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Epidermal growth factor based cancer vaccine for non-small cell lung cancer therapy: report from a phase I scale up trial

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Epidermal growth factor (EGF) plays an important role in the regulation of tumor growth upon binding to the EGF receptor (EGFR). Several strategies had been developed to disrupt the EGFR associated signal transduction cascade. Therapeutic approaches include monoclonal antibodies and small molecule tyrosine kinase inhibitors. Our method consists of active immunotherapy with human recombinant EGF (hu-EGF) intended to induce EGF immune deprivation. Previous studies have demonstrated that vaccination with EGF is immunogenic and safe in 70 advanced cancer patients. In 40 Non-small cell lung cancer (NSCLC) patients, those who developed a high antibody response had a significant increase in survival in comparison with bad responders. To optimize the immunization scheme, a phase I trial was designed to evaluate the effect of vaccine dose escalation on immunogenicity and survival. Twenty advanced NSCLC patients were randomized to receive 70 mg (single dose) or 140 mg (double dose) of hu-EGF, coupled to a carrier protein and adjuvanted in Alum. Thirteen patients (65 %) developed antibody titers against the EGF, defined at least 4 times above baseline. In the double dose group, 8 patients (80 %) achieved seroconversion, while 5 patients (50 %) developed anti-EGF antibody titers in the single dose arm. The geometric mean of the antibody titer was higher in the double dose group. No significant toxicity was seen after vaccination. Main adverse events consisted in chills, fever, nausea, vomiting and cephalea. As a surrogate endpoint of vaccination, EGF concentration was quantitated in sera using a commercial kit. After immunization, a statistically significant inverse correlation between antibody titers and EGF concentration was evidenced. Double dose treated patients showed a trend to increase in survival in comparison with the single dose immunized subjects. Patients with high antibody titers had a significant increase in survival compared with bad responders or a historical control group.

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Use of OvaRex(r) MAb-b43.13 as an immunotherapeutic treatment of epithelial ovarian cancer: experience as single agent post first-line therapy and in combination with chemotherapy in recurrent disease

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OvaRex(r) MAb-B43.13 (oregovomab) is a murine monoclonal antibody (MAb) studied in multiple clinical trials as adjunct immunotherapy for ovarian cancer. The i.v. infused MAb modulates immunity by complex formation with the circulating tumor-associated antigen CA125, followed by uptake of these immune complexes by antigen-presenting cells and preferential processing and presentation to T cells. Clinical experience with OvaRex(r) MAb-B43.13 across six Phase II studies has established several therapeutic principles. T and B cell responses were commonly induced after 3-4 doses. About 60-70% of pts developed robust HAMA and Ab2 responses, and 20% developed anti-CA125 antibodies during treatment. IFN-gamma ELISPOT analysis conducted in three trials demonstrated significant increases in MHC class I and II restricted T cell precursors specific for autologous tumor (63% of pts) and CA125 (41% of pts). Development of a T cell response was associated with highly significant advantage in time to progression and survival ($p < 0.01$)(Ehlen et al., Gyn Oncol 80:310, 2001; Schultes et al., Proc. AACR 43: 144, 2002). In two placebo controlled studies in pts with no evidence of disease following front line therapy, clinical benefit relative to placebo was demonstrated in the population with more optimal responses to front line therapy. The treatment effect was enhanced in patients afforded time to mount meaningful immune responses and with detectable levels of circulating CA125 at time of first injection (OvaRex(r): 20.2 months, placebo 10.3 months; p=0.022, log-rank test). Treatment of patients with residual tumor burden or recurrent disease is considered in conjunction with chemotherapy. Three trials with more than 50 actively treated patients in combination with chemotherapy have demonstrated preservation of both chemo-responsiveness and treatment-emergent immune responses. The safety experience in more than 500 treated patients has been similar in active and placebo populations. The combined data from all studies conducted to date clearly establish OvaRex(r) MAb as an efficient and well-tolerated immunotherapeutic approach to improve clinical outcomes as an adjunct therapy in ovarian cancer patients with more optimal responses to first line therapy and baseline CA125 values >5 U/mL as well as a combination therapy with chemotherapeutic agents in patients with residual or recurrent ovarian cancer. These findings will be further validated in confirmatory studies.

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Identification of a WT1 HLA A*0201-restricted CTL epitope using whole gene *in vitro* priming

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The Wilms tumor (WT1) protein is a self-protein recently identified as a candidate antigen for leukemia vaccine and T-cell therapy. The current study assessed the feasibility of generating WT1 specific T-cell responses using whole gene *in vitro* priming. The advantages of whole gene *in vitro* priming are 1) the entire spectrum of epitopes of a given protein is present, 2) selection and presentation of these naturally processed peptides are done by the antigen presenting cell. Monocyte derived dendritic cells (DC) of HLA A0201 positive normal donors were infected with replication-deficient recombinant adenovirus (Adeno) or vaccinia virus (Vac) expressing full length WT1. CD8+ T-cell cultures were restimulated every 7-10 days, alternating Adeno/WT1 infected autologous DC with Vac/WT1 infected DC. T cell responses were evaluated by measuring levels of interferon-gamma secretion by ELISPOT analysis in response to WT1 expressing target cells. After 4 stimulation cycles, CD8+ T cell lines that specifically recognized WT1 transduced autologous fibroblasts, but not control transduced fibroblasts, were identified and cloned. HLA A2 restriction of the clonal T-cells was documented by 1) antibody blocking experiments and 2) recognition of WT1 transduced fibroblasts derived from a second donor, who shares only the HLA A2 allele with the original donor. Recognition of leukemia cells "naturally" overexpressing WT1 by the CTL clone was shown by recognition of HLA A2 transduced WT1 overexpressing cell line K562 but not of HLA class I negative control transduced K562 cells. Using truncated WT1 retroviral constructs to transduce autologous fibroblasts the WT1 epitope was localized to the first 92 N-terminal aminoacids of the WT1 protein. Using overlapping WT1 peptides the epitope was further localized to aa37-47. All 9mer WT1 peptides within this region were synthesized. The CD8+ clone specifically recognized the 9mer VLDFAPPGA (aa37-45), demonstrating that this WT1 peptide is a naturally processed HLA A0201 restricted epitope. The ability to generate WT1 specific CD8+ T-cell clones and clone their T-cell receptor might allow treatment of malignancies associated with WT1 overexpression using genetically engineered T-cells. These data provide further validation of WT1 as a leukemia vaccine and T-cell therapy candidate.

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Administration of FGF-2 peptide vaccine leads to abrogation of angiogenesis and tumor development

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Basic fibroblast growth factor (FGF-2) is an important stimulator of angiogenesis that has been implicated in neoplastic progression. Attempts to neutralize or modulate FGF-2 have met with some success in controlling neovascularity and tumor growth. In this study, we developed a vaccine directed towards FGF-2. Peptides corresponding to the heparin binding domain [L(HBD)] or the receptor-binding domain [L(RBD)] of FGF-2 were incorporated into liposomes and used to vaccinate animals. Mice vaccinated with L(HBD) generated an antibody response to FGF-2, blocked neovascularization in a gelfoam sponge model of angiogenesis, and inhibited experimental metastasis by > 90% in two tumor models: the B16BL6 melanoma and the Lewis lung carcinoma (LLC-LM). These effects were not observed in mice treated with L(RBD). Investigation of immune status revealed that mice vaccinated with L(HBD) generated both a specific cellular response measured by a delayed type hypersensitivity response and IFNgamma production and a humoral response. Furthermore, antibody against FGF-2 was shown to inhibit FGF-2 binding to heparin sulfate. These data indicate that vaccination with L(HBD) leads to the induction of a cellular immune response, as well as a specific humoral response that may abrogate FGF-2 activity and tumor development. Studies to address the effects of the

L(HBD) vaccine on wound healing, reproduction and embryonic development were performed in mice. Vaccination with the L(HBD) vaccine did not result in alterations in mean time to wound healing when compared to unvaccinated animals or those treated with a liposome control. In addition, L(HBD) vaccinated female mice were not impaired in their ability to become pregnant, support the growth and development of embryos, or deliver viable offspring. Furthermore, these offspring did not demonstrate any alterations in organogenesis when compared with pups born to untreated mothers or those treated with liposome control preparations. Thus, while vaccination against FGF-2 inhibited angiogenesis and tumor development, it did not appear to adversely alter wound healing or reproduction. Taken together, the generation of an active immune response that targets FGF-2 to block angiogenesis and tumor development is a unique approach to vaccine development that warrants clinical investigation.

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MSI derived frameshift mutations represent novel tumor specific antigens

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Microsatellite instability (MSI) caused by defective DNA mismatch repair (MMR) is a hallmark of hereditary non-polyposis colorectal cancers (HNPCC) but also occurs in about 15% of sporadic tumors. If instability affects microsatellites in coding regions, translational frameshifts lead to truncated proteins often marked by unique frameshift peptide sequences at their C-terminus. Since MSI tumors show enhanced lymphocytic infiltration and our previous analysis identified numerous coding mono- and dinucleotide repeat bearing candidate genes as targets of genetic instability, we examined the role of frameshift peptides in triggering cellular immune responses. Using peptide pulsed autologous CD40-activated B cells we have generated cytotoxic T lymphocytes (CTLs) that specifically recognize HLA-A2.1-restricted peptides derived from frameshift sequences. Among 33 frameshift peptides predicted from mutations in 12 different genes, 9 peptides conferred specific lysis of target cells exogenously loaded with cognate peptide. Four peptides derived from a (-1) frameshift mutation in genes coding for TGF-betaIIR, OGT, MSH-3 and Caspase-5 gave rise to CTL capable to lyse MSI cancer cell lines, carrying this frameshift mutations. Given the huge number of human coding microsatellites and assuming only a fraction being mutated and encoding immunologically relevant peptides in MSI tumors, frameshift protein sequences represent a novel subclass of tumor specific antigens. It is tempting to speculate that a frameshift peptide directed vaccination approach not only could offer new treatment modalities for existing MSI tumors but also might benefit asymptomatic at-risk individuals in HNPCC families by a prophylactic vaccination strategy.

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Active and protective immunity induced by a protein based vaccine targeting the HER2/neu oncogenic protein

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More than 185 000 new cases of breast cancer are diagnosed each year. Among them, 30% are shown to overexpress the HER2/neu oncogene which plays a crucial role in the pathogenesis and contributes to a poor clinical outcome. The HER2/neu protein is an attractive therapeutic and immunogenic target. The existence of antibody, helper T cells and cytotoxic T cell immunity to HER2/neu have been demonstrated in patients with cancer. Moreover, passive transfer of HER2/neu specific monoclonal antibodies such as Trastuzumab, have been shown to be of clinical benefit in patients with HER2/neu overexpressing tumors. A vaccine targeting Her2/neu should be effective if serum antibody responses are elicited that mimic trastuzumab and is anticipated to show increased efficacy if T cell responses are elicited in addition to serum antibody responses. Approaches to elicit immunity to HER2/neu currently tested include peptide-based, DNA-based and protein-based vaccines. Our group (GSK/ Corixa) has developed a protein-based vaccine composed of both the extracellular domain (ECD) and the carboxyl terminal portion of the intracellular domain (ICD), formulated in a strong adjuvant. We show that after several vaccinations, mice develop both humoral and cellular responses to HER2/neu. This Her2/ neu specific immune response is able to protect mice against a tumor challenge with an HER2/neu expressing mouse tumor and implies both CD4 and CD8 T cells. HER2/neu specific antibodies were induced in rabbits and monkeys. The presence of functional antibodies that inhibit the *in vitro* growth of the human breast cancer cell line SKBR3 and the *in vivo* growth of human ovarian SKOV3 tumor xenograft was demonstrated in sera form vaccinated animals. These studies demonstrate that a vaccine based on a purified dHER2 protein formulated in a strong adjuvant can induce a systemic antitumor immune response with both humoral and cellular components directed against the extracellular and intracellular domains of the HER2/neu oncogene.

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Development of a WT1 protein vaccine

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The Wilms' tumor (WT1) gene is overexpressed in most human leukemias as well as in several solid tumors. There is substantial evidence that WT1 is immunogenic in humans and in mice. The current study (1) assessed the optimal WT1 protein formulation in terms of immunogenicity and toxicity in C57/Bl6 mice and (2) tested *in vivo* efficacy of this formulation in HLA-A2/Kb transgenic mice. Two immunological adjuvants (MPL-SE, ENHANZYN) were compared at 2 different dose levels (10ug, 25ug) with WT1 protein vaccine in C57/Bl6 mice. 10ug MPL-SE stood out as being especially effective for induction of IgG2a antibodies and potent IFN-gamma responses against WT1. A multiple dose titration study (doses ranging from 25ug, 100ug to 1000ug WT1 protein) in female C57/B6 demonstrated induction of antibody and T-cell immunity against WT1, without any signs of toxicity. To assess *in vivo* efficacy of the WT1 protein vaccine formulation a WT1 tumor model was established in the HLA A2 transgenic mouse strain. Mice were immunized with either saline (Gp 1, control) or 100ug WT1 protein using 10ug MPL-SE as adjuvant (Gp 2). Three weeks after the last immunization mice were inoculated with 2 million WT1 positive tumor cells. Histopathological analysis demonstrated that 10/10 (100%) animals in the control group developed tumors. In marked contrast only 4/9 (45%) animals in the WT1 protein immunized group showed tumor take. Given that 1) existent immunity to WT1 is present in some patients with leukemia 2) vaccination to WT1 protein elicits WT1 specific Ab, and T-cell responses in mice without toxicity to normal tissues and 3) WT1 protein immunization shows *in vivo* efficacy in the A2/Kb transgenic mouse model, human phase I trials are being contemplated testing WT1 protein with MPL-SE as adjuvants in patients with AML and MDS.

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Inhibition of tumor cell growth by antibodies induced after vaccination with peptides derived from the extracellular domain of Her-2/neu

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Monoclonal antibodies directed against Her-2/neu have inhibiting effects on tumor growth. The humanized murine anti Her-2/neu antibody Trastuzumab is successfully used in the clinical routine. However, an active vaccination inducing a long-term immunity to Her-2/neu is still a desirable goal. The aim of the presented study was to induce production of specific Her-2/neu antibodies and to test their efficacy to inhibit tumor cell growth. BALB/c were immunized with peptides derived from the extracellular domain of the human Her-2/neu, coupled to tetanus toxoid (TT). Seven days after the last immunization animals were sacrificed and antigen-specific antibody levels were measured. The IgG fractions from these sera were isolated and used for *in vitro* proliferation assay performed with the breast cancer cell line SK-BR-3. Moreover, hearts, lungs, livers, and kidneys were histopathologically screened for inflammatory infiltrations. Immunization with the peptides led to induction of anti-Her-2/neu antibodies - in particular IgG_1 – which were able to precipitate human Her-2/neu from cell lysates of SKBR-3. Incubation of the SKBR-3 cells with the IgG fractions from the mice sera led to a significant reduction of the cell growth, indicating the anti-tumor activity