of this observation needs further investigation. Around P11, GluR5 expression shifts to oligodendrocytes. The dynamic upregulation of GluR5 during the postnatal period may contribute to white matter development and may influence the vulnerability of developing white matter to excitotoxic insults.

BMS 9

Enhanced efficacy of bioactive compounds: Targeting isoprenylation in cancer cells to mediate apoptosis

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Aims: The rate limiting enzyme for mevalonate synthesis in eukaryotic cells is 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Hmger) and products of mevalonate synthesis play a key role in tumorigenesis (Li et al., 2002). Isoprenoids have the potential of inducing cancer cell death (apoptosis) by inhibiting Hmger. Although isoprenoids gave promising results in the treatment of cancer in pre-clinical trials (Bifulco, 2005), effective doses were poorly tolerated in cancer patients due to toxicity. The study aims to establish a combinatory treatment of isoprenoids with Rapamycin, an mTOR (mammalian target of Rapamycin) inhibitor, suggested to be a potential chemotherapeutic sensitizer (Shi et al., 1995). The combinatory treatment will serve to sensitize tumour cells for induction of apoptosis by isoprenoids, enhancing the therapeutic index of isoprenoids.

Methods: Cell culturing and pharmacological inhibitors: 3 cell lines (A549, PC3, C32) were chosen from a panel of cell lines. Pharmacological inhibitors used were Rapamycin, Limonene, Perillyl alcohol, alpha-pinene. Cytotoxicity assays (XTT) were performed on all cell lines alone or in combination and read at 490nm on microplate reader. Annexin V assay was used for apoptotic quantification by flow cytometry. Western blot analysis measures phosphorylation and activation of proteins using 4EBP and p70 S6 kinase antibodies.

Results: To evaluate the effect of growth inhibition and apoptosis, cells were cultured in the presence of Rapamycin and Isoprenoids. Preliminary data show that exposure of Rapamycin triggered response in PC3 and A549 whilst response was not observed for C32. All cell lines were differently sensitive to Isoprenoid exposure. Combinatory treatment with both drugs was performed, lowering the dose of Isoprenoids needed for apoptosis. Western blot analysis was carried out and kinase activity was shown to be abrogated in presence of Rapamycin.

Conclusions: We propose the synergistic combination of isoprenoids with the mTOR inhibitor, rapamycin (CCI-779) to enhance apoptosis and reduce toxicity. Hence this combinatory treatment might be an effective treatment option in patients with specific solid tumours. The identification of sensitive cell lines will allow further evaluation of molecular mechanisms that initiate apoptosis.

BMS 10

In vitro investigation of anti-osteoporotic bioactivity of extracts from indigenous plants and investigating whether these induce an oestrogen growth factor response

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Aims: The main aim of the study is the production of an extract from a local indigenous plant that induces the differentiation of the osteoblast cell line MC3T3-E1 without inducing an estrogen growth factor response in the breast cancer cell line MCF-7.

Methods: A wide range of different methodologies have been used. Methanol extraction has been utilized for the extraction process. Using the osteoblast cell line MC3T3-E1, titrations were performed so as to obtain the working concentrations for beta-oestradiol, the extracts, and an already commercialized product (Lignan). To determine the cytotoxicity, XTT assays were performed on the different compounds tested. A chemiluminescence method has also been utilized to directly measure the osteogenesis rate by measuring the uptake of the stain Alizarin red for calcium mineralization (bone matrix). Western blotting is being used to measure the activation of the Estrogen Receptor (ER-alpha) by using a specific phospho-antibody. Real time-PCR will be utilized for expression studies of identified oestradiol response genes.

Results: Using methanol reflux, two different extracts have been produced. The working concentrations of the test compounds have already been identified and XTT assays have showed us that none of the test compounds tested (beta-oestradiol, lignan, and carob pod extract) is cytotoxic. The osteogenesis rate has been quantified and after 15 days, a 3.6 fold increase in osteogenesis has been obtained with beta-oestradiol and a 7.6 fold increase in osteogenesis has been obtained with Lignan, when compared with a no factor control. Out of the 2 extracts produced, one of them did not produce any significant results. However, pilot studies conducted on the second extract have shown very promising results and the current experiments being performed will determine the ideal working concentration of this extract. We also confirmed the work done by Takamizawa et al (2004) where whilst ascorbic acid 2-phosphate stimulates osteogenesis, ascorbic acid showed a repressive effect depending on concentration used.

Conclusions: To date we can conclude that both beta-oestradiol, and Lignan increase the rate of osteogenesis. Although the primary extract did not produce any significant results, pilot studies on the secondary extract have shown us that the results are very promising. The effect of these test compounds on ER-alpha and on oestradiol response genes still needs to be determined.

BMS 11

The characterization of c-Kit mutations in Gastrointestinal stromal tumours

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Aims: Gastrointestinal stromal tumours (GISTs) are specifically mesenchymal tumours arising throughout the gastrointestinal tract. The incidence is of approximately 2/100,000, with a 5-year survival in 50% of the patients. Up to 80% of GISTs are CD117 positive due to a mutation in the c-Kit, whilst Platelet Derived

Growth Factor Receptor Alpha (PDGFRA) mutation is mostly characterized by CD117 negativity. Surgery is the main treatment in cases of benign and located GISTs, and imatinib mesylate is used in cases of metastasis or non-operable cases. The c-Kit mutations which respond to imatinib therapy are found in exons 11 and 9 whilst those resistant are found in exons 13 and 17. Considering the recent immunohistochemical discoveries and the increase in identification involving diagnosis and clinical approach in GIST, the aim of this retrospective study is to assess the sensitivity of various molecular techniques used to identify mutations in KIT hotspot exons 11, 9, 13 and 17 by using DNA and RNA isolated from formalin-fixed-paraffin-embedded (FFPE) tumour material from GIST patients. The main objective is to identify the genetic mutational profiles of c-Kit in GIST patients. The mutational state will also provide a molecular classification of patients and give information on prediction of therapy outcome that can be introduced in the diagnostic service.

Methods: 1. Microscopical examination will be performed on tumours which are diagnosed as leiomyoma, leiomyosarcomas and GISTs found throughout the gastrointestinal tract. 2. The pressure cooker antigen retrieval technique using Citrate buffer pH6 will be used for the immunohistochemical immuno-peroxidase technique using Avidin Biotin complex (ABC) to stain the slides with CD117. 3. After staining, CD117+ and CD117- samples will be identified, recorded and the CD117+ samples will be subjected to the study. From the formalin fixed paraffin embedded (FFPE) blocks, sections will be cut using a microtome. 4.

Mutation Analysis – Nucleic acid (DNA/RNA) will be isolated from the sections and Polymerase chain reaction (PCR) will be the method used to amplify the hotspot exons of c-Kit gene for patient samples that are CD117 positive (exons 9, 11, 13 and 17). The PCR products will be cloned to distinguish between neoplastic and non-neoplastic DNA/RNA material so as to select and identify the underlying mutation in the tumour tissue and to increase the sensitivity of the sequencing analysis.

Results: The project shall yield the percent CD117 positive GIST patients as diagnosed with the current classification. Currently the immunohistochemical detection of CD117 has been optimised and the retrospective analysis of 42 samples has commenced. Using a c-Kit positive sample, various DNA extraction protocols have been investigated to allow the efficient isolation of good quality DNA. The PCR for c-Kit exons 9 and 11 has been optimised on patient material. The mutations associated with imatinib sensitivity will be documented following molecular characterisation.

Conclusions: The identification of mutations that are associated with imatinib sensitivity shall be useful to implement this treatment regime in the current therapy. The introduction of molecular techniques to classify patients into therapeutic group allows individualised therapy for better patient quality of life.

BMS 12

Differentiation effects of biological extracts and histone deacetylase inhibitors on HL60 leukaemia cells in combinationwith retinoic acid

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Aims: Differentiation Therapy with retinoic acid has become a therapeutic solution for a previously commonly fatal disease, Acute Promyelocytic Leukaemia. It has the added benefit of reduced side effects by comparison to chemotherapy. This treatment is unfortunately limited to one rare leukaemia. Various biological extracts and known DNA modified are used to induce differentiation in leukaemia cell lines which are less susceptible to retinoic acid.

Methods: Leukaemia cells were exposed to the different biologicals followed by retinoic acid. Markers of granulocytic diffentiation were detected by means of nitro blue tetrazolium (NBT) reduction. The tested extracts were derived from Maltese endemic plant Darniella melitensis as well as a number of holometabolous insects.

Results: Protease treated insect conditioned medium was effective in inducing HL60 differentiation as a pre-treatment to retinoic acid. Certain histone deacetylase inhibitors were similarly effective. On the other hand, an organic fraction from the insect conditioned medium was very active in inducing HL60 differentiation, when used alone. Extracts of Darniella melitensis were effective in inducing differentiation in diverse leukaemia cell lines.

Conclusions: This research may help expand the spectrum of leukaemias susceptible to differentiation therapy. This may be both through enhancing the effect of retinoic acid as well as via other independent mechanisms.

BMS 13

Investigating the angiotensin converting potential of naturally occuring terpenes using in silico models

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Aims: Preliminary biological assays with naturally occurring terpenes are indicative of their Angiotensin Converting Enzyme (ACE) inhibitory potential. The aim is to establish a robust predictive in silico tool to qualify and quantify ACE inhibition of a series of terpenes, and to make sound recommendations as to their viability for inclusion in more extensive drug design strategies that seek to identify novel agents within this highly utilised antihypertensive pharmacological class.

Methods: Initial X-ray Crstallographic Models were identified from the Protein Data Bank. These described one holo- and two apo- forms of the receptor- one bound to captopril and the other to enalaprilat. Theterpene series included in the study were constructed and structurally optimised using SYBYL. They were then docked into the ligand binding pocket of the ACE using the bound co-ordinates of captopril and enalaprilat as templates. The binding affinity of each member of the terpene series to the receptor was calculated using SCORE, and compared to those of captopril and enalaprilat. Comparison of binding modality between the terpene series and captopril and enalaprilat was also carried out.