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.