1105 POSTER

Association of ABCC2 Genotype With Response and Progression-free Survival of First-line FOLFIRI in Japanese Patients With Advanced Colorectal Cancer

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Background: This hypothesis-generating retrospective study examined the effects of genetic polymorphisms in genes related to both irinotecan pharmacokinetics and pharmacodynamics on efficacy of combined therapy consisting of irinotecan, 5-fluorouracil, and leucovorin (FOLFIRI).
Material and Methods: Japanese patients with advanced colorectal cancer who received first-line FOLFIRI were studied, since this regimen is frequently used because of its high effectiveness for the treatment of such patients. All patients harbored *UGT1A1*1/*1*, **1/*6*, or **1/*28* genotypes that were proven to show similar irinotecan pharmacokinetics and efficacy of FOLFIRI, ensuring the subjects having similar genetic backgrounds of *UGT1A1*. Direct sequencing was conducted to analyze genetic polymorphisms in genes related to irinotecan pharmacokinetics including ATP-binding cassette, sub- family C, member 2 (*ABCC2*), and irinotecan pharmacodynamics such as topoisomerase 1 (*TOP1*).
Results: A total of 61 patients with advanced colorectal cancer received first-line FOLFIRI from June 2003 through April 2008. The overall response rate and median progression-free survival in FOLFIRI were 43% and 7.5 months, respectively. Overall response rate was higher in patients with CC genotype at −24 in A*BCC2* than others (P = 0.0313). Median progression-free survival was the longest in patients with CC at −24 in *ABCC2*, followed by those with CT and TT (P = 0.0091). Clear gene-dose effects were observed between −24C>T and median progression-free survival. Other polymorphisms in all genes tested were not correlated with the efficacy of FOLFIRI.
Conclusions: We thus found the association between −24C>T in *ABCC2* and efficacy of FOLFIRI. Our findings suggest that pharmacogenetics of *ABCC2* can be used to predict the irinotecan efficacy.

1106 POSTER

Cysteine Rich 61 (CCN1) Protein Expression as a Predictive Marker in Endometrial Cancer

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Background: Cysteine rich 61 (Cyr61/CCN1) is an important player in tumorigenesis due to its pro-angiogenetic activities. Cyr61 undergoes alternative splicing resulting in two different mRNAs. Hypoxia triggers the predominant expression of the solely protein-generating mRNA. Cyr61 expression studies in EC found both, a downregulation as well as an overexpression of the protein. We studied the expression of Cyr61 and its splicing isoforms in EC.
Methods: Cyr61 protein expression in 138 tissue samples originating from EC patients was evaluated by immunhistochemistry (IHC) and correlated to clinicopathologic factors separating histological types I and II. Survival of tumour patients was calculated by using Kaplan–Meier curves and Log-rank-test. Expression of both Cyr61 mRNAs was investigated by real-time PCR. Immunhistochemical results were correlated to expression levels of Cyr61 mRNAs.
Results: Cyr61 overexpression was detected in 15% of endometrial cancer samples. Multivariant-analyses confirmed correlation of high protein expression levels (IHC) with lymph node metastasis, lymphangioinvasion and tumour-grading. Patients with an overexpression of Cyr61 showed lower overall-survival and shorter relapse-free-survival compared to patients exhibiting low or moderate Cyr61 expression. We could not find any significant correlation between immunhistochemistry and expression of the protein-generating mRNA.
Conclusion: Overexpression of Cyr61 in EC correlates well with poor survival, lymph node metastasis, lymphangioinvasion and tumour-grading. Therefore, it could represent a new molecular marker in predicting survival of patients with estrogen-dependent EC. Posttranslational modifications may account for the discrepancy of Cyr61 protein expression examined by IHC and no significant correlation with expression levels of the protein-generating Cyr61 mRNA obtained by real-time PCR.

1107 POSTER

Phase I Study of Multiple Peptides Vaccination in Patients With Advanced Bile Duct Cancer

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Background: The prognosis of patients with advanced bile duct cancer is extremely poor and there is only few standard treatment. Recently, the safety and the clinical efficacy of vaccination with cancer-testis antigen derived peptides has been reported in some clinical trials. In ths study, we investigated the safery, immunological responses and anti-tumour effect of vaccination with four cancer-testis antigen derived peptides which we previously identified for patients with advanced bile duct cancer.
Material and Methods: Patients with advanced bile duct cancer who had unresectable tumours to be refractory to chemotherapy were vaccinated once a week for four weeks of a treatment cycle and continued vaccinations until their diseases were progressed. On each vaccination day, the HLA-A*2402 restricted epitope peptides, which were derived from four cancer-testis antigens, DEP domain containing 1 (DEPDC1), lymphocyte antigen 6 complex locus K (LY6K), insulin-like growth factor (IGF)-II mRNA binding protein 3 (IMP-3) and TTK protein kinase (TTK) mixed with Montanide ISA-51, SEPPIC were administered by subcutaneous injection. The adverse events were assessed by Common Terminology Criteria for Adverse Events (CTCAE) version 3 and the immunological responses were monitored by an enzyme-linked immunospot (ELISPOT) assay or a flow cytometry. The clinical effects were observed by CT scan, progression-free survival (PFS) and overall survival (OS).
Results: Nine patients (4 males, 5 females, median age 70 years, range 59−78) were enrolled and treated at doses of 0.5 mg each of four peptides (n = 3), 1 mg each (n = 3) and 2 mg each (n = 3). Eight of 9 patients developed grade 1 or 2 local skin reactions in the injection sites. No grade 3 or 4 adverse events were observed. Peptides-specific T cell immune responses were observed in seven of 9 patients and clinical responses (stable disease or objective response) were observed in six of 9 patients. The median PFS after the first vaccination was 156 days and the median OS was 380 days. The patients who developed grade 2 local skin reaction showed the longer survival time (p = 0.0027).
Conclusions: The cancer vaccine therapy using these four peptides was well tolerated and appeared to provide some clinical benefit. This result warrants further Phase II clinical study.

1108 POSTER

DEAD/H (Asp-Glu-Ala-Asp/His) Box Polypeptide 3, X-linked is a CD133+ Tumour-specific Protein and Induces Antitumour Immunity

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Background: Cancer cells that exclusively maintain the ability of self-renewal and differentiation are termed cancer stem cells (CSC). It is still controversial if the classical CSC hierarchy exists in all of solid tumours, however, accumulating evidence suggests that heterogeneity within cancer cells exists and that cancer survives as the cells with CSC features during potentially lethal stresses, including chemotherapy, radiation treatment, and molecular targeting therapy. Although it is necessary to eradicate CSC to obtain cure of cancer, effective treatment has not been elucidated. It has been demonstrated that most of immunogenic tumour-associated antigens belong to cancer/testis (CT) antigens. One of the reasons why CT antigens are immunogenic is that they are unlikely to maintain peripheral tolerance, owing to restricted expression in the testis and in immortal malignant cells. It was reported that CT antigens are mainly expressed in CSCs. Since CSC are highly immortal, it is possible that they possess immunogenic antigens that are not expressed in differentiated cancer cells or normal epithelial cells, and that these antigens may be ideal therapeutic targets for cancer treatment.
Materials and Methods: Tumour cells: B16F10 is a melanoma of B6 mice origin and was maintained *in vitro*. $CD133^+$ tumour cells were isolated with PE-conjugated anti-CD133 mAb and anti-PE microbeads™ and autoMACS™. All of the human cancer cell lines, including HCT116, 87.5, S2, A549, MCF-7, and WM115 were obtained from ATCC.
Proteome analysis: Proteome analyses of $CD133^+$ and $CD133^-$ tumour cells were carried out by two-dimensional protein gel electrophoresis. Expression of DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3, X-linked (DDX3X) was analyzed with immunoblotting assay.

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