Method: This bicentric clinical phase I study was conducted in an open-label setting and consisted of escalating single dose part (SD) followed by a multiple dose (MD) regimen. In case of safety of the SD, the MD was designed to evaluate safety as well as efficacy of the corresponding doses of twice weekly subcutaneously administered MGN1703 in a dose escalation scheme with 0.25 to 60 mg over 6−12 weeks. CT scans were performed every 6 weeks. Patients who were stable or who responded to MGN1703 according to RECIST after the first 6 weeks were treated for 6 further weeks. Immune assessment with flow cytometry of peripheral blood (PB) was performed before, during and after therapy to assess changes in the cellular compartments.
Results: A total of 24 patients with metastatic solid tumors (8 melanoma, 8 colorectal, 5 lung, 2 breast and 1 kidney cancer) were treated with 0.25 mg (n = 5), 2 mg (n = 3), 10 mg (n = 5), 30 mg (n = 8), and 60 mg (n = 3) MGN1703. Of them, 9 patients showed stable disease after 6 treatment weeks. Two of these 9 patients completed only 10 and 11 treatments, respectively (corresponded to 5 and 5.5 treatment weeks) and discontinued treatment due to administrative reasons. Further patients who dropped out before the completion of 6 weeks of treatment were 3 patients with progressive disease, 3 patients with protocol violation/ administrative reasons, and 1 patient with withdrawal of consent. Fifteen patients finished the first 6 treatment weeks according to the protocol. Of them, 7 patients (46.6%) showed a stable disease after 6 weeks and continued the treatment for further 6 weeks. All these 6 patients finished the 12 weeks treatment according to the study protocol. Three of them remained stable after 3 months. Median progression-free survival was 1.5 months.
The immune assessment of cell compartments suggested an increase of dendritic cells during therapy. Phenotypically, there was a shift in favour of plasmacytoid as compared to myeloid DC. Also, the relative number of naïve B-cells slightly increased with the number of memory B-cells reduced. T-cell frequencies mainly remained unchanged.
Conclusion: MGN1703 showed moderate efficacy in heavily pre-treated patients with metastatic solid tumors. MGN1703 induces changes in the cellular compartments of TLR-9 expressing cells such as plasmacytoid dendritic cells and naïve B-cells. A clinical phase 2 study for the maintenance therapy of patients with metastatic colorectal carcinoma responding to first-line chemotherapy has been initiated.

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Immunotherapy with the toll-like receptor 9 agonist MGN1703 in patients with metastatic solid tumors − safety results of a clinical phase I study

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Background: MGN1703 is a novel synthetic DNA-based immunomodulator, which acts as a toll-like receptor 9 agonist. The safety of MGN1703 has previously been shown in several animal toxicity studies models as well as in the first clinical observations of MGN1703 in form of Investigator-initiated-trials (ITT). A clinical phase I study was conducted in patients with metastatic melanoma and colorectal, breast, lung and kidney cancer without further treatment options. The results of the study are presented here.
Method: This clinical phase I study was conducted at two study sites in Germany in an open-label setting and consisted of escalating single dose regimen (SD) followed by a multiple dose part (MD) in a dose escalation scheme. Doses of 0.25 mg, 2 mg, 10 mg, 30 mg, and 60 mg were administered subcutaneously over 6−12 weeks twice weekly. When a dose level proved to be safe after SD, MD was initiated. Subsequently, responding patients were proposed to participate in the extension phase of the study (6 further weeks).
Results: 28 patients with metastatic solid tumors were included in this clinical study. Five patients received 0.25 mg, 3 patients received 2 mg, 5 patients received 10 mg, 8 patients received 30 mg, and 3 patients received 60 mg MGN1703. The most frequently reported drug-related adverse events (AEs) were fever, dizziness, fatigue, and headache. All drug-related adverse events were of mild to moderate intensity or ⩽CTC-Grade 2 and were reported in single patients. There were no dose-related changes in the incidence of AEs. No subject was withdrawn from the study due to AEs. Local reactions in form of mild redness were reported only in few patients (SD − 0.25 mg group: n = 2, 2 mg: n = 1, 60 mg: n = 1; MD − 30 mg: n = 4, 60 mg: n = 1).
Conclusion: According to our data, twice weekly subcutaneous application of MGN1703 in a dose of up to 60 mg is safe and well tolerated without dose-limiting toxicities. The dose level of 60 mg MGN1703 for twice weekly application is recommended for the phase II clinical trial, which has been initiated.

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A novel method to improve antigen presentation and immunopotency of RNA-loaded monocyte-derived dendritic cells

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Background: Monocyte-derived dendritic cells (DCs) provide a powerful vehicle for the stimulation of cell-mediated immunity through effects on both $CD4^+$ and $CD8^+$ T cells. Generation of antigen-specific cytotoxic T cells (CTLs) is an important factor for immune control of cancer. In the autologous DC-based therapy, AGS-003, antigen is delivered into DCs in a form of amplified autologous total tumor RNA. Such RNA must be translationally competent to assure the expression of protein it encodes for presentation by the DCs. Improvements made to the RNA structure that lead to greater levels of antigen expression and, therefore, higher cell surface epitope densities should improve the overall immune response initiated by the administration of AGS-003. Demonstration of the expression of any single specie captured in a population of the RNA amplified from the tumor cell is challenging due to the high complexity of the RNA and low absolute level of individual RNA.
Methods: We developed a model system to study the expression levels of the various RNAs in the electroporated DCs. The model system was used to evaluate whether the change in primers together with incorporation of animal product-free PCR enzyme into the amplification protocol as well as new approach (post transcriptional) capping of the synthesized RNA results in the increased antigen expression. The impact on the changes implemented into the RNA amplification protocol was also studied in T cell assays.
Results: We demonstrate that an improved RNA amplification process results in a fully translation capable RNA repertoire. Microarray studies demonstrated that the changes introduced into the amplification protocol result in an amplified RNA set which is more representative of the starting total tumor RNA. Furthermore, modification of the *in vitro* transcription method from co-transcriptional capping to post-transcriptional capping was implemented increasing both RNA yield and capping efficiency. Protein expression studies confirmed a correlation between increased capping efficiency of the improved RNAs with and increased levels of protein expressed in electroporated DCs. Finally, these DCs are more efficient at inducing antigen-specific $CD8^+CD28^+CD45RA^-$ effector memory T cells *in vitro*.
Conclusions: These studies predict that AGS-003 could demonstrate increased activity in cancer patients which is currently tested in a phase II clinical trial in RCC.

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A better immune reaction to Erbb-2 tumors is elicited in mice by DNA vaccines encoding rat/human chimeric proteins

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The Erbb-2 (neu in rat and Her-2 in humans) tyrosine kinase receptor is an oncoantigen, i.e. a tumor-associated molecule directly involved in cancer progression. Since oncoantigens are self-tolerated molecules, to trigger a response circumventing tolerance, we generated two plasmids (RHuT and HuRT) coding for chimeric neu-Her-2 extracellular (EC) and transmembrane (TM) proteins that are expressed on the cell membrane of the transfected cells and recognized by monoclonal antibodies reacting against neu and Her-2. RHuT encodes a protein in which the 410 amino-terminal residues are from the neu EC domain and the remaining residues from Her-2. Almost symmetrically, HuRT encodes for a protein in which the 390 amino-terminal residues are from Her-2 and the remainder from neu. The ability of RHuT and HuRT to elicit a protective response to neu and Her-2 in wild-type (wt) mice and in transgenic mice tolerant to neu and Her-2 proteins was compared with that of plasmids coding for the fully rat or fully human EC and TM domains of the Erbb-2 receptor (HuHuT and RRT respectively). In most cases RHuT and HuRT elicited a stronger response, though this chimeric benefit is markedly modulated by the location of the heterologous moiety in the protein coded by the plasmid, the immune tolerance of the responding mouse, and the kind of Erbb-2 orthologue on the targeted tumor. Moreover, the different ability of chimeric plasmids to elicit an effective protective immune response was evaluated by Winn type assay experiments in wt mice. When spleen cells