to low oxygen tension conditions was achieved through the impairment of ubiquitin-dependent HIF-1α degradation involving the molecular chaperone HSP90 but it was not dependent on the prolyl hydroxylation of HIF-1α protein. Notably, we also showed that bcl-2, HIF-1α and HSP90 proteins form a tri-complex that may contribute to enhancing the stability of the HIF-1α protein in bcl-2 overexpressing clones under hypoxic conditions.
**Conclusions:** We identified the stabilization of HIF-1α protein as a mechanism through which bcl-2 induces the activation of HIF-1 in hypoxic tumour cells, involving the molecular chaperone HSP90.

### 456 Identification of oncoantigens associated to breast cancer stem cells for preventive antitumour vaccination

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**Background:** Characterization of genes differentially expressed during the stages of tumour progression may lead to the identification of "oncoantigens", tumour-associated molecules that play important roles in driving tumour progression and constitute potential targets for preventive antitumour vaccination. Until now, we have identified putative oncoantigens (POAs) as molecules expressed by mammary cells in pre-neoplastic lesions and over-expressed in evident neoplastic lesions. However, many human malignancies, including breast cancer, are organized in a hierarchical network of rare slowly dividing cancer stem cells (CSCs), rapidly dividing amplifying cells and differentiated tumour cells. CSCs constitute the source of the tumour and could be responsible for tumour progression, metastasis, resistance to therapy and recurrence, so preventive vaccination should target them. Thus, analysis of CSCs transcriptional profiling may identify new POAs, more suitable for effective vaccination.
**Material and Methods:** Mammary tumour specimens were obtained from a cell line derived from BALB-neuT breast carcinomas (Ag12). Cells were plated in differentiative conditions to obtain tumour epithelial cells (e0) or under specific conditions to generate mammospheres (p1), which were then disaggregated and plated to obtain second (p2) and third (p3) passage mammospheres. Expression of CSCs markers on mammospheres was checked by cytofluorimetry. Transcription profiling was performed on e0 and p1−3 using Illumina microarray platform MouseWG-6 v2.0.
**Results:** Mammospheres generated from Ag12 displayed clonogenicity, self renewal, CSCs markers and ability to differentiate in mammary epithelial cells and maintained the tumourigenic potential. 452 deregulated transcripts were detected in mammospheres using rank product statistics, comparing e0 with p1−3. To detect CSC vaccination targets, a subset of 183 transcripts (POAs) which expression increased from p1 to p3 were selected by K-mean clustering. Vaccination targets for breast cancer prevention were selected ranking the 183 transcripts on the basis of the relation between their expression and survival in 7 public human breast cancer transcription profiles. The actual protein increase of some of these POAs in p1−3 was confirmed in ELISA and cytofluorimetric experiments.
**Conclusions:** Mammospheres transcription profiling led to the identification of new POAs. Future experiments will validate these POAs in preventive vaccination in BALB-neuT mice.

### 457 Lysophosphatidic acid induces cell-cell adhesion disassembly and actin cytoskeleton disorganization through an event that requires RhoA-Rock and Src signaling in colon cancer cells

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**Background:** Lysophosphatidic acid (LPA), an extracellular lipid mediator of multiple cellular responses, acts as a potent stimulator of tumour progression triggering different cell signaling pathways that stimulate cell proliferation, migration and survival in colorectal cancer (CRC). Adherens junctions (AJ) disassembly and actin cytoskeleton alterations are initial events of cancer development; however, the cellular mechanisms underlying these phenomenon remain to be defined. The aim of this study was to examine the cell signaling pathways triggered by LPA to mediate alterations of cell-cell adhesion and actin cytoskeleton reorganization during CRC progression.
**Material and Methods:** Cell monolayers of Caco-2, a colon adenocarcinoma cell line, were used as CRC model. Cells were serum starved for 24 h and then treated with 10 μM of LPA for 15 to 60 min or pretreated for 1 h with inhibitors of Rho GTPases, Rho-kinase (Rock), PI3K, PKA, EGFR and Src, before LPA treatment. Changes in the location of AJ proteins E-cadherin, b-catenin and p120-catenin were examined by immunofluorescence, and actin cytoskeleton organization by confocal microscopy using rhodamine-phalloidin. RhoA and Rac1 activation was assessed by the pull-down assay, and Src and FAK activation through immunoblotting of the phosphorylated protein forms. Cell migration was analyzed through the wound-healing technique, and cell viability through the crystal violet assay.
**Results:** LPA treatment induced cell-cell adhesion disassembly, alteration of the actin cytoskeleton organization with stress fibers formation. Pharmacological inhibition of Rho with toxin A from *Clostridium difficile* and Rock with Y-27632 prevented AJ disassembly and actin reorganization caused by LPA treatment. Additionally, Src inhibition with PP2 abrogated p120-catenin redistribution from cell-cell contacts to cytosol induced by LPA. We observed that LPA treatment caused RhoA, Src and FAK activation as evidenced by immunoblotting, however RhoA activation was not prevented by Src inhibition with PP2. Furthermore, by the wound-healing technique we demonstrate that Rho, Rock and Src chemical inhibition also prevented the increase in cell migration LPA-mediated.
**Conclusions:** Our finding indicates that LPA modulates AJ disassembly, actin disorganization and cell migration through a regulatory cascade that integrates RhoA-Rock and Src-FAK signaling pathways in colon tumour cells.

### 458 Inhibition of TGFβ2 production in mouse dedifferentiated hepatoma cells leads to decrease of their tumourigenic and metastatic potential

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**Background:** Cytokines of Transforming Growth Factor (TGF) β family are involved in regulation of cell proliferation, apoptosis, motility and differentiation. Also TGFβ plays dual role in carcinogenesis acting as tumour suppressor or promoter depending on stage of tumour progression and tissue context. Increased levels of TGFβ1 were detected in serum and urine of patients with advanced stages of hepatocellular carcinoma (HCC). While the role of TGFβ1 in hepatocarcinogenesis is actively investigated, the impact of other isoforms in this process is underestimated. Our aim was to investigate the role of TGFβ2 in HCC progression. We have shown that in experimental model of mouse HCC progression highly invasive fast-growing HCC (fgHCC) was characterized with overexpression of TGFβ2 and downregulation of HNF4α, liver enriched transcriptional factor playing a central role in maintenance of hepatocyte morphology and differentiation.
**Material and Methods:** To inhibit production of TGFβ2 in H33 cells culture obtained from fgHCC we used shRNA technique. The effects of TGFβ2 inactivation in H33 cells in vitro were studied by RT-PCR gene expression analysis, proliferation and cell motility tests. To analyze TGFβ2 effects on tumourigenic and metastatic potential of HCC tumour cells were injected subcutaneously into syngenic recipient mice.
**Results:** TGFβ2 inactivation in H33 cells induced re-expression of HNF4α and C/EBPα, transcription factor also essential for the maintenance of hepatic differentiation, and alterations in several TGFβ2 responsive genes expression. Inhibition of TGFβ2 in H33 cells led to growth retardation and decrease of cell motility in vitro. After subcutaneous injection into mice H33-siTGFβ2 cells showed delay in tumour formation and decrease of metastatic potential.
**Conclusions:** TGFβ2-induced activation of TGFβ signaling in HCC cells can contribute to tumour progression increasing tumourigenic and metastatic potential of tumour cells. It can be explained by involvement of TGFβ signaling in regulation of such key properties as proliferation, cell motility and differentiation, probably due to repression of HNF4α and C/EBPα.
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### 459 The FGF-2 binding domain of thrombospondin-1: functional characterization and exploitation to design antiangiogenic compounds

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New blood vessels formed by angiogenesis supply oxygen and nutrients to solid tumours and provide a gateway for metastatic cells to enter the bloodstream and disseminate to distant organs. Thrombospondin-1 (TSP-1), an endogenous inhibitor of angiogenesis, restrains angiogenesis through different mechanisms, including the direct binding to and sequestration of angiogenic factors, in particular fibroblast growth factor-2 (FGF-2). TSP-1 binds FGF-2 through a site located in its type III repeats domain.
We hypothesized that this domain might serve as a template for the development of inhibitors of angiogenesis. Using a peptide array approach, we identified a FGF-2 binding sequence in the type III repeats of TSP-1.

To develop inhibitors of FGF-2 based on this sequence, computational and MRI approaches were used to identify relevant residues and conformational determinants for the TSP-1/FGF-2 interaction. A pharmacophore model was then designed and used to screen a library of compounds leading to the identification of three FGF-2-binding small molecules, which inhibited angiogenesis in vitro and in the chicken chorioallantoic membrane assay. These lead compounds represent the starting point for the development of new TSP-1-mimetic inhibitors of angiogenesis.
To better characterize the functional role of the FGF-2 binding domain of TSP-1 in modulating tumour behavior, we engineered human tumour cells (1A9 human ovarian carcinoma) to over-express either the whole type III repeats domain or its N-terminal region containing the FGF-2 binding sequence, or its C-terminal region lacking the FGF-2 binding sequence. The coding sequences for the above TSP-1 portions were amplified by PCR and cloned into the p3XFLAG-CMV-13 expression vector for mammalian cells. Then, 1A9 stable transfectants which secreted the sequences of interest were obtained. *In vitro* and *in vivo* studies are ongoing to investigate the consequences of the expression of this TSP-1 domain on the malignant behaviour of the tumour cells and particularly on their angiogenic activity.
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### 460 Endothelial cell specific chemotaxis regulator (ECSCR) is a novel tumour endothelial marker

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**Background:** Angiogenesis is the formation of new blood vessels from pre-existing ones. It occurs during embryonic development and in the adult only during wound healing and the menstrual cycle. However, adult angiogenesis can be reactivated in pathological conditions such as cancer. This makes angiogenesis targeting therapies a very appealing research field. The endothelium lines the interior of all vasculature in the body and it is well documented that tumour associated vasculature differs from the normal one. Endothelial cell specific chemotaxis regulator (ECSCR) is a novel transmembrane glycosylated protein upregulated in cancer that is involved in chemotaxis and tube formation. The aims of this work were to elucidate the expression pattern of ECSCR in both developmental and tumour angiogenesis.
**Materials and Methods:** Paraffin fixed tumour and normal tissues were stained with ECSCR and CD31 or ULEX antibodies and analysed by immunohistochemistry and immunofluorescence. The same techniques were applied to analyse tissue arrays of tumour and normal matched adjacent tissues from the same patient. The expression pattern of zebrafish ECSCR was uncovered by RNA whole mount *in situ* hybridization.
**Results:** ESCR is differentially expressed in tumour and normal tissues. Furthermore, its location seems to be inside the endothelium line, facing the lumen of the vessels. In the developing zebrafish embryos ECSCR is endothelial specific, being expressed in the major trunk and head vessels.
**Conclusions:** These data suggest that ECSCR is a good tumour endothelial marker, present at sites of active angiogenesis.

### 461 Further studies on the antitumour activity of a hybrid synthetic antitumour ester in combination with adriamycin on murine melanoma B-16

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**Background:** NSC 290205 (A) is a hybrid synthetic antitumour ester which combines a D-lactam derivative of androsterone and nitrogen mustard. In this study cyclophosphamide in the standard CHOP chemotherapeutic regimen was replaced with NSC 290205 (AHOP) and the efficacy of these regimens against murine Melanoma B-16 was compared.
**Material and Methods:** Melanoma B-16 was used in this study. It was purchased from NCI (USA). Tumour was grown in C57 Bl mice and was transplanted subcutaneously with puncture in the inguinal region. The amount of the transplanted graft was 40−50 mg fragment. The acute toxicity of the compounds was determined and the lethal dose LD10 was used as a therapeutic dose. The antitumour activity was assessed from the inhibition of tumour growth by volume in $cm^3$ and T/C % oncostatic parameter, according to the protocol of the experimental evaluation of anticancer drugs of the NCI. Treatment was given as an intermitted dose on days 1, 5, 9.
**Results:** Results show that treatment with steroidal derivative (A) or cyclophosphamide (C) produced almost equal borderline activity. Moreover, both CHOP and AHOP regimens showed significant and comparable antitumour effect. AHOP caused the maximum effect inhibiting tumour growth by 83.0% and producing T/C values of 277.7%. CHOP was less effective producing 53.7% inhibition of tumour growth and T/C 181.9%.
**Conclusions:** Although the treatment of Melanoma B-16 with cyclophosphamide or NSC 290205 yielded equivalent results, AHOP showed higher antitumour potency than CHOP. It is very likely that the D-lactamic steroid (androstan) alkylator for A, containing the amide group – NH-CO – combined with adriamycin which intercalates between DNA base-pairs, is the explanation for the higher activity of AHOP as compared to CHOP. Preclinical research supports that the aza-steroidal alkylator NSC 290205 demonstrates favorable acute and sub acute toxicity, as well as superior antitumour activity which in combination with adriamycin against Melanoma B-16 justifies further clinical studies.

### 462 Serum N-glycome biomarker for monitoring progression of DEN-induced hepatocellular carcinoma in rat

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**Backgrounds and Aims:** The diagnosis and certainly the follow-up of liver diseases such as cirrhosis and HCC remains a heavily debated problem. So, a more sensitive and specific non-invasive serological marker is needed for the early diagnosis of HCC and for monitoring treatment. Because most serum N-linked glycoproteins are synthesized by the liver, any changes in serum total N-glycans could reflect an alteration of liver physiology. Thus, changes in the quantity and type of N-glycans in serum could be exploited for the non-invasive diagnosis of liver diseases. In the present study, we studied glycomics during development of HCC in rat model.
**Methods:** Rat HCC was induced by diethylnitrosamine (DEN), a hepatocarcinogen, which results in the sequence of fibrosis and cirrhosis encountered in human hepatocarcinogenesis. N-glycans were profiled using the DSA-FACE technique developed in our laboratory. Glycan structures and glycan genes associated with progression of HCC in liver were analysis by westen lectin blot and qPCR.
**Results:** In comparison with control rats, two glycans (R5a and R5b) in serum total N-glycans of DEN rats increased gradually but significantly during progression of liver cirrhosis and cancer, whereas a biantennary glycan (P5) decreased. The log of the ratio of R5a to P1(NGA2F) and R5b to P1, [log(R5a/P1) and log(R5b/P1)], were significantly ($p < 0.0001$) elevated in HCC rats, but not in cirrhosis, fibrosis and control animals. We thus propose a GlycoTest model using the above serum glycan markers for monitoring the progression of cirrhosis and HCC in the DEN-treated rat model. These serum glycan markers were validated in a rat model involving prevention of tumour development by using an antitumour drug (S-trans-trans-farnesylthiosalicyclic acid; FTS). DEN-treated rats were subsequently treated with FTS leads to prevent progression to HCC. We found that GlycoTest markers (P5, R5a and R5b) in the FTS treated DEN rats reverted towards non-DEN levels, while HCC-specific markers, log(R5a/P1) and log(R5b/P1), normalized completely. Moreover, we found an increase in core-α-1,6-fucosylated glycoproteins in serum and liver of HCC rats by western lectin blot, demonstrating altered fucosylation during progression of HCC.
**Conclusions:** By analyzing N-glycomics during progression of HCC, we identified serum N-glycan biomarkers (GlycoTest model) that can be used to monitor progression of HCC and to follow up treatment of liver tumours in the DEN rat.

### 463 Autocrine regulation of receptor for advanced glycation endproducts (RAGE) by S100A4 promotes migration and invasion in A375 melanoma cells

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**Background:** The calcium-binding protein S100A4 is associated with metastasis of different cancer entities, including melanoma. The multiligand receptor for advanced glycation endproducts (RAGE) has been suggested to interact with extracellular S100A4 protein. We hypothesized that the interaction between RAGE and S100A4 plays an important role in activation of growth, adhesion, motility and migration in a human melanoma cell line with high metastatic potential.
**Materials and Methods:** In order to investigate the cellular role of the RAGE-S100A4 interaction *in vitro*, we produced recombinant S100A4 and soluble RAGE (sRAGE). Furthermore, we established A375 melanoma cells stably transfected with S100A4 using vector pIRES2-AcGFP1 (A375-S100A4). The overexpression of S100A4 has been verified by western blot and flow cytometry. Assays for determination of migratory, invasive and adhesive behaviour of A375-S100A4 cells were performed. Furthermore, specific