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

marker, PCNA, and HER2/neu were significantly reduced in the mammary glands of protected mice.
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A phase I trial in patients with solid tumours using autologous dendritic cells loaded with mannan-conjugated recombinant MUC1 protein

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We report the results of a Phase I immunotherapy trial (1) aimed to elicit reproducible cellular immunity using autologous cultured dendritic cells (DC) loaded with Mucin 1 (MUC1) antigen *ex vivo* before injection. MUC1 is a glycoprotein frequently expressed in large quantities by adenocarcinoma cells. Recombinant MUC1 protein (FP) when conjugated to mannan (M-FP) is rapidly taken up by DC and macrophages. We previously reported trials using the direct injection of MFP to patients (pts), obtaining variable T-cell and antibody responses but no effects on disease progression (2).
Eligible patients had MUC1 positive solid tumours, age >18yrs, PS 0–1 with adequate haematological, renal and hepatic function, and no autoimmune disorders. Ten pts underwent leukapheresis on 3 occasions every 4 weeks with average yield $2.8x10^9$ PBMC. DC were derived from plastic-adherent PBMC by culture with 500 U/mL IL-4 and 500 U/mL GM-CSF for 6 days, being pulsed with 10 ug/mL M-FP on day 5. The harvested cells were injected at i.d. and s.c. sites.
Nine of the 10 pts completed the 12 week study, evaluable for toxicity, immunological endpoints and efficacy; 3 were followed-up for 6 months and another 5 for 12 months post-trial. Patients were injected three times with DC/M-FP. Two patients (renal and ovarian carcinoma) who were clearly progressive at study entry, received additional i.d. injections starting 9 and 11 months from initial treatment, first using freshly harvested DC/M-FP then thawed cryopreserved M-FP pulsed cells. They have had a prolonged period of stable disease (>30 months) with ongoing treatment at 3 monthly intervals.
There was no treatment-related toxicity although 2 pts developed marginal anti-thyroid and nuclear antibodies, respectively. Measured immune responses are Th1-type. All pts developed DTH-like responses at injection sites, confirmed by skin biopsies in 5, after the second or third DC/M-FP injections which were recurrent with each additional injection. Different from earlier trials using direct injection of M-FP (2), all pts showed significant vaccine-specific T cell immunity as IFNγ production by both CD4 and CD8 cells to MUC1 antigen (Elispot), and only 3 pts maintained or had an increase in low titre antibody responses. The results indicate that i.d. injection of DC loaded with mannan-conjugated cancer antigen induce consistent immune responses.

References

[1] Supported by PrimaBiomed Ltd, Victoria, Australia.
[2] Karanikas et al, J Clin Invest 100: 2783, 1997.

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Induction of anti-tumor immunity by an anti-idiotype antibody mimicking human HER2/Neu

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Our goal is to apply an anti-idiotype (Id) based vaccine approach for the treatment of HER2/Neu positive human cancer. Amplification and/or over-expression of HER2/Neu occurs in multiple human malignancies and is associated with a poor prognosis. The HER2/Neu proto-oncogene is a suitable target for cancer immunotherapy. We have developed and characterized a murine monoclonal anti-Id antibody, 6D12 that mimics a specific epitope of HER2/Neu and can be used as a surrogate antigen for HER2/Neu. In this study, the efficacy of 6D12 as a tumor vaccine was evaluated in a murine tumor model. In this model, the murine tumor cell line EL4 was transfected with the human HER2/Neu gene (EL4-Her) and injected into syngeneic, immuno-competent C57BL/6 ($H\text{-}2^b$) mice. Immunization of naïve mice with 6D12 conjugated with keyhole limpet hemocyanin and mixed with Freund's adjuvant or 6D12 combined with the adjuvant QS-21 induced anti-6D12 as well as anti-HER2/Neu immunity. The immune sera from mice reacted with the antigen positive SKBR3 cells by ELISA and FACS analysis. The anti-HER2/Neu specific antibodies in the mice sera also demonstrated strong reactivity with EL4-Her cells, but no reactivity at all with parental EL4 cells by FACS analysis showing specificity of the binding. In *in vitro* culture, immune sera killed HER2/Neu positive tumor cells by antibody dependent cellular cytotoxicity (ADCC). Mice immunized with 6D12 were protected against a challenge with lethal doses of EL4-Her, whereas no protection was observed when 6D12 vaccinated mice were challenged with HER2 negative EL4 cells or when mice were vaccinated with an unrelated anti-Id antibody and challenged with EL4-Her cells. These data suggest that the anti-Id 6D12 vaccine can induce protective HER2/Neu specific antitumor immunity and may serve as a potential network antigen for the treatment of patients with HER2/Neu positive tumors. Supported by the NIH grant R01CA91878.

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Allogeneic whole cell vaccination significantly delays disease progression in hormone-relapsed prostate cancer: final data from a phase II study

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Immunotherapy of cancer is under active development and is particularly appealing for patients with asymptomatic hormone-relapsed prostate cancer (HRPC), in whom conventional treatments offer no survival advantage.
We have evaluated a vaccine comprised of three irradiated allogeneic prostate cell lines ($8x10^6$ cells each) for intradermal injection into draining lymph node basins. The treatment period was one year; the first two doses were supplemented with BCG as vaccine adjuvant at a two-week interval, followed by monthly doses of cells alone. A total of 28 HRPC patients were enrolled on the study using conventional entry criteria of failed hormonal therapy, absence of detectable bone metastases plus the ability to mount a delayed-type hypersensitivity (DTH) response to at least one of a panel of common recall antigens.
Two patients were protocol violators and did not form part of the intention-to-treat (ITT) population. 11 of the 26 patients in the ITT population showed statistically significant decreases in their rate of prostate specific antigen (PSA) release. Median time to disease progression (TTP) was assessed using standard clinical and radiological parameters and was 58 weeks in the ITT population compared with historical control values of ca. 25 weeks. No significant side effects were recorded and quality of life remained unchanged throughout the entire course of treatment.
Immunological analysis showed clear evidence of immune activation after vaccination. Responding patients demonstrated a titratable T_H1 cytokine release profile in response to restimulation with a vaccine lysate, whilst non-responders demonstrated a mixed T_H1 and T_H2 response. An unvaccinated control group did not show any notable vaccine specific cytokine responses. Furthermore, immunological profile, as defined by cell surface markers, maximal cytokine production and proliferation, has been shown to correlate with PSA response using Artificial Neural Network (ANN) analysis.
In conclusion, this study represents evidence of the potential efficacy of whole cell allogeneic vaccination in HRPC and a randomised double-blind study is in preparation.

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Multi-epitope peptide vaccine and co-administration of IL-12 prevents tumor growth in Her-2 transgenic mice

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New approaches in cancer treatment are based on the development of vaccines directed against tumor-associated antigens, thereby guaranteeing effective antitumor immune responses.
In a previous study, we selected putative B cell epitopes (P4, P6, P7) derived from the extracellular domain of Her-2/neu. Immunization of BALB/c mice with these peptides gave rise to Her-2/neu specific antibodies, which elicited strong antitumor activity *in vitro*.
The aim of the present study was to evaluate whether peptide immunization also prevent tumor growth *in vivo*. Female FVB mice transgenic for c-*neu* were immunized with a multiepitope vaccine consisting of the three described peptides coupled to tetanus toxoid. The immunizations were performed with or without addition of the Th1 promoting cytokine