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Poster session 1: Target identification and validation

P1.1 DOWNREGULATION OF MIR-21 EXPRESSION INDUCES AUTOPHAGY AND INCREASES THE SENSITIVITY OF K562 CHRONIC MYELOID LEUKEMIA CELLS TO ETOPOSIDE AND DOXORUBICIN

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MicroRNAs (miRs) are small non-coding RNAs that regulate gene expression. miR-21 plays an important role in cancer (including leukemia1) drug resistance2. It has recently been reported to directly target and upregulate the expression of Bcl-23. AntimiRs are used to downregulate the expression of targeted miRs and some are already in clinical trials for treatment of certain diseases. The aim of this work was to use antimiRs to downregulate miR-21 expression in K562 chronic myeloid leukemia cells, in order to increase the sensitivity of these cells to conventional drugs and to further investigate the mechanisms involved in this sensitization.

K562 cells were transfected with antimiR-21 and in some experiments further treated with etoposide or doxorubicin (appropriate controls were included). Downregulation of miR-21 levels was confirmed by RT-qPCR. The effect of miR downregulation on cellular proliferation (BrdU assay), cell cycle (flow cytometry following PI labeling) and programmed cell death (TUNEL assay) was studied. The expression levels of proteins involved in apoptosis (Bcl-2, PARP) and in autophagy (Beclin and LC-3) were investigated by Western Blotting. Cellular sensitisation to the effects of doxorubicin or etoposide was confirmed by counting viable cell number (Trypan blue assay).

Downregulation of miR-21 per se caused a decrease in viable cell number, caused no alterations in the cell cycle profile but decreased cellular proliferation and slightly increased TUNEL labelling. Bcl-2 expression was downregulated but, unexpectedly, PARP cleavage was not afected. The expression of Beclin-1 was increased and that of LC-3-I was decreased, suggesting that the mechanism of cell death could, at least in part, involve autophagy. Finally, miR-21 downregulation increased the sensitivity to etoposide or doxorubicin.

In conclusion, we describe, for the first time, that miR-21 may influence drug sensitivity by regulating autophagy.

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P1.2 TARGETING ERG/DNA COMPLEX BY A SMALL SELECTIVE DNA LIGAND: IMPORTANCE OF THE SEQUENCE

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Conventional chemotherapies remain widely used in the treatment of cancer despite poor selectivity associated with strong toxicity. To increase selectivity and decrease toxicity, new therapeutic approaches need to be developed to treat cancer. We chose to develop an approach that target the functionality of oncogenic transcription factors implicated in oncogenesis. One of those is the ERG gene over-expressed or translocated in several cancers. To bypass the effect of this oncogenic transcription factor, our researches focus on the inhibition of ERG/DNA interaction by using small heterocyclic diamidines molecules (DB compounds) that interact in the small groove of the DNA helix in a sequence-selective manner and compete for protein binding. Previous in vitro studies selected compound DB1255 (a diamidine-diphenyl-dithiophene derivative) for its capacity to interfere with ERG/DNA interaction.

Belonging to the Ets family of transcription factors, ERG interacts with the DNA on the consensus sequence EBS (Ets-binding-site) with minimal (underlined) sequence being 5′-GGA(A/T)-3′, and enlarged sequence corresponding to the 5′-agCAGGAAGTtcg-3′ sequence. In vitro, ERG/DNA complex could be inhibited by the selected DB1255 compound through its interaction on the sequence indicated in italics that is located 3′ to the minimal EBS: 5′-agCAGGAAGTtcg-3′. To determine the selectivity of action of the DB1255 compound in connection with the ERG protein selectivity to its large consensus site, the EBS sequence was mutated at various positions. Each mutated sequences was first tested by EMSA for ERG/DNA binding and secondly evaluated by DNaseI footprint assays for DB1255 binding selectivity.

We also evidenced that the presence of a 5′-GT dinucleotide located in the 3′ extremity of the minimal EBS is necessary for an optimal interaction of ERG to the DNA and showed the importance of the 5′-GTT trinucleotide located 3′ to the minimal EBS sequence for an optimal DB1255 interaction and a subsequent decrease of ERG/DNA binding. These in vitro results were confirmed in cellulo using luciferase assays evidencing the decrease of ERG-induced transcriptional activity by DB1255.

In conclusion, our results highlight the precise consensus sequence for ERG binding to the DNA and the selectivity for binding to DNA of DB1255 as a functional inhibitor of ERG/DNA complex formation.

P1.3 SPECIFIC INHIBITORS OF CASEIN KINASE 2 (CK2) INDUCE A STRONG ANTI-LEUKEMIA EFFECT BY ENHANCING THE TUMOR SUPPRESSOR ACTIVITY OF IKAROS

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Casein kinase II (CK2) is a pro-oncogenic kinase whose increased activity in leukemia is associated with poor prognosis. We have demonstrated that CK2 directly phosphorylates the Ikaros protein *in vivo*. Ikaros is a DNA-binding protein that functions as a tumor suppressor in acute lymphoblastic leukemia (ALL). The use of Ikaros mutants that are phosphomimetic and phosphoresistant at CK2 phosphorylation sites demonstrates that: 1) CK2-mediated phosphorylation inhibits Ikaros' function as a transcriptional repressor; and 2) Hyperphosphorylation of Ikaros results in increased resistance of leukemia cells to conventional chemotherapy. Inhibition of CK2 in B-ALL leads to the restoration of Ikaros function as a regulator of transcription of genes that are essential for normal lymphocyte differentiation. Inhibition of CK2 in B-ALL also resulted in Ikaros-mediated repression of the genes that suppress apoptosis, which increased sensitivity of leukemia cells to radiation and doxorubicin. These results suggest that: 1) The use of CK2 inhibitors is a promising treatment option for ALL; 2) The mechanisms of action of CK2 inhibitors involves restoration of Ikaros tumor suppressor activity; and 3) Treatment with CK2 inhibitors enhances sensitivity of ALL cells to conventional chemotherapy by decreasing transcription of anti-apoptotic genes. These results establish biomarkers that can be used to monitor the effect of CK2 inhibitors on leukemia cells and provided a basis for the testing of CK2 inhibitors as a novel treatment for ALL.

P1.4 CD160130: A SELECTIVE INHIBITOR OF THE TUMOR-TYPE HERG1 CHANNELS, ACTIVE IN HUMAN LEUKEMIAS

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hERG1 channels, besides sustaining the cardiac Ikr (rapid K+current), are aberrantly expressed in human cancers, where they regulate several aspects of neoplastic cell behavior such as the triggering of pro-survival signals and the development of resistance to chemotherapy. In view of these actions, hERG1 is now considered as a potentially attractive target for antineoplastic therapy, particularly in leukemias. However, hERG1 is also considered as an anti-target and an obstacle for pharmacological development, due to the negative cardiac side-effects which follow its blocking. To reconcile these two contradicting features, we have identified a strategy based on the biophysical and structural differences between the 'cardiac' and the 'tumor' hERG1, noticeably by the preferential expression of the hERG1B isoform in leukemias.

We have subsequently investigated the pharmacological effects of CD160130, a phosphodiesterase-4 inhibitor which also demonstrates significant hERG1 channel binding affinity and antineoplastic activity on primary B-cell chronic lymphocytic leukemia cells. We first evaluated the killing ability of CD160130 on several human leukemic cell lines (FLG 29.1, REH, 697 cells). CD160130 displayed a good killing ability with an ED50 value between 2 and 4 μM and was also an inducer of apoptosis in leukemia cells also having a synergic effect with Fludarabine. CD160130 displayed

a ten-fold greater inhibition on currents of the hERG1B isoforms, expressed in HEK 293 cells, compared to hERG1A with an IC50 value (1.8 μM) similar to the ED50 obtained for leukemia cell killing. When tested on FLG 29.1 (a human myeoloblastic leukemia cell line which exclusively expresses the hERG1B isoform), CD160130 blocked hERG1 currents with an IC50 value almost identical to that determined in hERG1B transfected cells and, again, similar to the ED50 for leukemia cell killing therefore confirming the selectivity of CD160130 for the hERG1B tumor-type isoform. Interestingly, no killing effect was exerted by CD140793, a close analogue of CD160130 which possesses no hERG1 inhibitory activity. Further in vitro and in vivo experiments are still ongoing.

Overall, these findings point towards CD160130 as a potential first-in-class selective tumor-type hERG1 channel inhibitor for the treatment of leukemias.

Poster session 2: Preclinical drug profiles

P2.1 ANTITUMOR ACTIVITY OF NOVEL SMALL ANDROGEN RECEPTOR MODULATING MOLECULES (SARMS) IN EXPERIMENTAL HUMAN PROSTATE CANCER MODELS

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Prostate cancer is the second most common cause of cancer mortality in males. To date, the major challenge facing physicians is the management of castration-resistant prostate cancer (CRPC) and, in particular, the identification of effective long-term antiandrogen treatments. In the present work we analyzed the activity of two (*R*)-bicalutamide derivatives ((*R*)-9 and (*S*)-11) capable of acting as androgen receptor (AR) antagonists in hormone-sensitive or -refractory prostate cancers *in vitro* and *in vivo*. *In vitro* experiments were performed on one hormone-sensitive (LNCaP) and two hormone-refractory (PC-3 and DU145) human prostate cancer cell lines, and on one cell line (LNCaP-AR) engineered to stably express high levels of AR. Cytotoxic activity was evaluated by SRB assay, apoptosis by TUNEL method and PSA levels by ELISA. *In vivo* experiments, aimed to evaluate the toxicological profile and the antitumor activity of (*R*)-9 compound, were performed on SCID mice. In hormone-sensitive LNCap cell lines, the active enantiomer of the most widely prescribed non-steroidal antiandrogen, (*R*)-bicalutamide (Casodex ™), exhibited only cytostatic activity, whereas the two derivatives, (*R*)-9 and (*S*)-11, induced a cytocidal effect and up to 94% apoptosis. A similar apoptotic effect was also observed in LNCAP-AR and was triggered by the mitochondrial pathway. The antitumor activity of the two new SARMs was not affected by the concomitant presence of the synthetic androgen R1881. Moreover, both compounds completely inhibited the tumor growth of hormone-refractory PC3 and DU145 cells, in sharp contrast to the lack of activity induced in the same cells by (*R*)-bicalutamide. We also observed a strong reduction in PSA levels in LNCaP and LNCaP-AR culture medium after a 48-hr exposure to each derivative. Preliminary *in vivo* studies on the toxicological profile showed a good tolerance to (*R*)-9 and a high antitumor activity. In conclusion, our results seem to indicate that the new investigational SARMs, (*R*)-9 and (*S*)-11, are more active than the enantiomer, (*R*)-bicalutamide, in *in vitro* models and could represent an attractive option for CRPC patients. Further *in vivo* experiments are needed.

P2.2 OCID15314: A NOVEL HDAC INHIBITOR WITH POTENT ANTI-MULTIPLE MYELOMA EFFECTS

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HDAC inhibitors represent promising therapeutic candidates for the treatment of several cancers including multiple myeloma (MM). In the present study we investigated the preclinical activity of OCID15314, a HDAC inhibitor. OCID15314 shows potent inhibitory activities on HDAC1 and HDAC6 (IC50 of 0.8μM and 0.009μM respectively). OCID15314 was effective against several MM cell lines with GI50 values ranging from 0.5μM to 3.9μM. Additionally, OCID15314 was also effective against fibrosarcoma (HT1080; GI50 3.9μM), breast cancer (MDA-MB-231; GI50 4.2μM), colon cancer (HCT116; GI50 5.4μM), lung cancer (NCIH460; GI50 3.9μM and prostate cancer (PC3; GI50 4.93μM). In RPMI8226 MM cell line, OCID15314 induced acetylation of α-tubulin and lysine on histone H3, suggesting the inhibition of HDAC6 and HDAC1 activities, respectively. Importantly, OCID15314 was also found to be non toxic to human PBMCs. The anti-MM effects of OCID15314 were further confirmed in vivo in a mouse xenograft plasmacytoma model using RPMI8226 cells and a lung tumor xenograft model using NCIH460 cells. A significant inhibition in tumor growth was observed in both the xenograft models along with prolongation of overall survival. Analysis of OCID15314 treated RPMI8226 tumor samples also revealed significantly higher levels of acetylated α-tubulin thereby suggesting an inhibition of HDAC6 activity. No significant adverse effects on haematological parameters, biochemical parameters or spleen/body weight ratio were noted in either of the in vivo models. Taken together, OCID15314 is a HDAC inhibitor with potent anti-MM activity and is well poised for consideration as a developmental candidate.

P2.3 A NOVEL DRUG COMBINATION FOR THE TREATMENT OF KIDNEY CANCER

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Renal cell carcinoma (RCC) is one of the most lethal kidney cancers. Loss or inactivation of Von Hippel-Lindau (VHL) and Tuberous Sclerosis Complex (TSC) in RCC results in high levels of HIF-α activity. mTOR inhibitor (rapamycin) and the AMPK activator 5-aminoimidazole-4-carboxamide (AICA)-riboside (AICAR) are currently used separately to treat cancer patients. We investigated the effect of a novel combination of drugs on reducing kidney tumorigenesis. Our data show that the drug combination (1) significantly increased cell apoptosis (4-8 fold), (2) decreased cell proliferation (4-6fold), and (3) abolished Akt and HIF activity in primary proximal tubular kidney rat cells (TSC2+/- and TSC2-/-) and human VHL-deficient cells (786-O). We found that the drug combination decreased cell invasion and cell immigration by 75% and 80%, respectively. The combined drugs abolished the activity of Akt in all cancer cells. We have identified a putative HIF-1/2α binding site in the Akt1 promoter. Using electromobility shift (EMSA) assay, we found that the drug combination effectively abolished the HIF-2α protein in the nuclear extracts of 786-O cells available for binding to the putative Akt1 HIF site. Importantly, we have tested the effect of each drug and the combined drugs on tumor size and numbers in TSC2-deficient mouse model. Our preliminary data show that treatment with rapamycin, AICAR and Rap+AIC for 1 month decreased tumor size by 43%, 27% and 83% respectively, suggesting that the drug combination is more efficient in reducing tumor number and size compared to each drug alone. This is first evidence that a novel drug combination is effective in reducing kidney tumormigenesis. These data will provide a base-line data for clinical trials in patients with kidney cancer.

P2.4 METRONOMIC VINORELBINE COMBINED WITH ENDOXIFEN ON MCF7 CELL LINE

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Introduction: Metronomic chemotherapy has shown both clinical (Briasoulis, Pappas et al, 2009) and laboratory evidence (Biziota et al. TAT-2009) of antitumor activity and inhibition of tumor vasculature and endothelial cell function. However the effects of metronomic chemotherapy on cancer cells alone has not been studied enough. In the context of our research interests on metronomic chemotherapy we investigated low (metronomic) concentrations of vinorelbine (1 or 10nM) combined with endoxifen, the active metabolite of tamoxifen, in MCF7 cell line.

Materials and methods: MCF7 cells were cultured in phenol red-free MEM supplemented with 10% FBS which was replaced with stripped FBS 72h before the experiments. Cells were exposed in different time intervals with vinorelbine and endoxifen alone or combined. Total protein was extracted with RIPA buffer; for cytosolic-nuclear extraction hypotonic and high salt extraction buffer were used, respectively. Total RNA was isolated by using Quick mini RNA prep KIT (Zymo-Research).

Results: Endoxifen downregulates the levels of estrogen receptor-a (ERa) in cytoplasm and causes upregulation of ERa in nucleus due to translocation that is appeared from the 1st hour of exposure and remains even for 24 hours; total levels of ERa remain stable. Vinorelbine alone in metronomic concentration (1 and 10nM) does not change protein levels of ERa, while a higher concentration (100nM) causes downregulation of ERa mRNA. Addition of low doses of vinorelbine on endoxifen treatment does not antagonize the localization of ERa. Low endoxifen concentration reduces the levels of HEY1 mRNA without affecting Notch1 and HES1 mRNA levels remarkably. Co-treatment with metronomic vinorelbine diminishes this effect, while 100 nM of vinorelbine alone drop HEY1 mRNA. Low endoxifen concentration lowered TSP1 more potently than the high concentration, and IL-8 is upregulated in a dose response fashion by vinorelbine, and also when combined with both doses of endoxifen.

Conclusions: Vinorelbine as a chemotherapeutic agent has better anti-angiogenic profile in metronomic concentrations in terms of angiogenesis markers. Low endoxifen concentrations (1nM) which reflect the poor tamoxifen metabolizing patients, have an estrogenic like action in contrast to higher (100nM) concentrations. The addition of a metronomic regime to hormonal therapy for breast cancer deserves further scientific exploration.

P2.5 INVOLVEMENT OF AUTOPHAGY IN ENDOGENOUS AND NVP-AUY922-INDUCED C-KIT DEGRADATION IN BOTH IMATINIB-SENSITIVE AND -RESISTANT GASTROINTESTINAL STROMAL TUMOR CELLS

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Background: Heat shock protein 90 (HSP90) is a ubiquitously expressed chaperone involved in the post- translational maturation and stability of proteins. Inhibition of HSP90 leads to degradation of its client proteins, a process which is conceived to be mediated by the ubiquitin dependent proteasome machinery. Recent studies suggest that there are crosstalk between the two major protein degradation pathways within eukaryotic cells, the proteasome and autophagy pathways. However, the role of autophagy in degradation of HSP90 client proteins remains unclear.

Methods and results: Our previous study has demonstrated that NVP-AUY922 (AUY922), a highly potent, non-geldanamycin HSP90 inhibitor, induced both growth inhibition and apoptosis in gastrointestinal stromal tumor (GIST) cells that express either an imatinib-sensitive, exon 13 mutated c-Kit (GIST882) or an imatinib-resistant, exon 11/17 double mutated c-Kit (GIST48). Significant dose and time dependent reduction of both total and phosphorylated forms of c-Kit proteins in these cells were observed upon AUY922 treatment. This loss of c-Kit protein is due to accelerated c-Kit degradation as demonstrated by a shortened c-Kit half-life in the presence of AUY922 in cycloheximide treated GIST cells. AUY922-induced c-Kit reduction could be partially reversed by inhibiting either autophagy (by 3-MA, bafilomycin-A or silencing beclin-1 expression by siRNA) or proteasome (by MG-132 or lactacystin) degradation pathways, and almost totally reversed by concomitant blocking of both degradation pathways. Interestingly, western blotting analysis showed that autophagy inhibitor or beclin-1 siRNA alone increased c-Kit protein level as compared with control GIST cells suggesting autophagy plays a role in c-Kit turnover even in the absence of AUY922. The involvement of autophagy in endogenous and AUY922-induced c-Kit protein turnover was further confirmed by co-localization of c-Kit and autophagosome via immunofluorescence staining.

Conclusions: We demonstrated that AUY922 treatment induced down-regulation of c-Kit protein and apoptosis in both imatinib-sensitive and -resistant GIST cells. In addition to proteasome, autophagy mediated protein degradation may also play a pivotal role in both endogenous and HSP90 inhibitor induced c-Kit turn over in GIST. Our data support the potential use of AUY922 in treating TKI- refractory, c-Kit-expressing GIST and highlight a possible role for autophagy in HSP90 mediated client protein degradation.

P2.7 INHIBITION OF HISTONE DEACETYLASES (HDACS) IN HODGKIN LYMPHOMA CELL LINES: DIRECT CYTOTOXICITY AND IMPACT ON THE INTERACTION WITH LYMPHOCYTES

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Histone deacetylases (HDACs) are a class of enzymes which play a crucial role in regulating gene expression and activity of proteins responsible for cell-cycle progression, proliferation and cell survival. As the activity of HDACs is known to be highly dysregulated in malignant Hodgkin lymphoma (HL) cells, they represent an interesting therapeutic target in this disease.

Here we show that the novel HDAC inhibitor LBH589 (Panobinostat, Novartis) induced apoptosis in Hodgkin lymphoma cell lines at nanomolar concentrations. Furthermore, the combination with standard therapeutic agents showed synergistic effects.

Next, we studied the effects of HDAC inhibition on the cross-talk between HL cells and lymphocytes, that represent the main component of the HL tumor microenvironment and play a major role in tumor initiation and maintenance. We observed that LBH589 had potent effects on the expression of HL- and lymphocyte surface molecules known to be involved in HL cell survival and/or communication with lymphocytes. In HL cell lines, LBH589 treatment induced a decrease of surface molecules, including CD30. Overexpression and constitutive signalling of CD30, is a hallmark of HL cells and crucial for cell survival. Vice versa, treatment of immune effector cells resulted in an increase of surface activation markers.

To investigate the impact of HDAC inhibition on tumor cell- bystander cell interactions, IFNγ and TNFα secretion was measured (ELISA) in the supernatant of HL- and peripheral blood mononuclear cell co-cultures exposed to LBH589. IFNγ and TNFα are known to be important components of the HL cytokine network. Interestingly, a dramatic decrease of IFNγ was detected in response to LBH589 treatment while TNFα secretion was enhanced. The IFNγ release was dependent on direct cell-cell contact and mediated by CD3-positive cells, whereas the TNFα secretion was independent of cell-cell contact.

Our results show that the HDAC inhibitor LBH589 induces apoptosis in HL cell lines, targets surface molecule expression and alters the cytokine release pattern in lymphocyte co-cultures. Based on this data, we think that LBH589 mediated HDAC inhibition may have an impact on the cytokine network in the HL microenvironment that counteracts tumor cell survival.

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P2.8 ANTI-PROLIFERATIVE EFFECT OF CISPLATIN, DOXORUBICIN AND EPIRUBICIN IN TWO PROSTATE CANCER CELL LINES

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Introduction: Prostate cancer (PC) is one of the most common in men, representing a major cause of death worldwide. Hormone therapy has so far been used as one of the first therapeutic approaches in early stages of PC. However, in more advanced stages, PC doesn't respond to hormone therapy. Until now, no effective therapy was found for PC in more advanced stages. Chemotherapy has been shown to reduce pain and prolong life of patients with PC, offering them a longer and better quality of life. Several studies suggest that application of chemotherapy in PC should be further explored. The main goal of this study is to evaluate different responses induced in two PC cell lines that differ in hormonal receptors expression when treated with three different citotoxic drugs applied in clinical practice.

Material and methods: Studies were performed in two PC cell lines obtained in ATCC: LNCaP (androgen and estrogen dependent) and PC3 (androgen and estrogen independent). In order to evaluate cell proliferation after chemotherapy treatment, we used colorimetric test MTT. Cells were incubated with different concentrations of cisplatin, doxorubicin and epirubicin for different periods of time and cell proliferation was evaluated through MTT assay.

Results: Doxorubicin and epirubicin, compared with cisplatin, have demonstrated a more powerful anti-proliferative effect in both cell lines under study. For the same drug, the inhibition of cell proliferation is higher in the LNCaP than in PC3 cell line. On the other hand, as the incubation time with cisplatin increases, IC50 (half maximal inhibitory concentration) decreases for both cell lines. It was observed the same with doxorubicin and epirubicin up to 72 hours of incubation. However, it appears that after 96 hours of incubation with these two drugs, PC3 cell line shows an increase of the IC50 value.

Conclusion: Through this work it can be concluded that LNCaP cells (responsive to androgens and estrogens) are more sensitive to chemotherapy, namely the results obtained with doxorubicin or epirubicin. Thus, chemotherapy application in the context of the PC should be reassessed. However, more studies are required to clarify the effect of these drugs in PC.

P2.9 PRECLINICAL VALIDATION OF ANTI-AXL RECEPTOR TYROSINE KINASE MONOLOCLONAL ANTIBODIES FOR PANCREATIC IMMUNOTHERAPY

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Pancreatic cancer is the fourth leading cause of death in both men and women with a 5-year survival rate of 5%. Investigation on therapeutic targets has been increased in order to provide new treatments. The tyrosine kinase receptor Axl is an oncogene belonging to the TAM family and is up-regulated in about 63% of patient with a pancreatic adenocarcinoma.

We generated anti-Axl monoclonal antibodies to validate Axl-RTK as potential therapeutic target in this pathology. Among all the anti-Axl antibodies produced, we selected two by their capacity to inhibit Axl receptor phosphorylation induced by Gas6 ligand. Moreover, in vitro experiments with these antibodies demonstrated an inhibition of pancreatic cell lines proliferation and migration. In vivo, these two mAbs attenuate several pancreatic xenografts growth in nude mice. Our data suggest that anti-Axl mAb could be envisaged as a new therapeutic way against pancreatic cancer pathology.

P2.10 MUSHROOM EXTRACT INCREASES P53 EXPRESSION AND CAUSES CELL CYCLE ARREST AND APOPTOSIS IN A BREAST CANCER CELL LINE

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Mushrooms are part of the sexual life cycle of particular fungi with specific metabolic pathways, and therefore may contain a largely unexploited source of powerful new

pharmaceutical products with potential antitumor properties [1,2]. Furthermore, they may have potential as functional foods. *Suillus collinitus* is an edible mushroom found in European pine forests. The aim of this work was to study the cytotoxic potential of extracts from this mushroom in various cancer cell lines.

Different extracts (methanolic, ethanolic and aqueous) were prepared and extract-induced cell growth inhibition was assessed with the sulforhodamine B assay in four human tumour cell lines (lung, breast, colon and gastric cancer). The methanolic extract was further characterized in its phenolic composition by HPLC-DAD. The effects of the extract on cell cycle profile and apoptosis were evaluated by flow cytometry and the effect on the expression levels of proteins related to cell cycle and apoptosis was further investigated by Western blotting.

Regarding cell growth inhibition, the methanolic extract was the most potent one, particularly in MCF-7 cells (GI_{50} 25.2±0.2μg/ml). Moreover, the GI_{50} concentration ninduced a G1 cell cycle arrest, with a concomitant decrease in the percentage of cells in the S phase. Furthermore, it caused an increase in the percentage of apoptotic cells, from 6.0±0.2% in untreated cells, to 15.3±2.0% in treated cells. In addition, 48h treatment with the GI_{50} concentration caused a strong increase in the levels of p53, p21, cleaved caspase-3 and cleaved PARP, together with a decrease in Bcl-2. The main components identified in the methanolic extract were: protocatechuic acid (5.2±0.2mg/kg dw), p-hydroxybenzoic acid (14.1±1.2mg/kg) and cinnamic acid (1.3±0.2mg/kg).

Results indicate that *Suillus colinitus* is a promising source of bioactive compounds. Particularly, its methanolic extract appears to have a p53-mediated effect on the normal cell cycle distribution and apoptosis induction in human breast tumor cells.

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P2.11 THE IN VITRO AND IN VIVO EFFECT OF ASCORBIC ACID IN A MELANOMA CELL LINE: A NEW MECHANISM OF ACTION?

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Introduction: Malignant melanoma is a type of skin cancer that affects younger population. In the metastatic stage it is extremely difficult to treat and does not respond to current therapies. Ascorbic acid (AA) is the reduced form of vitamin C. As an antioxidant, the main role of vitamin C is to reduce oxidative stress. However, this nutrient may have a pro-oxidant activity, promoting the formation of reactive oxygen species (ROS) that can induce cancer cell death revealing a potential therapeutic of AA in cancer. The aim of this work is to evaluate, the effect of AA in a melanocytic melanoma cell line (A-375 cells).

Methods: A-375 cells were cultured in DMEM supplemented with 10% fetal calf serum and incubated with different concentrations of AA during 48 hours. The half maximal inhibitory concentration (IC50) was calculated after 24 and 48h by MTT assay. In order to evaluate cell survival and proliferation, clonogenic assays were performed. Flow cytometry was performed to determine cell viability and death, ROS production and cell cycle. The expression of protein kinase C alpha (PKC-α) was determined by western blot assay. In order to verify in vivo the evolution of tumor growth, Balb/c nu/nu xenografts were daily submitted to intraperitoneal therapy with AA.

Results: AA induces a decrease in cell proliferation and survival in a dose dependent manner, being the IC50, after 24h and 48h, of 1.34 mM and 1.01 mM, respectively. However, we didn't observe a cytotoxic effect when cells are treated with AA in lower concentrations, nor cell cycle or PKC-α expression alterations. Even when cells were treated with 10mM of AA, cell death only reaches 17.3%. This is accompanied by a small increase in intracellular peroxides and superoxide radical levels. The in vivo studies suggest that AA, daily administered, inhibits tumor growth.

Conclusion: AA induces a decrease in cell survival and proliferation in A-375 cells, that is supported by the in vivo studies. These results suggest that AA may have a potential anti-cancer effect in melanoma cell lines.

P2.12 THE ROLE OF GOSSYPOL IN THE TREATMENT OF HEPATOCELLULAR CARCINOMA: STUDIES WITH 18F-FDG

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Introduction: Hepatocellular Carcinoma (HCC) overexpresses GLUT1 which is related with a promotion of tumorigenesis. The suppression of GLUT1 expression by siRNA significantly impaired tumorigenicity of HCC cells, suggesting GLUT1 as an innovative therapeutic target for this aggressive tumor.

Positron Emission Tomography (PET) using the radiolabeled glucose analogue 18F-FDG enables the detection of increased glycolysis events in cancer cells. Glucose is transported into the cell mainly through the GLUT1 and 3.

Gossypol is a natural compound that has demonstrated anticancer effect in several tumor types. It is known that gossypol is a competitive inhibitor of the GLUT1. The aim of this study is to test the anticancer effect of gossypol in HCC cell lines, as well as check its effect on 18F-FDG uptake.

Methods: The cell lines used are HepG2 (wp53), HuH7 (mp53) and Hep3b2.1-7 (p53 null). These cell lines were propagated in the presence of gossypol in several concentrations. Cell proliferation was evaluated by the MTT test, in order to obtain dose-response curves.18F-FDG was incubated in a cell suspension with 2×106 cells/ml (25μCi/ml) in cells pre-incubated with gossypol and control cells. Samples were collected to Eppendorf tubes for tracer uptake calculation. Eppendorfs were then centrifuged and radioactivity of cell pellets and supernatants was measured with a well-type gamma counter.

Results: This study demonstrates that the concentrations of gossypol necessary to achieve the IC50 is higher for HuH7 cell line (IC50(24h)=7.5μM). Interestingly, the cell line more sensitive to this compound is Hep3B2.1-7 (IC50(24h)=2.6μM), which may be related to the fact that these cells do not express p53. The cellular response is dependent on time. for each of the cell lines studied. In addition, for the three cell lines studied, gossypol was able to decrease the percentage of 18F-FDG uptake in the similar way.

Conclusions: These results shown that gossypol has anti-proliferative effect on HCC. Gossypol also has shown ability to decrease the uptake of 18F-FDG in the three cell lines. More studies must be done to clarify the role of p53 in the anti-proliferative effect of this compound in HCC as well as the effect in the uptake of the 18F-FDG.

P2.13 FURTHER INSIGHT INTO COMBINING OMBRABULIN VASCULAR-DISRUPTING AGENT WITH DOCETAXEL IN EXPERIMENTAL MODELS

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Ombrabulin (AVE8062), a synthetic, water-soluble derivative of combretastatin, is a vascular disrupting agent (VDA) binding tubulin at the colchicine site and destabilizing microtubules in tumoral cells and endothelial cells of tumor vasculature. In a range of early- and advanced-stage solid tumors, ombrabulin selectively shuts down the tumor blood supply within the neoplastic tissue leading to extensive necrosis of the tumor core. As for other VDAs, the central tumor necrosis is surrounded by a narrow rim of viable tumor cells leading to tumor regrowth following treatment termination. To eradicate the entire tumoral cell population, ombrabulin is thus combined with cytotoxic agents. Here the combination of ombrabulin with the microtubule stabilizing agent docetaxel was studied in mice bearing mammary carcinoma xenografts, using caliper measurements to assess tumor volume, non-invasive contrast-enhanced ultrasound imaging (CE-US) to monitor tumor perfusion and quantitative whole-body autoradiography (QWBA) to follow tissue distribution of co-administered radiolabeled [14C]-docetaxel. In vivo efficacy studies showed that the schedule of administration for optimal antitumor efficacy in combination was an intermittent schedule with ombrabulin given 24 hours ahead of docetaxel repeated over 2 sequences 5 days apart (ie ombrabulin administered on day1 and day6 and docetaxel administered on day2 and day7). Therapeutic synergy in combination was obtained with this schedule of administration. Correlative studies using CE-US during the first sequence of combined therapy evidenced the early pharmacodynamic effect of ombrabulin on tumor perfusion, with blood-flow shutdown 24 hours after administration of ombrabulin followed by reperfusion from the periphery of the tumor at 48 hours. QWBA studies revealed limited tumor exposure to docetaxel after the first sequence in line with potent inhibition of tumor perfusion and extensive tumor necrosis, whereas high docetaxel concentrations were measured in the tumor rim after the second sequence. In vivo efficacy studies with alternative schedules confirmed that both sequences of combined treatment contribute to the overall antitumor activity as measured by tumor growth inhibition, although through seemingly distinct mechanisms.

P2.14 SUBCUTANEOUS ADMINISTRATION OF A THROMBOSPONDIN-1 MIMETIC PEPTIDE NORMALIZES TUMOR VASCULATURE, INCREASES DRUG UPTAKE AND INDUCES REGRESSION OF ADVANCED OVARIAN CANCER

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Epithelial ovarian cancer (EOC) is the most lethal gynecological cancer and is detected at late clinical stage in more than 80% of the cases. To study this disease, we have generated an orthotopic, syngeneic mouse model that results in the formation of large primary ovarian tumors, numerous secondary lesions throughout the peritoneal cavity, and abundant ascites, which are symptoms that closely replicate human EOC. Treatment of the disease typically involves cytoreductive surgery followed by chemotherapy. Alternative therapeutic approaches have focused on targeting the tumor vasculature through the use of anti-angiogenic compounds such as thrombospondin-1 (TSP-1).