DC vaccination: Dendritic cells (DC) were generated from bone marrow cells. DC co-cultured with the same number of irradiated tumour cells (5,000 cGy) or synthesized DDX3X were inoculated subcutaneously (s.c.) as vaccine.
Results: We found that vaccination with $CD133^+$ tumour cells evoked specific T-cell priming and that $CD133^+$ tumour-specific LN T cells mediated potent antitumour therapeutic efficacy, thereby curing parental melanomas that comprised <1% $CD133^+$ tumour cells. Proteome analyses revealed that DDX3X is one of $CD133^+$ melanoma-specific proteins. The LN T cells draining DDX3X vaccines exhibited specific IFNg and IL-17 release upon $CD133^+$ tumour stimulation. A DDX3X vaccination induced antitumour therapeutic immunity against parental melanoma. In contrast, vaccination with $CD133^+$ tumour cells that lost DDX3X expression failed to induce antitumour immunity. We examined DDX3X expression in human cancer cell lines and normal human cells. All of the examined human cancer cells expressed DDX3X. HCT116, 87.5 and MCF-7 cells that showed CSC-like phenotypes highly expressed DDX3X.
Conclusion: These results indicate that anti-CSC, especially anti-DDX3X, immunotherapy is a promising treatment option in the clinical setting.

1109 POSTER

Personalized Cancer Immunotherapy With Oncolytic Adenoviruses Armed With Immunostimulatory Molecules GMCSF or CD40L

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Background: The adenovirus genome is rather well characterized, easy to engineer and tolerates multiple modifications. Therefore, the approach lends itself well to individually personalized medicine including personalized immunotherapy. This has been recognized also by EU legislators and patient-by-patient treatments are regulated by the Advanced Therapies regulation (EU 1394/2007), which has allowed us to treat more than 250 patients in an Advanced Therapy Access Program (also known as "hospital exemption" or "named patient basis").
Materials and Methods: Following extensive preclinical testing, 10 different viruses have been used. The optimal virus capsid, tumour specific promoter and arming device are selected based on preclinical and clinical data, taking into account the nature of the clinical problem in each patient (local vs systemic), while capsid switching has been utilized to enhance systemic delivery. Three schedules of low-dose cyclophosphamide have been used to reduce regulatory T-cells, induce TH2 $\rightarrow$ TH1 switch and enhance anti-tumour immunity. Autophagy induction with low-dose pulse temozolomide has been used with or without low-dose cyclophoshamide. Injections have been performed in ultrasound, visual or CT guidance, intratumorally, intracavitary and/or intravenously on an individual basis. Both archival and fresh pretreatment tumour samples have been studied for selecting the optimal virus and for prediction of efficacy.
Results: Based on more than 250 patients treated, the side effect profile is generally mild with slight variation between different viruses. Serious adverse events possibly related to treatment are seen in circa 6% of treatments, while mild to moderate fever, flu-like symptoms, tumour pain and fatigue are common. There has been no treatment related mortality. Evidence of possible efficacy (radiological stable disease or better in patients progressing prior to therapy) has been seen in 48% of patients overall and up to 77% with the optimized schedule. With the best schedule, more than half survive for a year or longer which is unusual in this difficult patient population and compares well to historical controls. Preclinical, clinical and immunological data will be presented. A clinical phase 1−2 trial is in progress.
Conclusions: The EU Hospital Exemption allows personalization of oncolytic adenovirus therapy on a patient-by-patient basis.

1110 POSTER

BPR1K653, a Novel Aurora Kinase Inhibitor, Exhibits Potent Anti-proliferative Activity in P-gp170 (MDR1)-mediated VX680-resistant Cancer Cells in Vitro and in Vivo

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Background: Mitosis is a key step in cell cycle that is tightly regulated by many proteins. Abnormal expression or activation of these regulatory proteins could result in aberrant mitosis, leading to the development of cancer [1]. At the molecular level, Aurora kinases (Aurora-A, Aurora-B and Aurora-C) are serine/threonine kinases that function as key regulators of mitosis. In this study, a novel pan-Aurora kinase inhibitor entitled BPR1K653 was developed and its potency against various MDR1-negative and MDR1-positive cancer cells was evaluated. Our data revealed that unlike the well characterized Aurora kinase inhibitors VX680 and PHA-739358, BPR1K653 is effective in targeting both MDR1-negative and -positive cancer cells *in vitro* and *in vivo*.
Materials and Methods: *In vitro* kinase activity assay was used to determine the activity and target specificity of BPR1K653. Anti-proliferative activity of BPR1K653 was evaluated in various cancer cell lines. Flow cytomertic analysis, immunofluorescence microscopy, Western blot analysis, real-time caspase−3/−7 activity imaging, and the TUNEL assay were used to follow mechanisms of action of BPR1K653. Efficacy of BPR1K653 was determined in different xenograft mice models.
Results: BPR1K653 specifically inhibited the activity of Aurora-A/-B kinase *in vitro*. It showed potent activity in a variety of human tumour cell lines regardless to the tissue origin, p53 status, and expression of the common drug efflux pump MDR1 (P-gp-170). In contrast, clinically tested Aurora kinase inhibitors, VX680 and PHA-739358, were ineffective in targeting the MDR1-expressing cancer cells. Interestingly, MDR1-expressing cancer cells treated with BPR1K653, but not with VX680, showed reduced-MDR1 activity. BPR1K653 induced cell endo-replication and the reduction of phosphor-histone H3, which are classical phenotypes of Aurora kinase inhibition. BPR1K653 also showed potent activity against the growth of xenograft tumours of the human cervical carcinoma KB and KB-derived MDR1-expressing VX680/vincristine-resistant KB-VIN10 cells in nude mice.
Conclusion: BPR1K653 is a promising anti-cancer compound that has potential for the management of various malignancies, particularly for patients with MDR1-related drug resistance after prolonged chemotherapeutic treatments.

1111 POSTER

Adoptive T Cell Therapy Enhances the Secretion Ability of Cytokines to Th1 and Reduces the Number of Tregs

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Background: It was reported that not only the secretion ability of cytokines from PBMC, but also the number of peripheral blood Tregs are related to advanced cancer patient's prognosis. We investigated the secretion ability of cytokines and the number of peripheral blood Tregs before and after adoptive T cell therapy (CD3-LAK) to assess the correlation with the immunological responses and the effect of the treatment. In addition, we examined the effect on the population of Tregs in tumour-bering mice treated by adopted T cell transfer (ACT). We also evaluated the effect on the induction of cytokines caused with lymphokine-activated killer cells(LAK) in vitro model.
Method: Seventy six patients who were treated by CD3-LAK more than four times were enrolled this study. We conducted this study after having obtained the informed consent of the study for these patients. We measured the secretion ability of cytokines from PBMC using the peripheral blood collected from the patients before the initiation of CD3-LAK and two weeks later after the 4th CD3-LAK. The methods we measured the secretion ability of cytokines are shown as follows. IFN-alpha: We stimulated the whole blood by Sendai virus for 20 hours, and IFN-alpha of supernatant was measured by bioassay. The other cytokines (IL-2, IL-4, IL-10, TNF-alpha, IFN-gamma etc): We stimulated the whole blood by PHA for 48 hours, and the cytokines of supernatant were measured by BioPlex array. In terms of the change of the number of peripheral blood Tregs, we analyzed Foxp3 and CD4 positive T cells by flow cytometry. In vivo model: ACT treatments were performed on days 7 and 10 following the subcutaneous injection of 1.0×10^6 colon26 cells. The Treg phenotype of lymphocytes in the draining lymph nodes and splenocytes was analyzed by flow cytometry. In vitro model: LAK cells were transferred to above the membrane that separated each well and CD4-positive cells were cultured under the membrane. IFN-gamma in the culture supernatant was assayed by ELISA.
Result: The values of IFN-gamma and TNF-alpha were markedly increased after CD3-LAK. The number and the population of Tregs were significantly lower compared to pre-treatment values. There was a significant longer overall survival in patients who had increased IFN-gamma, TNF-alpha secretion after CD3-LAK. In vivo model, the accumulation of Tregs in the draining lymph nodes and tumour was significantly suppressed after LAK treatment. In vivo model, the concentration of IFN-gamma in the culture solution was increased by LAK treatment.
Discussion: We found that the therapeutic intervention of CD3-LAK enhances the secretion ability of cytokines shifts to Th1, and reduces the

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.
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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.