoxic and antitumor activity in preclinical models. Satraplatin showed activity in hormone refractory prostate cancer (HRPC) and other tumor types in Phase II trials. A pivotal Phase III trial evaluating satraplatin as 2nd-line therapy for HRPC completed accrual of > 900 patients in 2005. Satraplatin’s activity, safety profile and ease of administration make it attractive for use as a single agent or in combination regimens. Cytotoxic chemotherapy directly kills tumor cells. Alternative mechanisms of efficacy have been demonstrated, including antiangiogenic effects. The goal of this study was to evaluate the potential antiangiogenic effects of satraplatin and its active metabolite JM118.

Methods: Antiangiogenic effects of satraplatin and JM118 were assessed on human umbilical vein endothelial (HUVEC) and dermal microvascular endothelial (HDMEC) cells and compared to non-transformed human dermal fibroblast (NHDF) and umbilical artery smooth muscle (HUASMC) cells, and human prostate (PC3), colon (HT29) and breast (R36C) carcinoma cells. Cells were exposed to satraplatin for 24h and the effect for the different cell types was studied using real time RT-PCR. Immunofluorescence and cell proliferation were assessed in 3D cultures. The effect of satraplatin on endothelial cell proliferation was assessed using the colorimetric MTT assay, and the effect of satraplatin on human fibroblasts, arterial smooth muscle cells, and tumor cells. Satraplatin and JM118 target endothelial cells, suggesting their antitumor effects are a result of both cytotoxic and antiangiogenic mechanisms.

P.213 HERBAL MEDICINAL PRODUCT ON THE BASIS OF CHELIDONIUM MAJUS L. NSC-631570

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Chelidonium majus L. (greater celandine) is the only species of the tribe Chelidonieae of the Papaveraceae family. Chelidonium is the main alkaloid of greater celandine roots and makes up 80% of the total alkaloid content. Major alkaloids are: chelidonine, protopine, absinthiopyrone, chelerythrine, sanguinarine. They make up 60-80% of the total alkaloid content of the raw plant. The plant is for hundreds years well known due to its multiply biological activity, including anticancer, antiviral, antimicrobial effects. However, water-insolubility makes clinical administration of the constituents of the drug and its extract possible only in form of local or peroral administration. Alkaloids were made water soluble by a patented process. Starting material is greater celandine roots. The alkaloids are extracted from the dried drug and then purified. This fraction consists of water insoluble alkaloid bases; heating with Thio-TEPA BP in lipophilic solution, cleaning with active charcoal and the reaction with HCl gas lead to a defined mixture of water – soluble alkaloids. In this form the alkaloids may be regarded as ‘bioactive’ in the sense of the applied anti-cancer induction of the drug. The active substances in the celandine roots are Chelidonium alkaloids, qualitatively comparable to Greater Celandine herb (Ph. Eur. 5.0), but quantitatively different to the herb. NSC-631570 shows a wide spectrum of biological activity, including direct anti-tubulin action, inhibiting of DNA and RNA synthesis in malignant cells, modulation of MMP secretion status of tumour cells and stimulation of apoptosis. Additionally, the substance is fluorescent active under UV light and possessed over a high tumour-selectivity shown by several models. To the specific properties of the drug belong also radio modifying effect and immune modulation. Clinical effectiveness of the drug was proven in several clinical trials, both were direct antineoplastic activity as also immune modulating properties could be observed. The advantages of the whole sum of the alkaloids use are preserving of the whole spectrum of biological activity.
Additionally, the sum of alkaloids is less toxic than each alkaloid alone resulting in significant low toxicity of the drug.

P.214 AMIFOSTINE (WR-1065) PREVENTS RADIATION-ENHANCED AUTOPHAGY BY SEQUESTRATION OF LYOSOMAL IRON

H.L. Persson

Background: Recently, it was demonstrated that cells, initially exposed to a large but non-lethal dose of ionizing radiation (IR), were readily killed by a second much lower dose. The observed sensitization by the first IR was due to enhanced autophagy and lysosomal turnover of damaged cellular iron-containing elements, i.e., mitochondria and various metalloproteins. As a consequence, the lysosomal pool of redox-active iron was greatly increased, making lysosomes very sensitive to iron-catalyzed oxidative disruption and ensuing cell death initiated by a second IR. Interestingly, a high-molecular-weight desferrioxamine starch conjugate (HMW-DFO), which exquisitely localizes and acts within the lysosomal compartment, partially prevented radiation-enhanced autophagy and subsequent increase of lysosomal redox-active iron. This observation suggests that IR-induced autophagy partly involves iron-catalyzed lysosomal disruption and ensuing leakage of harmful lysosomal content into the cytosol. Previous studies have shown that the radio-protective agent amifostine (WR-1065), being a weak base, accumulates within lysosomes due to proton trapping. Since WR-1065 sequeseters lysosomal iron, thereby preventing oxidative damage upon this organelle, the effects of this agent upon radiation-enhanced autophagy were analyzed within the same experimental model.

Experimental procedure: Murine histiocytic lymphoma (1774) cells, pretreated with WR-1065 or not, were exposed (or not) to a large but non-lethal dose of IR. Following 24 h at standard culture conditions, cellular ‘loose’ iron and total iron were assayed using the iron-specific chromogenic reagent Ferrozine and by atomic absorption spectrophotometry, respectively. Cellular distribution of redox-active iron was demonstrated light microscopically using the sulphide silver method (SSM).

Results and conclusions: Following IR, cells stopped to proliferate for approx. 48 h. At 8 h post-IR, changes of cell morphology, i.e., spreading and vacuolization, became evident. ‘Loose’ cellular iron increased greatly in irradiated cells at 24 h post-IR (Table), and SSM at the same time-point revealed that most redox-active iron was deposited within lysosomes. In contrast, WR-1065 present in the medium for one hour prior to IR, and SSM at the same time-point revealed that most redox-active iron was deposited

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<th>Non-irradiated cells</th>
<th>Irradiated cells</th>
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<tr>
<td></td>
<td>Control</td>
<td>WR-1065</td>
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<tr>
<td>‘Loose’ iron</td>
<td>Control</td>
<td>WR-1065</td>
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<tr>
<td>Total iron</td>
<td>Control</td>
<td>WR-1065</td>
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<tr>
<td>100 ± 4%</td>
<td>100 ± 51</td>
<td>491 ± 26</td>
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<td>100 ± 7%</td>
<td>100 ± 5%</td>
<td>68 ± 2%</td>
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In conclusion, it was shown that the treatment of leukemia cells, which are VDR positive, with the combination of vitamin D derivatives and cytostatics decreases IC50 values more effectively than with cytostatics applied alone. Thus, because of their good biological properties (high antitumor and low calcemic activity), PRI-2191 and PRI-1906 seem to be good candidates for further preclinical and clinical studies, especially as partner compounds in combined anti-leukemia treatments.

This work was partly supported by grant PB2-KBN-091/P03/2003 from Ministry of Informatics and Science, Poland.

P.217 ANTITUMOR ACTIVITY OF MANNAN-METHOTREXATE CONJUGATE IN VITRO AND IN VIVO

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Methotrexate (MTX) is widely used in the treatment of the number of oncological and hematological diseases. However, MTX also has known limitations, due to its low plasma half-life, toxicity for normal proliferating cells and resistance to the drug. This motivated scientists to look for the ways of the drug improvement. Others and we have recently published several reports about coupling MTX to different macromolecular carriers. This strategy seems to be very promising in obtaining preparations with amantened antitumor properties. The aim of this work was to investigate anticancer activity of MTX conjugated with mannan carrier. The obtained conjugate was pure and stable at different conditions. Three cell lines were applied for in vitro studies: A549 (human non-small cell lung carcinoma), B16 (murine melanoma) and P388 (murine leukemia). The in vitro cytotoxic effect of all agents was examined after 72-hour exposure of the cultured cells to varying concentrations of the tested agents and computing average 50% inhibitory concentration (IC50) dose. The investigation was also performed in the in vivo murine leukemia model (P388). Mouse, randomly divided in groups, were injected with 10^6 leukemia cells i.p., and 24 hours later each mouse was injected with 80 mg/kg of appropriate agent.

Studies revealed that mannan-MTX conjugate had from 30 to 45-fold smaller in vitro antiproliferative activity in comparison with free MTX. IC50 for A549, B16 and P388 cell lines were 1.814 ± 0.104 microg/ml, 3.187 ± 2.355 microg/ml, 0.1193 ± 0.0373 microg/ml for mannan-MTX and 0.058 ± 0.002 microg/ml, 0.068 ± 0.021 microg/ml, 0.0036 ± 0.0006 microg/ml for free MTX respectively. We have also evaluated the antitumor properties of mannan-MTX conjugate in in vivo model. Results of the experiment revealed that mannan-MTX conjugate had the advantage over the free MTX in terms of antitumor effect in vivo. Mice treated by the conjugate benefited from the statistically significant longer survival in comparison with mice treated by the free MTX (p < 0.001) and non-treated leukemia-bearing animals in control group (p < 0.001). Median survival times were 11, 16 and 22 days for control, free MTX and conjugate treated groups respectively. Data on average weight changes during the run of the experiment allow us to conclude that mannan-MTX conjugate has somehow higher toxicity in comparison with free MTX. However, this did not result in
excess deaths in the group treated by the conjugate, since no mice died due to the toxicity. Altogether data allow us to conclude that mannan-MTX conjugate has stronger antitumor activity in comparison with parental drug against P388 and deserves further studies as prospective antitumor agent.

P.218 ANTI TUMOR ACTIVITY OF N-SULFONYLURACIL DERIVATIVE APPLIED ALONE AND IN COMBINATION WITH HYPER THERMIA ON THE GROWTH OF MOUSE MAMMARY CARCINOMA

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Background: N-sulfonylpyrimidine derivatives were designed and synthesized as activity of 5-bromo-1-(metanesulfonyl)uracil have been also studied on mouse mammary carcinoma in vivo. Influence of local hyperthermia on the antitumor activity of 5-bromo-1-(metanesulfonyl)uracil on the tumor cells. Within the scope of antitumor investigation of compounds that have shown potent inhibitory activity on the growth of human tumor cell lines in vitro, at concentrations of 10-7-10-5 M (25–75%). In comparison with 5-fluorouracil some of N-sulfonylpyrimidine derivatives showed 10 times stronger activity and induction of apoptosis in treated tumor cells. Within the scope of antitumor investigation of compounds that have shown potent inhibitory activity on the growth of human tumor cell lines in vitro, we have investigated antitumor activity of 5-bromo-1-(metanesulfonyl)uracil on the mouse mammary carcinoma in vivo. Influence of local hyperthermia on the antitumor activity of 5-bromo-1-(metanesulfonyl)uracil have been also studied.

Material and methods: In this study we used a transplantable mouse mammary carcinoma (TM1/Ga). Tumour cells (106) were injected into the footpad of the right hind leg (intraosseously) of CBA mice. Tumour bearing mice have been treated with 5-bromo-1-(metanesulfonyl)uracil or with 5-fluorouracil (positive control) with and without hyperthermia (43,0 oC/60 min). The end point was tumour growth time (TGT). TGT is the time needed for tumor volume to grow five times over the treated volume measured by caliper and calculated by the formula AxBxC/6. 5-bromo-1-(metanesulfonyl)uracil has been synthesized in Rudjer Boskovic Institute.

Results: The results show that examined 5-bromo-1-(metanesulfonyl)uracil had good antitumor activity, i.e. the tumor growth time in this group was two times longer than tumor growth time in control group. When 5-bromo-1-(metanesulfonyl)uracil has been combined with local hyperthermia the antitumor activity of this derivative was enhanced, i.e. was additive. In comparison with positive control 5-bromo-1-(metanesulfonyl)uracil showed slightly better antitumor activity.

P.219 IN VITRO ANTI PROLIFERATIVE EFFECT OF N-ACETYL D-LACTOSAMINE SPECIFIC LECTIN FROM ALOCASIA INDICA ON HUMAN CANCER CELL LINES

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Alocasia indica lectin (AIL) is known to polyclonally activate human T-cells. AIL agglutinated rabbit, rat, guinea pig and sheep erythrocytes but was unable to agglutinate human ABO blood group erythrocytes. N-acetyl-D-lactosamine and serum glycosprotein asialofetuin were found inhibitory in the hemagglutination inhibition assay. The lectin was purified by affinity chromatography using asialofetuin linked amino activated silica gel. Chemical modification of AIL with pyridoxal, Diethylpyrocarbonate and Bis-dithionitrobenzoic acid did not affect its activity, suggesting the absence of arginine, histidine and cysteine respectively or in near the ligand-binding site of the lectin. Modification of tyrosine with N-acetylmalidazole led to 75 % inactivation of AIL. However, complete inactivation was observed only upon N-bromosuccinimide modification of tryptophan residues of the lectin. Antiproliferative activity of AIL was tested on seven human cancer cell lines DU145 (Prostate), PC-3 (Prostate) A549 (Lung), HCT15 (Colon), 502713 (Colon), KB (Oral) and IMR32 (Neuroblastoma). A 50% inhibition of proliferation was observed in DU145, PC3, HCT15 and 502713 at the lowest concentration (10 µg/ml or less) of the lectin tested. KB cell line showed 50% inhibition at 50 µg/ml of AIL. While very less inhibitory effect of AIL was observed on the proliferation of A549 and IMR32.

P.220 ANTI PROLIFERATIVE EFFECT OF CHR-2797, A NOVEL AMINOPEPTIDASE INHIBITOR, IN THE HUMAN BREAST CANCER XENOGRAFT, MDA-MB 468

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Aminopeptidases are members of the M1 family of metalloenzymes that remove aminoacids from the N-termini of proteins. The N-terminal aminoacids of cellular proteins are believed to play a substantial role in the turn-over of malignant cell intermediates in such a way that they impact cancer cell survival or proliferation. Members of this class of enzymes are overexpressed in human cancers. CHR-2797 is a novel, orally available aminopeptidase inhibitor currently in early clinical development for the treatment of advanced cancer and haematological malignancies. It exerts a powerful inhibitory effect on intracellular metalloenzymes, resulting in anti-proliferative, pro-apoptotic and anti-angiogenic effects. Both CHR-2797 and its active metabolite, CHR-79888, have shown pleiotropic activity against a broad range of human cancers in vitro and in vivo.

The human breast carcinoma cell line, MDA-MB 468, was used for testing the activity of CHR-2797 in a murine xenograft model. MDA-MB 468 cells were injected into the mammary fat pads of female nude mice (day 0). The animals (58 in total) were randomised on day 7 in a 1:1 fashion to receive vehicle (10 ml/kg, n=28 per group) and CHR-2797 (100 mg/kg, n ≥ 14 per group). CHR-2797 and vehicle were administered intraperitoneally (IP) and once daily. Half of the animals were treated between days 21 and 49, and the remaining mice were dosed between days 39 and 70 after tumour inoculation. Tumour length and width were measured at regular intervals. Tumour weight was calculated as length x (width)2/2.4. Weight increase of the tumours was inhibited by 31% (p<0.05) when therapy commenced on day 21, at which time the calculated mean tumour weight was 19 mg, whereas the inhibition was reduced to 22% when treatment began on day 39, in animals with a calculated mean tumour weight of 41 mg. The extent of inhibition was greater when treatment commenced at a lower tumour burden. Subsequent experiments showed that the degree of inhibition achieved after oral dosing was similar to IP administration. The long term administration (up to 30 days) of CHR-2797 to nude mice was not associated with significant toxicity, although a small decrease in body weight gain was occasionally observed.

Our results show that CHR-2797 had a small, but significant effect on the size and weight of well-established, subcutaneously implanted MDA-MB 468 human breast carcinoma in a mouse model, even when therapy was initiated as late as day 39, at which time the tumours were of considerable size.