the value of p53 activation in preclinical and clinical models before nutlin-3 like drugs are approved. The mechanism by which actinomycin D acts at these doses appears to be by the release of ribosomal proteins that bind to and inhibit MDM2 function. This mechanism explains why the drug can phenocopy the affects of Nutlin3.

42 INVITED

New inhibitors targeting critical cancer dependencies: Progress and challenges

P. Workman. *United Kingdom*

Abstract not received

43 INVITED

Targeting PI3K: where are we?

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Background: The Phosphoinositide 3-Kinase (PI3K) pathway is activated in a large fraction of human cancers due to activating mutations in PIK3CA, PIK3R1 or AKT and loss of function mutations in PTEN or INPP4B. This pathway plays a major role in controlling glucose uptake and metabolism in cancer cells and the ability of these mutations to provide a survival signal is in part due to increased nutrient availability.
Materials and Methods: Mice were genetically engineered to express mutant PIK3CA or to delete PTEN in specific tissues.
Results: Mice with activating mutations in the PIK3CA gene develop cancers that have high rates of glucose uptake and metabolism and pharmacological inhibitors of PI3K block glucose uptake and cause tumor shrinkage. Based on endothelial cell specific deletion of PI3K genes, PI3K signaling is also critical for neovascularization of tumors, raising the possibility that PI3K antagonists could block tumor growth by disrupting the vasculature.
Conclusions: The characterization of drug effects on tumors from genetically engineered mice is likely to provide a background for identifying biomarkers that predict which patients will benefit from treatment with PI3K pathway inhibitors.

Wednesday, 17 November 2010

Poster Sessions

Cancer vaccines

44 POSTER

Vaccination of dendritic cells pulsed with tumor endothelial cells inhibits tumor growth

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Background: Angiogenesis is required for the growth of solid tumors. Therefore, the breaking of tumor-induced endothelial cells (TEC) should be a useful approach for cancer therapy. Endothelial cells (EC) in the angiogenic vessels in solid tumors express proteins on their surfaces that are absent or barely detectable in normal vascular endothelium, including $\alpha v\beta 3$ integrin and receptors for certain angiogenic growth factors. Here we show that immunotherapy of solid tumor using dendritic cells (DC) pulsed with the TEC.
Material and Methods: Human umbilical vein endothelial cells (HUVEC) were cultured with mice tumor cells (B16 melanoma or Colon26 colon carcinoma)-conditioned medium and were used as a model of TEC (mTEC). TEC were isolated by Percoll gradient centrifugation from collagenase digested tumor tissue. Angiotensin-converting enzyme activity and CD34 were used as a marker of EC. Bone marrow-derived murine DC were incubated with lipofectin containing lysate of mTEC or TEC. Mice were immunized by intradermal injection of DC. After one week, B16 cells were injected intravenously, or Colon26 cells were injected intradermaly. Two weeks after the transfer of tumor cells, visible metastatic colonies of B16 on lung were counted, or the volume of Colon26 was measured.
Results: The number of the colonies in the lung was dramatically decreased in the mice immunized with mTEC pulsed DC compared with the mice immunized with none pulsed DC. DC pulsed HUVEC cultured with no tumor-conditioned medium had no inhibitory effect of the lung metastasis. The metastasis of B16 was decreased by the treatment of DC pulsed with the endothelial cells cultured with Colon26-conditioned medium. The colonies of B16 metastasis in lung were inhibited by vaccination of TEC isolated from solid B16 tumor. The tumor volume was also decreased in the mice immunized with Colon26-derived TEC pulsed DC. In mouse dorsal air-sac chamber method, angiogenesis induced B16 cells was inhibited by the treatment of TEC pulsed DC. No significant difference in wound healing (normal physiological angiogenesis) was observed between TEC pulsed DC and control mice.
Conclusions: Vaccination of DC pulsed tumor-induced endothelial cells can inhibit tumor growth and metastasis. Tumor-induced angiogenesis-targeted immunotherapy offers the potential for new approach to treatment of cancer.

45 POSTER

Preliminary results of a phase 1 study of intravenous administration of GL-ONC1 vaccinia virus including green-fluorescent protein real time imaging in patients with advanced cancer

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Background: GLV-1h68/GL-ONC1 is a genetically engineered vaccinia virus attenuated by insertion of the ruc-gfp (a luciferase and green fluorescent protein fusion gene), beta-galactosidase (LacZ) and beta-glucuronidase (GusA) reporter genes into the *F14.5L, J2R* (thymidine kinase) and *A56R (*hemagglutinin) loci respectively. Impressive anti-tumour activity was observed in preclinical models.
Material and Methods: GL-ONC1 was administered as an intravenous infusion in escalating doses (1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 plaque forming units) with three patients in each cohort on day 1 of a 28 day cycle for the first 5 cohorts. Cohort 6−7 will receive $1{,}667\times10^7$ and $1{,}667\times10^8$ pfu on days 1−3 and cohort 8 will receive 1×10^9 for 5 consecutive days. Green-fluorescent protein (GFP) imaging was performed at baseline and during each cycle on patients with superficial or mucosal lesions. Endpoints were safety, tolerability, viral replication, tumour delivery, neutralizing antibody development, anti-tumour activity and recommendation of dose for future trials.
Results: To date, 15 patients (11 males, median age 57 years) have been treated with no dose limiting toxicities (DLT) observed. Toxicities were mild (grade 1 or 2) including fatigue (n = 3), fever (n = 7), rigor (n = 1), myalgia (n = 2), flu-like symptoms (n = 2), vaccinia rash (n = 2), anemia (n = 2), oily skin/hair (n = 1) and moderate leukocytosis (n = 1). The rash comprising of vaccinia pustules was asymptomatic in one patient (grade 1) and symptomatic with itching and discomfort in the other patient (grade 2). In both patients the rash appeared in cycle 1 during the first week and resolved without treatment at the end of cycle 1. It was positive for GL-ONC1 viral plaque assay (VPA) and GFP expression. VPA of blood, urine, stool and sputum were negative for viral shedding in all except one patient which had positive viral shedding in blood, rash, stool and sputum. Blood (1 pfu) was only positive for viral shedding on day 2. Highest amount was seen in sputum (120 pfu) on day 9; all viral shedding were negative by day 13. There was an increase in neutralizing anti-GL-ONC1 antibodies in all but one patient. Best response was stable disease by RECIST observed in four patients for 3 to 6 months but one patient received 8 months of treatment.
Conclusion: GL-ONC1 is well tolerated with minimal toxicity and preliminary evidence of anticancer activity.
Trial is sponsored by Genelux. Trial identifier NCT00794131.

46 POSTER

Immunotherapy with the toll-like receptor 9 agonist MGN1703 in patients with metastatic solid tumors − clinical efficacy and immunological results of a 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 antineoplastic activity of MGN1703 has been previously shown *in vitro* and in several animal models. In this clinical phase I study patients with metastatic cancer without further treatment options were treated with MGN1703.

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.

47 POSTER

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

M.R. Weihrauch[1], H. Richly[2], M.S. von Bergwelt-Baildon[1], U. Hacker[1], A. Shimabukuro-Vornhagen[1], B. Nokay[2], M. Tschaika[3], M. Schmidt[3], B. Wittig[4], M.E. Scheulen[2]. *[1]University of Cologne, Department I for Internal Medicine, Cologne, Germany; [2]University of Essen, Department of Medicine (Cancer Research), Essen, Germany; [3]Mologen AG, Berlin, Germany; [4]Charité Universitätsmedizin Campus Benjamin Franklin, Institute for Molecular Biology and Bioinformatics, Berlin, Germany*

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.

48 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.

49 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