number of Tregs. And it suggests that adoptive T cell therapy influences immunoescape mechanism in patients with cancer. It will be necessary to clarify the mechanism of the effect and to develop an adoptive immunotherapy which has more beneficial clinical effect.

**1112** POSTER

**Imbalance in VEGF-A/sFLT-1 Enables Malignant Ascites to Resist Dendritic Cell-based Immunotherapy**

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**Background:** Malignant ascites (MA) is an intractable and immunotherapy-resistant state of advanced gastrointestinal and ovarian cancers. Dendritic cells (DCs) have a potential for DC-based immunotherapy as a new therapeutic modality for cancers. We recently proposed a unique and powerful method to activate DCs for cancer immunotherapy, 'immunostimulatory virotherapy', using a new DC-activating modality, the replication-competent, as well as fusion (F)-gene-deleted, nontransmissible recombinant Sendai viruses (rSeVs). The objective of this study was to explore the validity of immunostimulatory virotherapy for MA.
**Material and Methods:** An immunocompetent murine model of MA was generated using CT26 colon cancer cells, and DCs were generated from mouse bone marrow.
**Results:** Although we found a significant prolongation in the survival of the tumour-bearing mice by DC-rSeV/dF-GFP treatment, the outcome was nevertheless unsatisfactory. We determined that the imbalance between the vascular endothelial growth factor-A/vascular permeability factor (VEGF-A/VPF) and its decoy receptor, soluble *fms*-like tyrosine kinase receptor-1 (sFLT-1), was a major cause of the resistance to dendritic cell (DC)-based immunotherapy in the murine model of MA. We found that the ratio of VEGF-A/sFLT-1 was increased not only in murine, but also in human MA, and rSeV/dF-mediated secretion of human sFLT-1 by DCs dramatically improved the survival of tumour-bearing animals and inhibited the increase in their body weight. The improvement of survival was associated with enhanced CTL activity and the infiltration of these cells into peritoneal tumours. These findings were not seen in the immunodeficient mice. In vitro, while rSeV/dF-GFP infection did not affect DC expression of the typical co-stimulatory molecules, DC-rSeV/dF-hsFLT1 showed significant increases in positive cell numbers of, at least, CD40, CD83, and CD86 cells. Furthermore, the mIL-1b, mIL-6 and JE/mMCP-1 restoration of proinflammatory cytokine expression was observed in the mice treated with DC-rSeV/dF-hsFLT1.
**Conclusions:** The imbalance between VEGF-A/VPF and its soluble decoy receptor, sFLT-1, is responsible for the resistance of MA to DC-based immunotherapy, and the correction of this ratio by gene transfer of hsFLT-1 into DCs dramatically augmented not only DC function itself, but also the tumour-specific immune response. Therefore, this new concept, 'targeting VEGF-A/VPF activity during intraperitoneal DC vaccination', could represent a significant strategy to treat MA in the clinical setting.

**1113** POSTER

**Activation of Checkpoint Kinase 2 (Chk2) Contributes to the Antitumour Synergy Between IGF1 Receptor Kinase Inhibitor NVP-AEW541 and Sunitinib in Hepatocellular Carcinoma**

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**Background:** Insulin-like growth factor (IGF) signaling pathway has been demonstrated an important regulatory mechanism of tumorigenesis and drug resistance in many cancers. Previous studies have shown that inhibition of IGF signaling may induce apoptosis and reverse resistance to cytotoxic agents in HCC cells. The present study explored whether the efficacy of sunitinib or sorafenib can be improved by IGF receptor kinase inhibitor NVP-AEW541 (Novartis) in HCC cells and human umbilical venous endothelial cells (HUVECs).
**Materials and Methods:** HCC cell lines tested included Hep3B, PLC5, and SK-Hep1. The potential synergistic growth inhibitory effects were measured by MTT and median dose effect analysis. Apoptosis was measured by flow cytometry. The activity of pertinent signaling pathways and expression of apoptosis-related proteins were measured by Western blotting.
**Results:** IGF can activate IGF receptor and downstream AKT and ERK signaling activities in all the HCC cells and HUVECs. Addition of IGF increased resistance of HUVECs to the multi-kinase inhibitors sorafenib and sunitinib. Resistance of HCC cells to sunitinib, but not sorafenib, was also increased with the addition of IGF. The IGF1 receptor inhibitor NVP-AEW541 (Novartis) significantly enhanced the apoptosis-inducing effects of sunitinib, but not sorafenib, of HCC cells both *in vitro* and *in vivo*. The synergistic effects between sunitinib and NVP-AEW541 were independent of inhibition of IGF receptor, AKT, and ERK activities by NVP-AEW541. Activation of Chk2, which played important roles in regulation of DNA damage response, was found when NVP-AEW541 was combined with sunitinib but not with sorafenib. Knockdown of Chk2 expression by small interfering RNA partially abrogated the synergistic apoptosis-inducing effects of sunitinib and NVP-AEW541.
**Conclusions:** IGF in tumour microenvironment may increase resistance of HCC to molecular targeted therapy. The apoptosis-enhancing effects of IGF1 receptor inhibitors in HCC cells may be drug-specific, and Chk2 activation may be one important downstream mediator of the anti-cancer synergy between IGF1 receptor inhibitors and molecular targeted agents.
Supported by grants NHRI-EX99–9911BC, NHRI-EX100–9911BC and NSC99–3112-B-002–038.

**1114** POSTER

**The Anticancer MTOR-inhibitor Temsirolimus Induces Cardiotoxicity in a Mouse Model**

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**Background:** Cardiotoxicity is a major drawback and social problem linked to many anticancer treatments. Early identification of signs of this adversity would certainly benefit the management of oncologic patients. The mTOR-inhibitor temsirolimus is currently being evaluated for anticancer efficacy in hundreds of clinical trials and is approved for treatment of advanced renal cell carcinoma. However, the PI3K/Akt pathway converges on mTOR, which is a central regulator of cell growth, including cardiomyocyte growth. Here, we aim at evaluating the cardiac effects of the anticancer mTOR-inhibitor temsirolimus in a mouse model in vivo.
**Materials and Methods:** Left Ventricular (LV) fractional shortening (FS) was assessed by M-mode echocardiography in sedated C57BL/6 mice (2−4 mo. old) at day 0, and after 2, 7, 14, 21 days from a single i.p. injection of temsirolimus (0.1 mg/kg, a dose comparable to the one used to treat cancer in humans) or vehicle. Doxorubicin (Doxo, 2.17 mg/kg/day for 7 days) was used as a positive control. With Speckle Tracking echocardiography (ST) we also evaluated radial myocardial strain (%), a very sensitive parameter which can detect subtle changes in cardiac function.
**Results:** After 2 days, there was no change in FS with temsirolimus, but FS was already reduced with Doxo: 52±0.2%, $p = 0.0000001$ vs sham (60±0.4%). With temsirolimus, FS was reduced only after 21 days: 50±3%, $p = 0.009$ vs sham. Interestingly, with Speckle Tracking echocardiography we found that in the temsirolimus group radial strain was already decreased at 7 days: 42±5%, $p = 0.01$ vs sham (59±1%).
**Conclusions:** The antineoplastic mTOR-inhibitor temsirolimus induces LV dysfunction in mice. Such dysfunction occurs later than the one observed with Doxo, but speckle tracking echocardiography is more sensitive than conventional echocardiography and can detect early signs of myocardial alteration that may prelude to overt LV dysfunction. The clear mechanisms of temsirolimus cardiotoxicity are to be elucidated in further experimental studies. We also plan to apply speckle tracking echocardiography to clinical studies, in order to evaluate the impact of early identification of temsirolimus cardiotoxicity in the treatment of renal cell carcinoma.

**1115** POSTER

**Impaired Autophagy Contributes to Resistance to Metronomic Cyclophosphamide Chemotherapy**

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**Background:** Autophagy is a cellular stress response that is emerging as an important determinant of response to a wide range of anticancer therapies. Specifically, autophagy is usually thought to contribute to tumour cell survival, and thus therapeutic resistance, in tumours subjected to conventional chemotherapy (i.e., intermittent cytotoxic drug administration at maximum tolerated doses). Conversely, the role of autophagy during chronic anticancer therapy such as low-dose metronomic (i.e., antiangiogenic) chemotherapy is unknown.

**Material and Methods:** We studied the autophagic properties of human PC-3 prostate and MDA-MB-231 breast cancer models of acquired, stable resistance to metronomic cyclophosphamide therapy, compared to their parental counterparts. We also analyzed the anti-tumour effects of metronomic versus conventional cyclophosphamide ± chloroquine (autophagy inhibitor) therapy on PC-3 xenografts. Furthermore, we compared the *in vivo* growth properties of paired autophagy-competent and autophagy-deficient (i.e., beclin1 haploinsufficient) baby mouse kidney epithelial cells treated with either metronomic or conventional cyclophosphamide.
**Results:** LC-3 Western blotting and acridine orange flow cytometry of parental PC-3 and MDA-MB-231 revealed strong autophagy induction under conditions of metabolic stress mimicking the microenvironment in tumours undergoing metronomic cyclophosphamide therapy (i.e., hypoxia, low pH and reduced nutrients). In contrast, the autophagic response was reduced in a number of metronomic cyclophosphamide resistant PC-3 and MDA-MB-231 variants. Chloroquine impaired the response of PC-3 xenografts to metronomic cyclophosphamide. Similarly, the impact of metronomic cyclophosphamide was reduced in autophagy-deficient versus autophagy-competent baby mouse kidney epithelial cell allografts. In contrast, both pharmacological and genetic autophagy deficiency enhanced the antitumour effects of conventional cyclophosphamide.
**Conclusions:** Our studies suggest that impaired autophagy contributes to resistance to metronomic cyclophosphamide chemotherapy, and possibly to other forms of antiangiogenic or chronic anticancer therapies. In other words, chronic metabolic stress associated with metronomic chemotherapy may favor cell death promoting autophagy effects. In contrast, cell survival promoting autophagy effects prevail during acute cellular stress due to conventional chemotherapy. Thus, the impact of autophagy modulators in clinical development may vary dramatically depending on the nature of concomitant anticancer therapies.

**1116** POSTER

**The Influence of the Combined Treatment With Vadimezan (ASa 404) and Taxol on the Growth of U251 Glioblastoma Xenografts**

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**Background:** One of the most important biological characteristics of Glioblastoma multiforme (GBM) is high vascular density and targeting the vasculature in this tumour could be an attractive therapeutic strategy. Vadimezan (ASA 404, DMXAA) belongs to the class of small molecule vascular disrupting agents (VDA) that cause disruption of established tumour vessels and subsequent tumour hemorrhagic necrosis. Selective antivasculogenic effects of ASA 404 are mediated by intratumoral induction of several cytokines including tumour necrosis factor-α (TNF-α), granulocyte-colony-stimulating factor (G-CSF), interleukin 6 (IL-6) and macrophage inflammatory protein 1α (MIP-1α). Preclinical studies have demonstrated that ASA 404 acts synergistically with Taxanes, which at lower concentrations inhibit angiogenesis. In this study, we investigated if treatment of mice bearing U251 human glioblastoma xenografts with ASA 404 and taxol may be synergistic. Therapy response was evaluated also by FDG-PET imaging.
**Material and Methods:** $1.5\times10^6$ U251 cells were inoculated s.c. into the right hind limb of NMRI-Foxn1$^{nu}$ athymic female nude mice. Animals were randomly assigned in 4 groups (7−9 animals/group) for treatment: control, taxol, ASA 404 and ASA 404 plus taxol. The animals received either a single dose of taxol (10 mg/kg), ASA 404 (27.5 mg/kg), or taxol (10 mg/kg) plus ASA 404 (27.5 mg/kg) administered i.p.; ASA 404 was administred 24 hours after the treatment with taxol. 4 hours after treatment with ASA 404 (28 hours after treatment with taxol) FDG-PET scans were performed.
**Results:** The treatment with taxol did not affect the tumour growth in comparison to untreated controls. The treatment of animals with single dose ASA 404 alone or in combination with taxol caused a significant decrease in tumour volume. The combined treatment did not decrease the growth of the xenografts significantly more than ASA 404 alone. The final tumour weights were: control = 764±168 mg, taxol = 651±148 mg, ASA 404 = 283±127 mg, ASA 404 + taxol = 180±56 mg. FDG-PET imaging corelated with tumour response. SUV values were: control = 1.21±0.39, taxol = 1.14±0.19, ASA 404 = 0.36±0.08 and ASA 404 + taxol = 0.50±0.14.
**Conclusion:** The treatment with ASA 404 alone or in combination with taxol showed antitumoral effects in our glioblastoma model probably through destruction of blood vessels. The implications for the anticancer effect of this compound warrant further preclinical studies.

**1117** POSTER

**Overcoming the Acquired Resistance to Afatinib (BIBW2992) in HCC827, a Non-small Cell Lung Cancer Cell Line**

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Understanding of the pharmacological responses to drug treatment in cancer cells is essential for discovery and development of novel anti-cancer therapies. In this study, drug resistant cell lines, HCC827-BR1 and HCC827-BR2, were developed by treatment of HCC827 cells with escalating concentration of afatinib (BIBW2992). The $CC_{50}$ of BIBW2992 in HCC827 ranges from 2 to 10 nM while the $CC_{50}$s of BIBW2992 in HCC827-BR1 and HCC827-BR2 are approximately 10 uM. Gene expression analysis revealed that the epithelial-mesenchymal transition (EMT) may be involved in resistance to BIBW2992. The drug-resistant cells are more invasive as evaluated under in vitro assays. Results from this study have also identified that the drug-resistance cells are more sensitive to another kinase inhibitor; indicating that an oncogenic shift has occurred. When this drug is combined with BIBW2992 in treatment of HCC827 cells, much less colonies survived compared to cells treated by BIBW2992 alone. The clinical ramifications of these observations will be discussed.

**1118** POSTER

**Doxorubicin and Taxol Induce Apoptosis in Breast Cancer Cells by Activating Foxo3a Transcription Factor**

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**Background:** Foxo3a is a member of the forkhead box O class of transcription factors (foxO), which are key regulators of apoptosis, cell cycle arrest and cell division. Foxo3a phosphorylation by kinases, such as Akt, leads to its nuclear exclusion, cytoplasmic accumulation and subsequent degradation. Foxo3a inactivation has been associated with tumorigenesis and poor survival in breast cancer, what turns this protein into a possible target for anticancer drugs. In this study, we aimed to investigate the role and regulation of Foxo3a in response to taxol and doxorubicin (doxo) treatment in breast cancer cells.
**Materials and Methods:** The human breast carcinoma cell lines MCF7 and MDA-MB-231 were exposed to clinically relevant concentrations of taxol and doxo and citotoxicity was assessed by the MTT assay. Cell morphological changes were microscopicaly photographed. Western blot and Annexin V/PI by flow cytometry were used to detect caspases activation and apoptosis, respectively.
**Results:** After taxol exposure for 24h, there was an 85% cell viability inhibition, which was also observed after doxo treatment for 72h ($p < 0.01$), showing that both drugs display high toxicity against breast cancer cells. Morphological analysis of non-adherent cells revealed that the drugs induced 65% of cell death ($p < 0.05$), indicating that citotoxicity was not resulted from cell proliferation inhibition. Taxol and doxo could effectively induce apoptosis, as detected by the Annexin-V/PI method and caspases-3, -7 and -9 activations. Western blot analysis showed that there was a 16 and 12-maximum fold increase in Foxo3a levels after treatment with taxol and doxo, respectively. However, the Real Time PCR analysis of mRNA Foxo3a expression in cells exposed to the drugs showed that Foxo3a levels were not increased, indicating that Foxo3a expression is not transcriptionally activated. This finding suggests that taxol and doxo may induce cellular mechanisms which prevent Foxo3a degradation. Data analysis was done using the t student test and a $p < 0.05$ was considered statistically significant.
**Conclusion:** The association between the increase in Foxo3a expression and cells sensitivity indicates that this transcription factor may act mediating taxol and doxo-induced apoptosis. These data point Foxo3a as a cellular target for anticancer drugs in breast cancer cells.
**Acknowledgements:** FINEP, Swissbridge Foundation and INCT para Controle do Câncer, CNPq 573806/2008−0; FAPERJ E26/170.026/2008.

**1119** POSTER

**Influence of Soluble CD40 Ligand on Colorectal Cancer Cells: a Flow Cytometric Study**

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**Background:** The cell surface costimulatory molecule CD40 is a member of the Tumour Necrosis Factor Receptor family widely expressed on various