

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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POSTER

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 \leq 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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POSTER

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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POSTER

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

(SPC) from RHuT or RRT vaccinated mice were mixed with neu+ tumor cell lines and injected subcutaneously into the mammary fat pad of wt mice, a significant difference in tumor incidence was observed, being SPC from RHuT vaccinated mice the most effective. Immunohistochemical analysis revealed a clear differences in the type of the immune infiltrate in the tumor site. Mechanisms responsible for this kind of phenomena are under investigation.

Wednesday, 17 November 2010

Poster Sessions

Late breaking posters

4LB LATE BREAKING POSTER A first synthesis of [18F]-lapatinib: a new agent for positron emission tomographic studies of kinase receptors

For full abstract, see p. 4.

5LB LATE BREAKING POSTER Anti-tumor activity of MPC-9528, GMX1778 and APO866: Namp1 inhibitors of three different structural classes

For full abstract, see p. 4.

6LB LATE BREAKING POSTER The Namp1 inhibitor MPC-9528 and the PARP inhibitor olaparib synergize in killing a BRCA-deficient cancer cell line

For full abstract, see p. 5.

Molecular-targeted therapies – preclinical

50 POSTER A phase I study of the safety, tolerability and pharmacokinetics of pazopanib (P) in combination with gemcitabine (G) for advanced solid tumors

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Background: P (Votrient®), an oral angiogenesis inhibitor targeting VEGFR-1, -2, and -3, PDGFR- α and - β , and c-Kit, is FDA approved for advanced RCC. The combination of P + G is a novel regimen that may have broad clinical applicability.

Methods: Eligible pts had progressed on standard therapy, had adequate organ function, and received no prior anti-VEGF(R) therapy. A 3 + 3 dose escalation design was followed by an expansion phase. G was administered as a 30-min IV infusion on days 1 and 8 of each 21-day cycle at escalating dose levels (DL) of 1000 or 1250 mg/m². P was administered orally once-daily at escalating DLs of 400 or 800 mg. In the dose escalation phase, sparse sampling was performed to estimate peak and trough concentrations of P (C1D1: pre-dose and 3.5 h; C1D8 and C2D1 at pre-dose), and of G and its metabolite dFdU (C1D1 and C1D8 at pre-dose and end of infusion). In the cohort expansion phase, serial PK sampling was performed to characterize full PK profiles of G and dFdU with G given alone (C1D1) and in combination with P (C2D1). 24-hour P PK was determined on C2D1.

Results: 22 pts were enrolled; common tumor types were melanoma n = 8, NSCLC n = 4; CRC n = 4. The most frequent drug-related AEs (as a % of all pts) were fatigue 68%, neutropenia 59%, nausea 55%, anorexia 50%, and thrombocytopenia 41%. Most common Gr 3/4 AEs (as a % of all pts) were neutropenia 45% and thrombocytopenia 18%. Gr 3 (without Gr 4 observed) AEs were ALT increase 18%, and 9 % each for: lymphopenia, fatigue, diarrhea, abdominal pain, hyperbilirubinemia. A non-neutropenic pt in DL 0 with sarcoma metastatic to lungs died of pneumonia at Day 105. Mean DLs of P and G for each DL are listed in Table. DL 2 was expanded to 13 pts to further assess tolerability. PK analysis indicates that systemic exposures of P, G, and dFdU in both dose escalation and cohort expansion phases appeared consistent with historical data. Comparison of G and dFdU PK

parameter ratios (C2D1/C1D1) in expansion phase indicates P has no effect on the PK of G or dFdU. Best responses were: 1 PR (melanoma), 14 SD, 4 PD, 3 UNK. Durable disease control was observed with various tumor types; cholangiocarcinoma (cycle 17), melanoma (cycle 14), CRC (cycle 12).

Conclusions: P + G appears clinically active and tolerable for extended periods. Although an MTD was not reached, based upon analysis of tolerability as a function of DI, P 800 mg daily + G at 1000 mg/m² on days 1 and 8 every 21 days will be tested in phase II studies.

Dose level (n)	P (mg) daily	G (mg/m ² d1, 8 q21 d cycle)	Mean (%) P/G dose intensity (DI)	No. of subjects/ type of DLT
0 (n = 6)	400	1000	92.4/79.7	1/Gr 4 thrombocytopenia
1 (n = 3)	800	1000	81.7/83.1	0/
2 (n = 13)	800	1250	94.9/86.1	1/Gr 3 fatigue

51 POSTER Deacetylation and inactivation of peroxiredoxin by SIRT2 increases sensitivity of breast cancer cells to oxidative stress

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SIRT2, the mammalian ortholog of yeast *Hst2*, is a predominantly cytosolic and nuclear member of the class III, NAD⁺ dependent, histone deacetylase family. In the cytosol, SIRT2 co-localizes with microtubules and deacetylates α -tubulin at the lysine-40, indicating a role in proper cytokinesis. Here, we determined the other biologically important SIRT2 targets and their functions. In HEK293 cells, knockdown of SIRT2 followed by differential in-gel electrophoresis of the cytosolic extract and mass spectrometric analysis identified several significantly hyperacetylated proteins, including peroxiredoxin 1. Peroxiredoxins (PXD) are ubiquitous family of evolutionarily conserved, thiol-dependent peroxidases, which catalyze the reduction of hydrogen peroxide (H₂O₂). SIRT2 co-immunoprecipitated with PXD1, and ectopic over-expression of SIRT2 deacetylated PXD1 in HEK 293 cells. While hyperacetylation activates, deacetylation is known to inhibit the antioxidant activity of PXD1. Ectopic over-expression of SIRT2, but not the catalytically dead SIRT2 mutant, markedly increased intracellular reactive oxygen species (ROS) levels and increased ROS-induced DNA damage (determined by comet assay), as well as increased the sensitivity of the human breast cancer MCF7 and MDA MB231 cells to oxidative stress induced by H₂O₂. SIRT2 over-expression also significantly increased the sensitivity to ROS-inducing agents such as arsenic trioxide and menadione. Ectopic over-expression of SIRT2 induced FOXO3a and BIM levels, which was associated with increased caspase-3 activity and apoptosis of breast cancer cells. Additionally, wild type zebrafish embryos 48 hours post-fertilization were treated with 1.0 mM of splice-blocking morpholino (MO) targeting the exon 6 of the SIRT2 pre-mRNA versus the mismatch-control MO and/or 3.0 mM of H₂O₂. Exposure of zebrafish embryos to control MO and H₂O₂ increased ROS and produced cardiac edema and abnormal body curvature. In contrast, knockdown of SIRT2 in the zebrafish embryos decreased H₂O₂-induced ROS levels and abrogated ROS mediated cardiac edema and abnormal body curvature. These in vitro and in vivo findings demonstrate that SIRT2 regulates PXD1 acetylation and sensitivity to oxidative stress. These results also highlight the possibility that increased SIRT2 levels and activity can be therapeutically exploited for augmenting antitumor effects of agents that induce cancer cell death by increasing intracellular ROS levels.

52 POSTER Discovery of novel fused thiadiazoles as potent inhibitors of phosphoinositide-3-kinase (PI3K) and/or the mammalian target of rapamycin (mTOR)

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Several inhibitors of phosphoinositide-3-kinase (PI3K) are currently being evaluated in early clinical studies for their ability to block PI3K/Akt pathway signaling that is found to be activated in many tumors. Here, we disclose a novel series of PI3K inhibitors based on fused thiadiazole bicyclics and present their synthesis, structure-activity and structure-property relationships. We identified analogs with potent PI3K activity in the low nanomolar range and discuss their cross-reactivity with Flt3 as well as their cellular activity.

The mammalian target of rapamycin (mTOR), a class IV PI3K protein kinase, is – like PI3K itself – an important regulator of cell growth