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