

multitarget tyrosine kinase receptor inhibitor including those important for angiogenesis (vascular endothelial growth factor receptors) with both antiangiogenic and antitumor properties. However, if targeting the function of VEGF leads primarily to significant tumor regression it could also be followed by tumor relapses. One possible strategy to overcome this therapeutic obstacle might be to overexpress anti-vascular factors to destroy tumor vasculature, rather than inhibiting the formation of new vessels. Recently, the angiopoietin (Ang) family has been identified to cooperate with VEGF to control angiogenesis. Among Angs, Angiopoietin-2 (Ang2) is involved in vessel remodelling leading to angiogenesis or vessel regression depending on VEGF activity. Accordingly, it is of interest to investigate whether an Ang2 overexpression may impair tumor growth, in particular in the brain. The aim of this study was to evaluate and compare the effect of an anti-angiogenic strategy (by the use of SU treatment), an anti-vascular strategy (by a tumoral overexpression of Ang2) or the combination of both Ang2/SU, on glioma vasculature, angiogenesis and growth.

**Materials and methods:** 9L glioma cells were implanted in the striatum of Fischer 344 rats. Ang2 was overexpressed by 9L cells from a stable transfection. SU treatment was administered daily 3 days after 9L cell implantation. Four groups of animals were studied: 9L (n=10); 9L-SU (n=7); 9L-Ang2 (n=13); 9L-Ang2/SU (n=11). Tumor volume was analysed by MRI (7T, Bruker). Tumor vascularization was assessed by immunohistochemistry using antibodies against RECA-1.

**Results:** MRI results showed that, at day 18, each treatment leads to a significant reduction of the tumor volume in comparison with non-treated animals, i.e. 72% (9L-SU), 86% (9L-Ang2), 95% (9L-Ang2/SU) suggesting that combined inhibition of VEGF and overexpression of Ang2 enforces glioma regression. Immunohistochemistry study showed a significant decrease in tumor vessel density and length in the 9L-SU group in comparison with the other groups. Interestingly, this anti-angiogenic effect of SU treatment totally disappeared in presence of an Ang2 overexpression.

**Conclusions:** Collectively, our data reinforce the interest to consider anti-vascular strategies for glioma treatment, in parallel to anti-angiogenic strategies.

288

Poster

#### Role of E2F1 in sporadic Burkitt lymphoma formation

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Treatment of Burkitt Lymphoma (BL), an aggressive non-Hodgkin's lymphoma, requires intensive combination chemotherapy that is associated with systemic toxicity. A better knowledge of the molecular basis of BL lymphomagenesis might help to develop less toxic treatments. The molecular mechanism underlying sporadic BL (sBL) formation still remains largely unknown. No single genetic lesion, besides c-myc translocation has been associated to the pathogenesis of most sBL cases to date. Patients would benefit from novel therapies aiming at specific molecular markers that have yet to be identified. To gain insight into the molecular basis of sBL tumor formation we looked for genes whose expression was deregulated in most sBL cases and may therefore have a role in lymphomagenesis. Because c-myc induces the expression of activators E2F family members and they can behave like oncogenes in mouse models, we compared their expression in BL and lymphoblastoid B-cell lines (LCLs) and found that both mRNA and protein E2F1 levels, but not those of E2F2 and E2F3, were higher in BL than in LCL cell lines. Using qPCR and immunohistochemistry we determined that the relative expression of E2F1 mRNA and protein was also much higher in tumor samples derived from sBL patients than in control tissues or in samples from other B cell lymphomas. The elevated E2F1 expression is not a consequence of the high proliferation rate of these cells, because other lymphoma samples with a similar proliferation index showed no increased E2F1 expression. To assess whether elevated E2F1 is required for tumorigenicity of sBL we reduced E2F1 expression by specific shRNAs in DG75 sBL cell line. Reduction of E2F1 expression greatly decreases their ability to form colonies in soft agar and their tumor formation capacity when inoculated in immunodeficient SCID mice, reduces their proliferation capacity, causes their accumulation in the G2/M phase of the cell cycle and leads to hyperploidy. These findings indicate that E2F1 is involved in sBL formation and suggest that it might collaborate with c-myc in sBL lymphomagenesis by eliciting progression through G2/M. Since E2F1 is not required for proliferation of most normal cells and, in fact, E2F1-/- mice are viable until old age, our results suggest that E2F1 is a promising target for developing novel and less toxic treatments of sBL. Moreover, our

data show for the first time that over-expression of any E2F family member might play a positive role in the formation of a human tumor.

289

Poster

#### siRNA mediated downregulation of Bcl-2 in small cell lung cancer cell lines - impact on protein level and cell viability

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**Background:** The identification of activated proto-oncogenes has made possible to consider such genes as targets for gene silencing-based therapies. One of such targets is the BCL2 gene, known for its anti-apoptotic activity and overexpressed in most cases of small cell lung cancer (SCLC). The success of any gene silencing strategy depends on three main factors: the achievement of a suitable pharmacokinetic and biodistribution profile of the gene silencing drug; relevance of the target gene for tumor viability; and the potency and selectivity of the gene silencing drug. In this context, the objective of the present study was to evaluate the in vitro potency of different gene silencing molecules in terms of Bcl-2 downregulation and cytotoxicity against SCLC cells, as well as to evaluate the relevance of BCL2 as a molecular target in this type of tumor.

**Materials and methods:** An antisense oligonucleotide (ODN) and two small interfering RNA (siRNA) sequences targeting specifically the BCL2 mRNA at different positions, were transfected into SCLC cells that overexpress the Bcl-2 protein. The levels of Bcl-2 protein were determined through flow cytometry. Cell growth inhibition was evaluated by the resazurin reduction method, whereas cell viability was evaluated by flow cytometry with 7-aminoactinomycin D staining in direct correlation to Bcl-2 protein signal. Combined treatments of anti-BCL2 ODN or siRNA and the cytotoxic drug cisplatin were evaluated using both the Bliss Independence and Loewe criteria, and error propagation applied to infer about the uncertainty of the predictions of additivity.

**Results:** In experiments where tumor cells were submitted to 1 or 2 treatments with G3139 ODN or anti-BCL2 siRNAs, a significant sequence-specific downregulation of Bcl-2 protein levels was observed that, surprisingly, did not cause any sequence-specific inhibition of cell growth. Under treatment conditions resulting in the strongest Bcl-2 downregulation, anti-BCL2 siRNA-treated SW2 cells presented higher viability than cells treated with a non-targeted sequence. Combined treatments of anti-BCL2 ODN or siRNA and the cytotoxic drug cisplatin were merely additive on cell growth inhibition.

**Conclusions:** Overall, these results point out for the higher potency of siRNA, as compared to ODN, in silencing the BCL2 oncogene, but most importantly, question some previous established concepts on the role of Bcl-2 protein in SCLC cell lines.

290

Poster

#### Towards combined treatments targeting Met and downstream survival signals: from cell biology to preclinical trials

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Studying the nature of Met oncogene signals and establishing therapeutic approaches to target Met-triggered neoplasia represent two major aims of our laboratory.

The Met oncogene plays a major role during tumorigenesis, by conferring to neoplastic cells growth advantage, invasive abilities and decreased sensitivity to therapeutic agents. It is hence not surprising that Met is a prognostic marker for a variety of tumours. More recently Met received further attention as it can confer resistance to EGFR inhibitors in lung, medulloblastoma multiforme and breast tumours, by substituting EGFR activity. We recently uncovered novel mechanisms involving well-known proto-oncogenes, which are activated by Met to promote hepatocyte survival in developing livers. First, Met acts through the PI3K-Akt pathway to induce the translation and nuclear translocation of Mdm2, which is an endogenous antagonist of p53 activity and cell death. Besides, Met triggers Mdm2 transcription through a novel molecular mechanism currently under investigation. Interestingly, we found that these pathways also contribute to the survival and the anchorage-independent growth of cancer cells with aberrant Met.

Based on these findings, we are developing new therapeutic approaches combining drugs targeting both Met and its survival signalling targets. With