marker, PCNA, and HER2/neu were significantly reduced in the mammary glands of protected mice.
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258 POSTER

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

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259 POSTER

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.

260 POSTER

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.

261 POSTER

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

IL-12. In untreated mice, the tumor growth started 120 days after study initiation. In contrast, in mice immunized with the multi-epitope vaccine the tumor free interval was extended up to 140–190 days; the tumor free interval in mice immunized with the peptides and IL-12 was prolonged even up to 235 days. Once tumors developed, those mice immunized with peptides+IL-12 showed a significantly slower tumor progression than mice not or sham immunized. Characterization of the immune responses revealed that mice immunized with peptides+IL-12 displayed higher IgG2a levels in serum and Th1 biased immune responses (IFN γ) *in vitro*.
From our data, we conclude that immunization with a multi-epitope vaccine in conjunction with IL-12 is very effective in preventing progression of Her-2/neu overexpressing tumors. Such a vaccine could be used in humans together with chemotherapy and/or for prevention of metastases.

262 POSTER

Immunization with genetic vectors expressing rhesus CEA efficiently breaks immune tolerance in mice and rhesus monkeys

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Background: CEA is a 180KDa glycoprotein over-expressed in a high percentage of adenocarcinomas, particularly those of the colon, pancreas, breast and lung. For this reason, it is currently under evaluation in clinical trials as target for immunotherapy in the treatment of colorectal cancer.
Materials and Methods: To demonstrate that genetic vaccination with vectors expressing this tumour antigen is capable of specifically breaking immunotolerance in non-human primates, it is necessary to use the equivalent of human CEA. Since the rhesus monkey (*macaca mulatta*) homologue of this human tumour associated antigen was not available, we have identified and cloned rhesus CEA (rhCEA) from colon tissue samples. rhCEA is an open reading frame of 2118 nucleotides encoding for a 705 aa polypeptide with 78.9% homology to human CEACAM-5 protein.
Results: Vaccination protocols using rhCEA expressing vectors were designed both for mice and rhesus monkeys.. To demonstrate the capability of xenogeneic vaccination to elicit an immune response against CEA as self-antigen in this model, we immunized CEA. Tg mice with vectors encoding either human (homogeneic) or rhesus CEA (xenogeneic).
After treatment of mice with DNA followed by EGT (Electro Gene Transfer) and adenovirus boosting, cross-reactive antibodies against human CEA protein were measured only in rhesus CEA immunized groups. Importantly, cellular immune-response against human CEA was observed upon immunization with rhesus CEA both in wild type and transgenic mice.
To further increase the level of antigen expression, we have constructed a synthetic codon usage optimized rhCEA cDNA (rhCEAopt). *In vitro* studies showed 10–50 fold greater protein levels than a similar vector carrying the native cDNA. Similarly, intramuscular injection of a DNA vector followed by EGT or Adenovirus expressing rhCEAopt in CEA. Tg mice resulted in greater protein levels than those detected upon injection of vectors encoding for rhCEA. Mice immunized with plasmid/ adenovirus vector mixed modality, both containing the cDNAopt showed strong cross reactive human CEA-specific antibody response, 300-fold higher than hCEA containing vectors. Cell mediated responses were two- and three-fold higher against rhesus or human protein, respectively, than using the vectors containing the native rhCEA.
To assess the efficiency of immunization of rhesus macaques with rhesus CEA, we injected vectors encoding for rhCEA or rhCEAopt in twelve monkeys. Both Ad vectors alone or in combination with DNA were efficient in breaking immune tolerance to CEA in immunized rhesus monkeys and maintain over time elicited immune response.
Conclusions: Our data show that use of rhesus CEA and development of modified expression cassettes that result in increased potency of Adenovirus, plasmid DNA and other gene delivery vaccine approaches may have significant impact on vaccine development against neoplastic malignancies expressing CEA.
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263 POSTER

Synergistic antitumor activity of interleukin 23 and interleukin 2

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The recently discovered IL-23 shared with IL-12 similar heterodimeric structures and overlapping but distinct functions in the regulation of both innate and adaptive immunity. IL-12 has been shown to confer potent antitumor activity in a variety of murine tumor models. In our previous study, we showed that IL-23 also possessed potent antitumor activity. CT26 colon adenocarcinoma cells transduced with retroviruses carrying a single-chain IL-23 gene (CT26/IL-23) grew progressively until day 26 to an average size of 521 ± 333 mm^3 and then tumors started to regress in most animals, resulting in a final 70% rate of complete tumor rejection. In the present study, we seek a possible cooperative antitumor effect of IL-23 and IL-2. CT26 cells engineered to secrete both IL-23 and IL-2 (CT26/IL-23/IL-2) produced only a transient tumor growth, followed by complete rejection in all animals. Most significantly, transduction of both IL-23 and IL-2 resulted in significant reduction of lung tumor metastasis and led to 60% of mice survived the challenge, while all animals challenged i.v. with IL-23- or IL-2-transduced CT26 cells eventually died of lung metastasis. *In vivo* depletion experiment showed that rejection of CT26/IL-23/IL-2 tumor cells required both $CD4^+$ and $CD8^+$ T lymphocytes. Immunohistochemical analysis revealed tumor moderate infiltration of $CD4^+$ and $CD8^+$ T cells, and abundant infiltration of granulocytes ($Gr\text{-}1^+$) and macrophages ($Mac\text{-}1^+$) when tumors were in regression. We are currently investigating whether granulocytes and macrophages play a role in the IL-23/IL-2-mediating antitumor activity.

264 POSTER

Strong Melan-A/MART-1 specific CD8+ T cell responses to peptide vaccination in young melanoma patients

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Tumor vaccines aim to induce strong specific T cell activation in vivo, which may well result in enhanced immune protection. It remains poorly understood why T cell responses are detectable in some but not all patients. We have previously shown that tumor-driven CD8+ T cell pre-activation is one of the factors associated with increased T cell responsiveness to peptide vaccination. In search for further parameters, we analyzed whether patient age may play a role. Eight stage III/IV melanoma patients (34–75 years old) were treated with four monthly low dose vaccinations with CpG oligodeoxynucleotide 7909, mixed with Melan-A analog peptide and Incomplete Freund's Adjuvant. We used fluorescent HLA-A2/Melan-A multimers (tetramers) to measure T cell frequency ex vivo in circulating blood by flow cytometry. High percentages (between 0.07 and 3.42%) of Melan-A peptide specific CD8+ T cells were found after vaccination, revealing strong T cell responses in all eight patients. Interestingly, we found a statistically significant (P <0.01) inverse correlation between T cell responses and patient age. Thus, besides tumor-driven T cell pre-activation, young patient age is an additional parameter predicting T cell responsiveness to vaccination with tumor peptides.

265 POSTER

First clinical evidences of antigen spreading in metastatic melanoma patients treated with a NGcGM3/VSSP/Montanide ISA 51 vaccine: A Phase I/IIb study

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Background: N-glycolyl GM3 ganglioside immunodominant epitope is expressed on human breast and melanoma tumours but absent from normal melanocytes. This unique feature renders this antigen very attractive for immunotherapy. NGcGM3 was non-covalently incorporated in the natural outer membrane vesicles of *Neisseria meningitidis* to form very small sized proteoliposomes (VSSP) and emulsified with Montanide ISA 51. In previous studies the immunogenicity and safety of this vaccine have been documented but in breast cancer patients. With this phase I/IIb study we intended to evaluate, in metastatic melanoma patients, the immunogenicity and safety of the preparation at two different dose levels. Patient's monitoring for clinical responses was also planned.
Methods: Twelve and nine metastatic melanoma patients received 0.2 or 0.4 mg of the vaccine in each of the 9 immunisations, respectively. The first 5 IM doses (induction phase) at two weeks intervals, while the remaining