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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