Thursday 30 September

Poster Sessions

Cancer vaccines

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Frequencies of tetramer+ T cells specific for HPV16 E7 epitopes in the circulation of patients with squamous cell carcinoma of the oropharynx

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In previous studies we have described T-cell responses against p53 epitopes in patients with head and neck cancer (Hoffmann et al. Cancer Res. 2002). In order to overcome limitations for the clinical applicability of the "self-antigen" p53, we are currently focusing on immune responses against virus associated antigens. Since oropharyngeal carcinomas have been frequently described to be positive for the high risk human papilloma virus (HPV) 16, we investigated T-cell responses against HLA-A2.1 restricted epitopes of the HPV 16 E7 oncoprotein. T cells of 20 HLA-A2.1+ patients and 20 HLA-A2.1+ healthy individuals were evaluated by multicolor flow cytometry analysis using peptide-HLA-A2.1 complexes (tetramers) specific for three HPV 16 E7 peptides: E711–20, E782–90, E786–93. T cell clones specific for peptides of influenza matrix (a model recall antigen) and HIV reverse transcriptase (a model novel antigen) were studied in parallel. The HPV 16 E7 and, as a surrogate marker for HPV infection, p16 status was assessed immunohistochemically in frozen tumor sections, and in vitro stimulation experiments were performed with autologous dendritic cells. Patients with oropharyngeal cancer had significantly higher frequencies of CD8+ T cells specific for all three HPV 16 E7 peptides if compared to those of normal donors. A correlation between HPV 16 E7 specific T-cells and expression of the corresponding antigen in the tumor is currently determined. Furthermore, preliminary experiments revealed an increase of HPV 16 E7 specific T-cells upon in vitro stimulation with peptide pulsed dendritic cells and will be tested for reactivity against HPV 16 E7 tumor cell lines. The enumeration of T-cells specific for HPV 16 E7 epitopes helps to characterize the interaction of the cellular immune system with oropharyngeal tumors harboring oncogenic HPV 16 E7. In future, this might lead to the development of immunotherapeutical approaches comparable to those being developed in HPV positive cervical carcinoma.

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MVA-MUC1-IL2 vaccine immunotherapy for advanced non-small cell lung cancer (NSCLC): interim phase II data

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Background: Over-expression, non-polarity and under-glycosylation of the mucin glycoprotein molecule, MUC1, are associated with many cancers, making MUC1 an attractive target antigen for vaccine immunotherapy of cancer. MVA (Modified Vaccinia Ankara), a highly attenuated Vaccinia virus, is non-propagative in most mammalian cells and has an excellent safety profile. We have produced a recombinant MVA expressing MUC1 and IL2 (TG4010). Murine studies have shown that TG4010 can induce a MUC1 specific immune response associated with the elimination of MUC1 expressing tumors.
In a Phase I study of TG4010 in patients with late stage, MUC1 expressing cancers, TG4010 was well tolerated and showed some evidence of efficacy. We have initiated a Phase II study in which TG4010 is assessed alone or in combination with chemotherapy in NSCLC patients.
Methods: A randomized, two stage Simon design, two arm (18/33 patients stage 1/2 per arm), phase II study in stage IIIb/IV NSCLC is described. Arm 1: TG4010 is combined upfront with cisplatin and vinorelbine. Arm 2: patients are treated with TG4010 alone, followed by TG4010 + cisplatin and vinorelbine upon disease progression.
Results: 6 5 patients (15 stage IIIb-50 stage IV) have been enrolled. In arm 1, interim data show an objective response rate (ORR) of 7/15 patients (47%) (5 so far validated and 2 to be validated by independent central review). Response duration is between 114–195 days. Clinical benefit is observed in 12/15 patients (80%). ORR has satisfied the criteria to move forward to the second stage of the study. In Arm 2, clinical benefit is observed in 2 / 16 patients with TG4010 alone (2 until 211 days). An ORR of 3/14 in subsequent combination with chemotherapy is observed. TG4010 is well tolerated. Injection site reaction is the most frequent adverse event.
Conclusion: TG4010, combined with standard chemotherapy, is being evaluated further in stage 2 of the study.

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Structural mimics of heat shock protein70 (Hsp70) associated peptides from breast tumour cells can prime T cells to respond to tumour antigens

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Hsp70 plays an important role in tumour immunity. It can bind peptides derived from intracellular proteins and become endocytosed by macrophages and dendritic cells (DC), allowing the peptides to be presented on major histocompatibility complex molecules to T cells. Hsp70 can also stimulate DC maturation thereby enhancing their function as antigen presenting cells. Vaccination of humans with hsp70-peptide complexes (Hsp-PC) isolated from autologous tumour cells can induce protective anti-tumour immune responses. We have investigated whether structural mimics of Hsp-PC extracted from the MDA-MB-231 breast tumour cell line can mimic the ability of Hsp-PC to stimulate T cells. Peptide "recognisers" of Hsp70-PC were isolated from an M13 phage display peptide library by their ability to bind to Hsp-PC extracted from MDA-MB – 231 cells. By repeating the selection using one of the recogniser peptides as bait, we isolated a potential Hsp-PC peptide mimic. Using autologous immature dendritic cells as antigen presenting cells, purified Hsp-PC or its mimic peptide were capable of priming human T cells to release interferon-g (IFN-g) upon stimulation 10 days later with an MDA-MB-231 cell extract (6094pg/ml and 3416pg/ml of IFN-g, respectively). In contrast, the Hsp-PC recogniser peptide primeD T cells to respond to the breast tumour cell extract (714pg/ml of IFN-g) to a considerably lesser extent. There was notable variation in IFN-g production levels from donor to donor. These results demonstrate the potential of Hsp-PC and structural mimics of these complexes as tumour vaccines.

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Genetic immunization against ratHER2/neu in tumor challenge and spontaneous mouse tumor mode

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The efficacy of rat HER2/neu vaccination against exogenous tumor challenge and spontaneous tumor development was investigated. In transgenic Balb/NeuT mice, the transforming rat HER2/neu oncogene provokes the spontaneous development of an invasive carcinoma in all mammary glands. Female NeuT mice were immunized with Adenovirus 5 (Ad5) and /or plasmid-DNA followed by electrostimulation (DNA+ES), both expressing the rat p185 HER2/neu protein. The immune response to genetic immunization was monitored by antibody titration and IFN-g intracellular cytokine staining (ICS). A codon optimized rat HER2 cDNA sequence was used in this study. Several Ad/DNA combinations and immunization schedules confirmed the superior contribution of Ad-ratHER2.opt in inducing a strong Th1-skewed humoral and CD8+ cell-mediated response. These results prompted us to evaluate the protective response induced by vaccination against an exogenous tumor challenge in syngeneic or xenogeneic fashion. The mouse mammary tumor line D2F2 expressing rat HER2/neu (D2F2neu+) was injected subcutaneously in female Balb/c or NeuT mice immunized with Ad or DNA+ES. The masses of transplanted and spontaneous mammary gland tumors were monitored on the same mice. We showed that in a syngeneic approach, that adenoviral vaccination is better than DNA in protecting mice from spontaneous tumors. Subsequently, we evaluated that two Ad5-ratHER2.opt injections of 10exp9 viral particles at week 10 and 12 were sufficient to induce the highest immune response. They also had a significant impact on tumor progression. At 52 weeks, 45% of the mice were completely protected from tumors and the mean tumor number was < 3. Additional injections of Ad and/or DNA did not affect this result. Histological analysis revealed that in control mice, duct hyperplasia (detected at 10week of age when vaccination was started) was followed by atypical hyperplasia and, subsequently, adenocarcinoma. In vaccinated mice, however, spontaneous mammary tumor development was arrested in the duct hyperplasia stage till 52 weeks of age. Immunohistochemical analysis showed that the expression of the proliferation