PO-013 A NOVEL PEPTIDOMIMETIC TARGETING NRP1 INCREASES RADIOSensitivity OF MEDULLOBLASTOMA STEM CELLS

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Material and methods Several HCC cell lines were used to compare phenotypical features, susceptibility to platelets and drug-mediated effects. To determine effects on cancer cell features, in vitro cell growth and transwell invasion assays were performed. Furthermore, the underlying mechanism of a platelet-modulated drug response on the molecular level was explored.

Results and discussions In HCC cells, the anti-proliferative potency of sorafenib was counteracted by platelet factors and the mesenchymal phenotype. However, resminostat alone and in combination with sorafenib effectively triggered an anti-proliferative response independently of platelets or the mesenchymal phenotype. Therefore, resminostat determined the anti-proliferative response of the drug combination. Moreover, recent reports highlight HCC cell subpopulations which express cancer stem cell genes and harbour clonogenic growth and cell invasive capacities as critical for metastasis. Intriguingly, we found that platelets induced the cell-invasive capacity in HCC cells with detectable levels of several cancer stem cell markers and which featured a mixed epithelial-mesenchymal phenotype. Importantly, only the combination of resminostat with sorafenib, but not the mono-treatments, significantly reversed the platelet-induced cell invasion.

Conclusion Our pre-clinical data provide evidence on how platelets mediate pro-tumorigenic effects and modulate the therapeutic response to the resminostat/sorafenib drug combination. Platelet factors negatively modulated the drug response to sorafenib. This was overcome by the anti-proliferative activities of resminostat. Importantly, providing an explanation for the clinical benefit of the combination therapy, the platelet-induced invasive capacity was reversed only by the combination of resminostat with sorafenib, but not the mono-treatments.

PO-014 DIANHYDROGALACTITOL (VAL-083) REDUCES GLOBLASTOMA TUMOUR PROGRESSION IN VIVO, UPON BEVACIZUMAB-INDUCED HYPOXIA

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Introduction Medulloblastoma (MB) is the most common paediatric malignant brain tumour. Recurrences occur in more than 40% of cases and sequelae are very important due to aggressiveness of the treatments. Cancer stem cells (CSCs) generate tumours through the stem cell patterns of self-renewal and differentiation into multiple tumour cell types and have better DNA repair capability inducing tumour resistance to radiotherapy (RT) and chemotherapy. Neuropilin-1 (NRP1) is involved in the progression of MB and seems to be in relation with the differentiation state of cancer cells. Recent molecular research has provided a better understanding of tumour development for the purpose of more targeted treatments. MR438 is a new sugar-based peptidomimetic targeting NRP-1. Our first results showed that MR438 seemed to induce the differentiation of MB stem cells. The objectives were therefore to demonstrate the effect of MR438 on in vitro and in vivo radiosensitivity.

Material and methods DAoy, D283Med and D341Med cell lines were used for obtaining cancer stem cells by in vitro enrichment. Clonogenic assays were performed on MB stem cells exposed to 0, 2, 4, 6, 10 Gy of RT in combination with MR438. For in vivo experiments, xenografted nude mice with 3 subgroup tumours were treated by RT at 2 Gy x 5 days in combination with MR438 and compared to Tufts in in 6 groups (control, MR438, Tufts, RT, RT +MR438, RT +Tufts, n=6). Tumour volume was measured by caliper until a maximum of 45 days post-treatment, and then tumours were removed at the set end-points for clonogenic assay and cell viability.

Results and discussions Inhibition of NRP-1 via MR438 increased radiosensitivity of CSC models especially at the dose of 2 Gy. The DMF2 were 0.74, 0.89 and 0.88 for DA0Y, D283-Med and Med-D341 cells respectively. In heterotopic models, a significant improvement of tumours radiosensitivity was also observed in the MR438 +RT group by comparing RT alone or MR438 alone (p<0.01). In an interesting way, the self-renewal capacity for CSCs after tumour dissociation was also decreased significantly when tumours were treated by MR438 +RT versus RT (p<0.05).

Conclusion This work showed the interest of targeting NRP-1 in association with radiotherapy to limit MB progression in decreasing the stem cells number in these tumours. Moreover, our in vivo experiments proved the possibility to use MR438 peptidomimetic as a radiosensitizing agent for treatment of MB.
Results and discussions VAL-083-treated mice showed significantly smaller tumours compared to control (−83% for VAL-083, p<0.001) and bevacizumab (−75% for VAL-083, p<0.001). Additionally, analysis of tumour growth in time showed significantly reduced tumour progression for VAL-083 + bevacizumab compared to VAL-083 alone (p<0.01).

Conclusion These results show strong in vitro efficacy of VAL-083 alone in reducing tumour growth of an MGMT-unmethylated, recurrent GBM tumour and potentially even better for VAL-083 + bevacizumab.

PO-015 POTENTIATING ANTI-NEOPLASTIC EFFECT OF CISPLATIN BY A PROTEIN ARGinine METHYLTRANSFERASE 5 SELECTIVE INHIBITOR IN LUNG ADENOCARCINOMA CELLS

Introduction Protein arginine methyltransferase 5 (PRMT5) is an enzyme that is greatly implicated in diverse cellular processes, including transcriptional regulation, RNA metabolism, and cell-cycle regulation. It was found in previous studies to be highly expressed in cancers including lung adenocarcinoma, Hepatocellular Carcinoma (HCC), and melanoma; in amounts much more significant as compared to benign tissues; raising evidence that PRMT5 is involved in tumorigenesis. Recent studies have proven that inhibition of PRMT5 in HCC cells through the use of AMI-1, a water soluble selective protein arginine methyltransferase inhibitor significantly reduces the proliferation and migration of HCC cells. It was also found that β-catenin is a target of PRMT5, therefore inhibiting PRMT5 resulted in the silencing of β-catenin; as well as its downstream cell-cycle regulator cyclin D1. The aim of our study is to investigate the effect of PRMT5 inhibitor (AMI-1) on the proliferation, migration and survival of lung adenocarcinoma cells.

Material and methods We compared the effect of AMI-1 on lung adenocarcinoma (A549 cell line) suppression with the standard chemotherapeutic agent, cisplatin, by measuring cell viability using MTT assay, PMRT5/β-catenin expression via Western Blotting, the extent of cell migration through wound healing assay, and survival of cancer cells by performing cell cycle progression and Annexin-V staining assays.

Results and discussions Treating the cells with a combination of 10 μM AMI-1 and IC50 of Cisplatin (23.4 μM) significantly decreased cell viability at 24 and 48 hours. Moreover, treatment with both drugs at 48 hours led to a reduction in cell migration measured by the migration rate. AMI-1 induced G2/M arrest in A549 cells at 24 hours. This effect was enhanced in the combined treatment. Furthermore, A549 cells were unable to recycle again as they arrested at G1 after 48 hours of combination treatment. There was a minor cell death induction after 48 hours of treatment with both drugs. Neither PRMT5 nor β-catenin protein levels were affected due to the treatment. However, 48 hours treatment with AMI-1 alone or in combination with Cisplatin reduced the methylation of PRMT5- downstream target, Histone 4.

Conclusion Inhibition of the intracellular enzyme protein arginine methyltransferase 5 by AMI-1 potentiates the anti-cancer effect to cisplatin in the lung adenocarcinoma, with a promising potential role as an adjuvant therapy in lung cancer. The effect is likely mediated by methylation reduction of histone-4.

PO-016 INHIBITION OF CELL PROLIFERATION BY ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) APTAMER CONJUGATED CHITOSAN/SIRNA NANOPARTICLES

Introduction Gene silencing mediated by small interfering RNA (siRNA) has been widely investigated as a potential therapeutic approach. Its use, however, is hampered by its rapid degradation and poor cellular uptake into cells. Therefore, the success will depend on the design of effective systems able to selectively and efficiently deliver siRNA to target cells/organs. Our strategy relies on the use of a biocompatible biopolymer (chitosan) as carrier of siRNA coupled with specific anti-EGFR aptamers for cell targeting which is overexpressed in various cancer cells. Finally, poly (ethylene glycol) (PEG) and diethylaminoethyl (DEAE) will be covalently-linked with chitosan, in order to improve blood residency and transfection efficiency.

The selected siRNA will be directed to silence receptor activator of nuclear factor-kB ligand (RANKL). Its levels are elevated in numerous cancers. Blockage of EGFR by aptamer and knockdown of RANKL by siRNA inhibit cancer cell proliferation in vitro.

Material and methods
1. Synthesise and characterise chitosan conjugates: poly (ethylene glycol) (PEG), diethylaminoethyl (DEAE), and anti-EGFR-aptamer are covalently-linked with chitosan.
2. Synthesise, purify and characterise DEAE/PEG/anti-EGFR-aptamer-chitosan/siRNA nanoparticles
3. Optimise the nanoformulations (adjusting polymeric and charge ratios) through tests in vitro including transfection efficacy and cell proliferation assays in different cancer cell lines.

Results and discussions Nanoparticles were produced on the basis of our previous results. Particle size and zeta potential were measured by Zetasizer Nano ZS90 (Malvern Instruments Ltd., Malvern, UK). The sizes of synthesised nanoparticles were around 259±3 nm for Chitosan-DEAE15/siRNA with a zeta potential of +28.3±0.8 mV. The average cell viability of free siRNA or nanoparticle-treated cells was 89%–97% compared to nonrelated cells. The results showed that anti-EGFR-aptamer-chitosan/siRNA nanoparticles had a dose-dependent inhibition of cell proliferation. These nanoparticles had a significant inhibition effect of RANKL mRNA expression (RT-PCR) assay.

Conclusion Conventional cancer treatments such as chemotherapy have severe side toxicity on both tumour and host cells. Biological targeted agents such as monoclonal antibodies are available, but costly. EGFR and RANKL are two of major targets for drug development for cancer treatment. Non-viral