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Effects of androgen and valproic acid treatment on androgen-dependent cell line (LnCaP-SF)

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Prostate carcinoma (PCa) originates as an androgen-dependent hyper-proliferation of the epithelial cells of the gland and it evolves in an androgen-independent, highly aggressive cancer for which no cure is available to date. Studies in the last two years have corroborated that the short chain fatty acid valproate (VPA) potently modulates the biology of prostate cancer cells by inducing differentiation, inhibiting proliferation and increasing apoptosis. Hence we studied the effect of VPA in combination with dihydrotestosterone (DHT) on the human prostate cancer cell line, LNCaP-SF.

LNCa-SF is an androgen-deprivation induced human prostate cancer cell line, generated from the androgen-sensitive LNCaP cells, cultured in RPMI-1640 media containing charcoal-stripped FBS for a prolong period (more than four months). LNCaP-SF cells express considerably lower levels of androgen receptor than LNCaP cells and grow faster in androgen restricted condition in vitro.

In the human prostate cancer androgen-dependent cell line (LnCaP-SF) the responsiveness to androgen and valproic acid in vitro was exanimate, we observed that VPA was able to down-regulate both AR gene and protein expression, decreasing PSA levels, even in DHT presence. Moreover, LNCaP-SF proliferation was inhibited by VPA treatment of about 45%, with the G1 phase arrest (70%) and induction of apoptosis (29%). In addition we observed that after 72 hours of VPA (2 mM) treatment, cells switched into oblong cells with long dendritic processes losing the rounded up phenotype.

The hormones homeostasis, hence steroids metabolism, in prostate is guaranteed by UGTs which glucuronidate DHT metabolites through an irreversible process. Because VPA is it same a substrate of UGTs, we investigate the effect of VPA on some UGTs expression.

We found that VPA treatment modulates expression of such enzymes (UGT2B7, UGT2B11, UGT2B15) in LNCaP-SF cells, hence we cannot exclude a competitive effect of VPA in steroid catabolism. Although, VPA can reduce PSA and AR expression, decreasing cell proliferation rate, the action of VPA on prostate cancer should be further investigated. Therefore, in view of VPA ability in modulate hormone homeostasis of prostate cancer, LNCaP-SF cell line results a valuable tool for studying its molecular mechanisms.

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Synergistic activation of JAK/STAT and NF-kB pathways by GMCSF/IL2 fusion protein induces robust NK cell proliferation and activation

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NK cells constitute a potential candidate for cancer cell therapy because they express a diverse array of inhibitory and activating receptors, which recognize and kill infected cells or tumor cells without prior immune sensitization. However, autologous NK cell mediated adoptive immuno-therapy is restricted due to insufficient cytolytic activity of NK cells from patients with aggressive malignancies. In contrast, the infusion of alloreactive NK cells has shown more successful outcomes in the treatment of cancer, but this approach also presents difficulties such as the high doses of cytokines required to induce NK cell expansion ex vivo, which may also sensitize NK cells to apoptosis. Therefore, a critical issue for NK cell based therapy is the use of appropriate growth factors or cytokines that promote NK cell expansion and activation. We have previously shown that a murine GM-CSF/IL-2 fusion protein (aka GIFT2) displays novel antitumor properties in vivo compared to both cytokines in combination with regards to tumor site recruitment of significant functional NK cell infiltration. In the present work, we have generated the human counterpart of GIFT2 (hGIFT2). The functionality of both cytokines as part of the fusion protein was verified by performing proliferation assays in vitro with GM-CSF and IL-2 dependent cell lines (TF1 and CTLL-2 respectively). The effect of hGIFT2 on immune cells was analyzed by culturing PBMC with hGIFT2, as well as with both cytokines alone and in combination, and the number of immune cell types was quantified by flow cytometry. As result, hGIFT2 leads to a substantial four folds increase of human blood-derived NK cells which is significantly ($p<0.05$) superior to either IL2 or GM-CSF single cytokine treatment or both cytokines combined at equimolar concentrations. In addition, hGIFT2 induces robust expression of NK-cell activation markers: CD69 and CD107a as well as IFN gamma expression. As mechanism underlying hGIFT2 dependent effects, we determined that IL-2 and GM-CSF as part of the fusion act synergistically to induce greater activation of JAK/STAT and NF-kB pathways than single or combined cytokine treatment. Consequently, hGIFT2 induces significant expression of STAT5 and NF-kB target genes. In conclusion, the human hGIFT2 fusokine is a novel and potent tool for ex vivo expansion of activated NK cells which may be of use in cell-based immunotherapy of cancer.

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Aerosoltherapy in lung cancer

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Background: Although airways route might be an attractive alternative route to systemic administration, increasing concentration of the drug in the lungs while reducing whole-body toxicity, it is not often exploited for lung cancer treatment. In this study, we evaluated the nebulization of an anti-EGFR antibody (cetuximab) and established an animal model of broncho-pulmonary tumour sensitive to this antibody to compare the efficiency to target lung tumors and the pharmacokinetic of cetuximab through pulmonary and systemic routes.

Materials and methods: Cetuximab was nebulized with IA-1C MicroSprayer™ (PennCentury Inc., USA) connected to a FMJ-250 high pressure syringe, a device used to administrate, directly inside the trachea, aerosol in mice. The effect of nebulization on cetuximab was assessed in terms of its affinity for membrane EGFR (using flow cytometry), inhibition of cell growth and inhibition of EGFR phosphorylation. Then, tolerance to cetuximab delivered through airways was studied in mice without tumors. A model of lung cancer sensitive to cetuximab was established and consists in the instillation of human epidermoid carcinoma cells endotracheally in nude mice. Cetuximab was labelled either with a fluorescent dye or 64Cu and then, affinity to EGFR was evaluated in a competition assay by FACS. Optical imaging and microPET were used to follow biodistribution of labelled cetuximab administered through systemic or pulmonary routes in tumor-bearing animals. Blood samples were collected at different time point to analyze pharmacokinetic of cetuximab delivered through different routes.

Results: Firstly, our results showed that MicroSprayer™ did not alter cetuximab integrity, immunological and pharmacologic properties. Secondly, airway administration of cetuximab in mice seemed to be well-tolerated and did not induced additional toxicity in lungs, kidney, colon, skin, liver or spleen. Finally, FACS analysis demonstrated that labelled cetuximab displayed the same affinity to EGFR than the unmodified antibody. And higher, more rapid and prolonged tumor uptake was observed in animals receiving cetuximab through the pulmonary route.

Conclusions: These results highlight the potential of the pulmonary route for delivery of anticancer antibody in lung cancer.

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Investigation of the potential of mesenchymal stem cells (MSCs) for gene delivery to breast tumours

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Background:The use of Mesenchymal Stem Cells (MSCs) as systemic delivery vehicles for therapeutic genes has been proposed as a result of their combined ability to home to the tumour site, and evade the host immune response. Previous studies from this laboratory and others have shown tumor specific migration and engraftment of MSCs, highlighting their potential role as delivery vehicles for therapeutic genes. The sodium iodide symporter (NIS) confers upon a cell the ability to concentrate iodide, and adenovirus mediated expression of this gene in cancer cells has previously been shown to permit imaging and subsequent ablation of transfected tumors using radiolabeled iodide. NIS expression in MSCs could potentially provide for noninvasive imaging of MSC migration and engraftment in vivo, and due to the pathlength of ^{131}I, support ablation of surrounding tumor cells through bystander effect. The aim of this study was to establish membrane bound, functional expression of NIS in adult MSCs derived from healthy volunteers. Materials and Methods:MSCs were infected with an adenovirus containing NIS under the control of the CMV promoter. NIS gene expression was quantified using RQ-PCR, and protein expression and localization was detected by immuhistochemistry. The ability of transduced MSCs to concentrate iodide was determined at a variety of timepoints (1-10 days) following infection using radiolabelled iodide (^{125}I), with levels measured on a ÿ-counter. Potassium perchlorate ($KCLO_4$), a known inhibitor of NIS, was included in control wells. Results:There was an

average 4-fold increase in NIS gene expression in MSCs following infection. Immunohistochemistry showed positive staining for NIS throughout the cytoplasm as well as at the cell membrane. Iodide uptake studies revealed very efficient NIS function, with a 27-60 fold increase in iodide uptake at MOIs ranging from 50-200. Inclusion of the NIS inhibitor perchlorate in wells resulted in 70-85% inhibition of iodide uptake, confirming that it was specifically mediated by NIS. It is noteworthy that NIS expression and function remained significantly elevated 10 days following infection. Conclusion:The preliminary results presented here clearly demonstrate that adenoviral transduction is capable of inducing robust NIS expression and functional iodide uptake in MSCs. This study is an important initial step investigating the potential for use of radiolabeled iodide as an imaging agent to track MSC migration in vivo.

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Evaluation of tumor response to carmustin and sorafenib with magnetic resonance imaging in orthotopic human glioblastoma models xenografted in nude rats

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Despite aggressive therapeutic protocols, malignant glioblastomas remain uniformly fatal. Monitoring changes in gliomas microvasculature should help to evaluate the efficacy of new anti-tumor therapy. The aim of this study was to assess the sensitivity of magnetic resonance imaging (MRI) biomarkers to the anti-tumor activity of Carmustin and Sorafenib in human glioblastoma model.

Nude rats were orthotopically injected at D0 with U87-MG glioma cells. Rats were randomized at D14 to receive either one injection of 10 mg/kg Carmustin (BCNU) i.v. or 14 daily administrations of 100 mg/kg Sorafenib (SORA) p.o. or no treatment (CTL). Rat survival was monitored daily. Blood volume (BV), vessel size index (VSI), apparent diffusion coefficient (ADC) and blood brain barrier permeability to a contrast agent (BBB perm.) were mapped, in tumor, at 2.35T one day before treatment and 1, 4 and 14 days after treatment onset (respectively D13, D15, D18 and D28). Tumor volumes were measured on T_2-weighted images. VSI/BV and BBB perm. parameters were computed from T_2, T_2* and T_1-weighted images using an intravascular contrast agent (ferumoxtran-10, Sinerem®/Combidex®) and P846, a Gd-based contrast agent, both provided by Dr P. Robert, (Guerbet/AMAG Pharmaceuticals). In each group, the same four rats were imaged at each time point. Four additional rats were also imaged per time point and euthanized at the end of the imaging session for histological studies.

Tumor growth in the SORA and BCNU groups were strongly inhibited when compared to the CTL group (4 and 20 fold less, respectively). At D28, ADC in SORA and BCNU groups were 21 and 23% higher than in the CTL group, respectively. At any time, VSI did not differ between BCNU ant CTL groups. VSI in SORA group was significantly increased by 22 to 37% when compared to CTL group at D15 and D28, respectively. BV was not modified by BCNU treatment but was strongly decreased by SORA treatment (5±0.85 at D13 to 2.6±0.99% at D28). While BBB remained permeable in BCNU and CTL groups, SORA-treated tumor became impermeable to P846 as early as 4 days after treatment onset. Despite tumor growth inhibition and vasculature modification, BCNU and SORA displayed a moderate increase of U87-MG tumor-bearing rats survival (ILS=16% and 23%, respectively).

Despite the poor effect of SORA and BCNU treatments on the survival of U87-MG-bearing rats, MRI demonstrated a tumor growth inhibition induced by these 2 treatments. ADC appeared sensitive to both treatment but VSI and BV were sensitive to the effect of SORA only. This is consistent with the anti-angiogenic activity of SORA. Histological data (to be analyzed) will provide further information on SORA efficacy. Together, these results indicate that VSI, BV and ADC parameters measured by MRI would be of value to combine anti-angiogenic with cytotoxic therapies in glioblastomas.

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Poisoning of human topoisomerase II alpha by acridinecarboxamides and related cytotoxins

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Type II topoisomerases govern the topological state of DNA and play an essential part in DNA transactions involved in cell proliferation. They are targets for important cancer drugs that inhibit their DNA cleavage and religation functions by a mechanism known as topoisomerase poisoning. Poisoning involves the formation of a cytotoxic ternary complex between drug, DNA, and enzyme that stabilizes the cleavage complex. We have used recombinant human topo IIa and enzymatic and sequencing methods to explore the molecular interactions that stabilize the ternary complexes of acridinecarboxamide and related topo II poisons. We used agarose gels to assay inhibition of the relaxation of supercoiling, and the production of single and double strand DNA breaks, in pBR322 DNA, and acrylamide gels to determine the nucleotide sequence preferences of the trapped enzyme. All three assays confirm the biologically active acridinecarboxamides DACA, AS-DACA, and 9-amino-DACA as topo IIa poisons. By contrast, inactive analogues bearing morpholino and piperidine sidechains fail to trap the cleavable complex, despite the fact that their sidechains are known to form hydrogen bonding interactions with guanine in DNA in the same manner as 9-amino-DACA. These findings emphasize the specific nature of the molecular interactions between drug, DNA, and protein in the ternary complex, and suggest that the N,N-dimethylamino groups of the active compounds occupy a cavity within the enzyme that is too small to accommodate the larger cyclic sidechains. All measurements indicate that the acridinecarboxamides, as a class, are less effective at trapping the cleavable complex than amsacrine or mitoxantrone. The consensus sequences for enzyme trapping by DACA, AS-DACA, 9-amino-DACA, amsacrine and mitoxantrone, reveal greater similarities in site selectivity between the acridinecarboxamides and mitoxantrone, than between these agents and amsacrine. Nevertheless, there are clear similarities and differences in site preferences for each agent, which if replicated in vivo, implies possible differences in cellular response to different topo II poisons, even of the same structural class. The consensus sequences for the acridinecarboxamides and mitoxantrone contain a GC basepair at the cleavage site, suggesting that in the ternary complex the drug is bound to DNA with its sidechain making its normal interactions with guanine, and that these interactions are essential for topo II trapping.

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Insights into histone deacetylase inhibitors-induced apoptosis in cancer cell lines

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Histone deacetylase inhibitors (iHDACs) are able to induce apoptosis in many chemoresistant cellular models, suggesting that iHDACs could be an alternative treatment in chemoresistant cancer. However, the molecular mechanism regulating the iHDACs-induced apoptosis is far to be clear. The aim of our study is to obtain some insights into the iHDACs-induced apoptosis trying to determine whether there is an universal specific molecular mechanism for this apoptosis, and if this is the case, which are the hallmarks of this molecular mechanism.

We have studied the iHDACs-induced apoptosis in several cellular models of different origin including mouse and human leukaemia cell lines and human pancreatic, glioblastoma, bladder, colon and mammary carcinoma cell lines. In all cases a dose response and a time course for Trichostatin A have been performed. The iHDACs-induced effect on many apoptosis related phenomenon were evaluated (caspases activity, cytochrome c release, bcl-2 family member expression and modification, AIF release and translocation to nuclei, mitochondria depolarization, etc). Our studies have also included DNA microarrays analysis of iHDACs-induced changes on gene expression, followed by RNA interference analysis of putative genes candidates.

In all the different cellular model analysed, we have demonstrated that serine proteases activity, triggers the iHDACs-induced apoptosis. Also, intracellular calcium mobilization seems to be an universal requirement together with the induction of proapoptotic members of the Bcl-2 family (mostly Bax and Bak) and the decrease of antiapoptotic members of this family (mostly Bcl-2 and Bcl-xl). Apoptosis-inducing factor (AIF) is also a mediator of iHDACs-induced apoptosis, but not p53, p21, Bid and caspases, as previously suggested. Our studies also show that in many of the cellular models analysed there is a common pattern of iHDACs-induced changes in expression levels of the signal transduction related dual phosphatases (mostly DUSP-1, 3, 10), suggesting that these enzymes could be putative new targets for the development of new antineoplasic agents suitable for chemoresistant tumors based on the iHDACs-induced apoptotic pathway.