increases in BAP (p<0.001) and BSI scores (p=0.08) were suppressed when compared to placebo treatment.

Bone Alkaline Phosphatase*			Bone Scan Index*		
Baseline	N	median TTP (days)	Baseline	N	median TTP (days)
< 1.25 X ULN	165	199	< 1.4%	118	202
1.25 - 5 X ULN	66	102	1.4 - 5.1%	66	169
>5 X ULN	34	78	> 5.1%	53	83

*p < 0.001, log-rank test

Conclusions: In HRPCa patients, 10 mg atrasentan delays progression as measured by clinical, biochemical, and imaging criteria. The extent of skeletal tumor burden, assessed by biochemical and bone scan measures can provide prognostic information about patients clinical disease course.

578 ORAL

Preliminary results of carbon-11 acetate pet imaging in prostate cancer patients with rising PSA after radical therapy: clinical impact to choose appropriate further treatment strategies

S. Wachter[1], A. Kurtaran[2], B. Djavan[3], A. Becherer[2], T. Lorang[4], J. Schmaljohann[2], R. Pötter[1], R. Dudczak[2], K. Kletter[2]. *[1] General Hospital Vienna, Radiotherapy and Radiobiology, Vienna, Austria; [2] General Hospital Vienna, Nuclear Medicine, Vienna, Austria; [3] General Hospital Vienna, Urology, Vienna, Austria; [4] General Hospital Vienna, Computer Sciences, Vienna, Austria*

Purpose: Patients after radical therapy for prostate cancer (radical prostatectomy, radiation therapy) with rising PSA continue to be a diagnostic and therapeutic challenge. Recent studies reported promising data of C-11 acetate in visualization of recurrent tumor site as well as metatastatic spread of prostate cancer. In particular, this PET tracer does not undergo urinary tract excretion and seems therefore to be suitable for evaluation of locoregional disease. This study aims to evaluate the potential role of C-11 acetate PET imaging in patients with rising PSA after radical therapy in choosing the most appropriate treatment option (local and/or systemic therapy).

Methods: 13 clinically asymptomatic patients with rising PSA and evidence of recurrent/metastatic disease by standard imaging procedures (SIP) including bone scan, CT, MRI have been evaluated. C-11 acetate dynamic imaging of the prostate region has been performed after i.v administration of 400 MBq of C-11 acetate followed by whole body scans with a PET ring scanner. In case of abnormal C-11 acetate uptake in previously unknown localizations, additional radiological work-up has been done. C-11 acetate images were analyzed by visual interpretation followed by 3D image fusion of PET with CT and/or MRI for a better anatomical localization of suspected tumor sites.

Results: In 11 out of 13 patients (85%) increased C-11 acetate uptake was found. Seven of C-11 acetate positive patients (64%) demonstrated local and/or systemic manifestations which could be confirmed by SIP. In addition, previously unknown lesions could be detected by C-11 acetate imaging in 5 of 7 patients leading to modification of the treatment strategy. The remaining 4 patients demonstrated increased C-11 acetate uptake only locally and were therefore selected as candidates for local radiotherapy with potentially curative intention. In 2 patients (PSA level 0.6 and 1.4) no tumor sites were detected in accordance to SIP.

Conclusion: Our preliminary data demonstrate the feasibility of C-11 acetate whole body PET as a promising new imaging modality to localize the tumor sites in patients with rising PSA after radical therapy. These data indicate that C-11 acetate PET scan may be helpful to select patients with local disease from those having distant metastases to choose the most appropriate therapeutic option.

Immunobiology and biological therapies

579 ORAL

Pharmacodynamic studies of the specific oral EGFR tyrosine kinase inhibitor (EGFR-TKI) zd1839 ('Iressa') in skin from cancer patients participating in phase I trials: histopathological and molecular consequences of receptor inhibition

J. Albanell[1], F. Rojo[1], S. Averbuch[2], A. Feyereislova[3], R. Herbst[4], P. LoRusso[5], D. Rischin[6], J. Gee[7], R. Nicholson[7], J. Baselga[1]. *[1] Hospital Vall d'Hebron, Oncology Service, Barcelona, Spain; [2] AstraZeneca, Wilmington, DE, USA; [3] AstraZeneca, Alderely Park, UK; [4] MD Anderson Cancer Center, Oncology Service, Houston, TX, USA; [5] Harper Hospital, Oncology Service, Detroit, MI, USA*

Aim: The specific oral EGFR-tyrosine kinase inhibitor (EGFR-TKI) ZD1839 'Iressa' is under clinical development as an anticancer agent. Since receptor inhibition by ZD1839 is required for optimal antitumour activity, we have studied in vivo the pharmacodynamic (PD) effects of ZD1839 on EGFR activation and receptor-dependent events in the skin, an EGFR-dependent tissue, in cancer patients participating in ZD1839 Phase I clinical trials.

Methods: We studied the histopathological and molecular consequences of escalating doses of daily oral ZD1839 in 104 pre- and/or on-therapy (at approximately day 28 of therapy) skin biopsies from 65 cancer patients. We measured ZD1839 effects on EGFR activation by immunohistochemistry, using an antibody specific for the activated-phosphorylated-EGFR; effects on receptor signalling (activated MAPK), proliferation, p27KIP1 and maturation were also assessed. All statistical tests were two-sided.

Results: Histopathologically, the stratum corneum of the epidermis was thinner during therapy (p<0.001). In hair follicles, prominent keratin plugs and microorganisms were found in dilated infundibula. On the molecular level, ZD1839 suppressed EGFR phosphorylation in all EGFR-expressing cells (p<0.001). In addition, ZD1839 inhibited MAPK activation (p<0.001) and reduced the keratinocyte proliferation index (p<0.001). Concomitantly, ZD1839 increased the expression of the cyclin dependent kinase inhibitor p27KIP1 (p<0.001) and of maturation markers (keratin 1 and phospho-STAT3) (p<0.001) and increased apoptosis (p<0.001). These effects on the target and EGFR-dependent molecular endpoints were observed at all dose levels, before reaching dose-limiting toxicities.

Conclusions: Oral daily ZD1839 inhibits EGFR activation and affects downstream receptor dependent processes in vivo. The observed effects may be responsible for the acneiform rashes and desquamation that are seen in some patients. Effects of receptor inhibition were profound at doses well below the one producing unacceptable toxicity, a finding that strongly supports the use of PD assessments to select optimal doses, instead of a maximum tolerated dose, for definitive efficacy and safety trials. In addition, our studies show an important role for the EGFR in normal adult skin biology and provide a rationale for the investigation of ZD1839 in EGFR-dependent skin disorders, such as psoriasis or epithelial tumours.

'Iressa' is a trade mark of the AstraZeneca group of companies.

580 ORAL

Double suicide gene therapy for locally recurrent prostate cancer

J.H. Kim[1], M.S. Khil[1], J.O. Peabody[2], S.O. Stricker[2], M. Deperalta[3], A. Aullar-Cordova[4], S.O. Freytag[5]. *[1] Henry Ford, Radiation Onc, Detroit, USA; [2] Henry Ford, Urology, Detroit, USA; [3] Henry Ford, Pathology, Detroit, USA*

Purpose: We have demonstrated the potential of the HSV-1 TK/GCV and E.coli CD/5-FC enzyme/prodrugs systems as cancer therapies extensively in animal model systems. The present clinical study was carried out to determine whether the intraprostatic injection of E1B-attenuated adenoviral vector containing a double suicide gene together with two prodrugs administration would be safe and exhibit any therapeutic activity in patients with locally recurrent prostate cancer following radiation therapy.

Methods: A total of twelve patients with locally recurrent prostate cancer (biopsy proven and rising serum PSA on three consecutive measurements) were entered into the trial. Three cohorts of patients were used to escalate the viral dose administration ranging from 10 to the 10vp to 10 to the 12vp. The vector used was a E1B-attenuated adenoviral vector containing HSV-1 thymidine kinase/E.coli cytosine deaminase fusion gene. Two days after the TRUS guided viral injection into the prostate, patients received a 7 day course of two prodrugs, ganciclovir and 5-fluorocytosine. Regular follow-up tests including serum PSA, prostate biopsy to determine the transgene

expression, serum adenoviral antibody were done on all patients up to one year.

Results: All patients tolerated the prostate gene therapy well with minimal toxicities. None of the patients exhibited irreversible grade 3 and 4 toxicities directly related to the therapy. Six out 12 patients show varying degrees of tumor response in terms of serum PSA for duration from three weeks to one year. Histological evidence was obtained in selected patients for the transgene expression up to two weeks after the viral administration.

Conclusion: This prospective phase I study of double suicide gene therapy using E1B-attenuated replication competent adenoviral vector shows a therapeutic efficacy in patients with recurrent prostate cancer with minimal normal tissue toxicity.

581 ORAL

Enhancement of cisplatin-induced apoptosis by infection with adeno-associated virus type 2

V. Duverger[1], U. Sartorius[2], P. Klein-Bauernschmitt[1], P.H. Krammer[2], J.R. Schlehofer[1]. [1] *German Cancer Research Center, Applied Tumor Virology, Heidelberg, Germany;* [2] *German Cancer Research Center, Department of Immunogenetics, Heidelberg, Germany*

The non-pathogenic human adeno-associated virus, AAV has been shown to sensitize human cancer cells and experimental tumors towards chemotherapeutic agents, such as cisplatin. Since these drugs induce apoptosis, we investigated whether one mechanism of AAV-mediated sensitization of human tumor cells may result from an enhancement of cisplatin-induced apoptosis. In HeLa and A549 cells, infection with AAV type 2 (AAV-2) increased cisplatin-induced DNA fragmentation but had no cytotoxic effect by itself. This enhanced apoptosis appeared to be mediated by a component of the viral capsid since empty or UV-inactivated AAV-2 particles were also able to boost cisplatin-induced DNA fragmentation. The effects were AAV-2-specific since they were not observed after infection with AAV type 5 (AAV-5) or the autonomous parvovirus, H-1. AAV-2-mediated enhancement of apoptosis was not associated with a modification of the expression of CD95 ligand, CD95 receptor or other death receptors, as shown by RT-PCR and RNase protection assay. In contrast, using the mitochondrial fluorescent dye, JC-1 in flow cytometry, AAV-2 infection was found to further reduce the mitochondrial transmembrane potential after treatment with cisplatin in a caspase-independent manner, suggesting that increase of apoptosis by AAV-2 occurred at the mitochondrial level. In contrast, in cells of the small cell lung cancer line, P693, an enhancement of cisplatin-induced DNA fragmentation was not observed after infection with AAV-2. In these cells, sensitization to cisplatin-toxicity was associated with cell cycle arrest in G2/M. The data indicate that in the absence of viral gene expression, AAV-2-mediated sensitization to cisplatin involves multiple cellular pathways promoting cell death signals, in a cell type-dependent manner. The results further support that AAV-2 particles may be appropriate adjuvants for improving cancer chemotherapy, and may also have consequences regarding AAV-2-based vectors for gene therapy.

582 ORAL

Gene expression under control of the radiation-inducible Egr-1 promoter in an adenoviral vector: vector optimization for reduction of unspecific gene expression in the absence of irradiation

F. Wuerschmidt[1], I.E.O. Gomaa[1], M. Anton[2], T.V. Lukowicz[1], M. Molls[1], B. Gaensbacher[2]. [1] *Radiotherapy, TU Munich, Radiotherapy, TU Munich, Munich, Germany;* [2] *Exp. Oncology, TU Munich, Munich, Germany*

Purpose: Construction of a replication defective adenoviral vector expressing a cytotoxic gene under the control of a radiation inducible promoter, and determination of susceptibility to low doses of irradiation.

Methods: A reporter gene encoding the EGFP or the HSV-TK cytotoxic gene were placed in the E1 region of the Ad-genome under the control of the mEgr-1 promoter/enhancer. Replication defective adenoviruses were used to deliver either of the transgenes to rhabdomyosarcoma R1H tumour cells in vitro.

Expression of the reporter gene (EGFP) was detected by fluorescence microscopy; cytotoxicity of Ad.Egr-1.TK+GCV (10-2 to 10-4 mg/ml) was determined by crystal violet staining. Doses of 0 to 8 Gy were given 4hrs post infection and 1hr post GCV. For vector optimization, either insulating sequences of BGHpA were introduced at the upstream or at the upstream and downstream regions of the expression cassette; or the expression cassette with/without the insulating sequences was placed in an anti-parallel position to the E1 region of the Ad genome.

Results: The Ad-Egr1-EGFP and Ad-Egr1-HSV.TK were successfully constructed and the viral DNA was analysed. R1H cells were infected with the constructed viral vectors at different MOIs. If IR was combined with Ad-Egr1-TK and GCV, a significant decrease ($P<0.0001$) in cell survival was found after 4Gy (mean 25%, 95% C.I 20-29%) as compared to 4Gy alone (74%; 61-87%). Irradiating cells at 6Gy was comparable to 4Gy+Ad-Egr.1-TK + GCV. However, Ad-Egr.1-TK + GCV without IR also significantly decreased cell survival (47%; 42, 52%) indicating considerable leakiness of the system. This is supposed to be due to activation of the Egr-1 promoter by viral enhancers in the absence of IR. Preliminary results with EGFP reporter gene suggest that leakiness of the system can be reduced by introduction of the BGHpA insulating sequences and/or inversion of the expression cassette.

Conclusions: Radiation induced gene expression under control of the Egr-1 promoter can be achieved with low doses of IR. The combination of gene therapy with radiotherapy is significantly more effective than IR alone. However, induction of gene expression without IR is considerable. Currently, we are introducing insulating sequences and/or invert the expression cassette with the aim of achieving a tighter temporal and spatial control of gene expression by low doses of IR.

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583 ORAL

Phase II trial of HLA-b7 plasmid dna/lipid (allovectin-7®) immunotherapy in patients with metastatic melanoma

J. Gutheil[2], M. Atkins[1], P. Schwarzenberger[1], J. Lutzky[1], J. Rubin[1], A. Deisseroth[1], R. Blum[1], L. Hutchins[1], R. Gonzalez[1]. [1] *Allovectin-7® Phase II Study Group;* [2] *Vical Incorporated, Clinical Research, San Diego, USA*

This phase II study evaluates the response rate, duration of response and toxicity of direct intratumoral injections of Allovectin-7® (a plasmid DNA-based therapy encoding the genes HLA-B7 and β2-microglobulin) in patients with metastatic melanoma.

Seventy-eight adult pts have been enrolled to date with 73 in the intent to treat (ITT) population (at least one dose of Allovectin-7®) and 54 pts evaluable for response (completed evaluation at week 10). Entered pts have stage III or IV disease with visceral metastases limited to the lung; good performance status (KPS * 80%); adequate organ function; and an injectable lesion * 1 x 1 cm. Treatment consists of 10 μg Allovectin-7® administered by intratumoral injection weekly x 6 followed by a 4-week observation period. Stable or responding patients may receive additional treatment cycles. Median age is 58 (range 33-82) with 45 males and 28 females. Mean Karnofsky performance status is 94%. All patients received prior systemic therapy. The overall response rate is 11% (8/73) among the ITT population and 14.8% (8/54) in the evaluable population. Responses include 2 CR's and 6 PR's with a median duration of response of 21 weeks (range 6 to 26+ weeks). Stable disease was seen in 19.2% (14/73) of the ITT population and 25.9% (14/54) of the evaluable population.

The most common side effects are mild to moderate injection site reactions and flu-like symptoms, all of which resolved rapidly and decreased in incidence after the first injection. Five Grade 3 treatment-related adverse events have been reported and include ascites, pain and dizziness. No injection-related serious adverse events have been noted. Three drug-related serious adverse events occurred. All 3 events were seen in a single patient (abdominal pain and two episodes of ascites).

Preliminary results of this ongoing trial indicate that Allovectin-7® is active and well-tolerated in patients with advanced malignant melanoma. Future studies will include an evaluation of higher Allovectin-7® doses and injection of multiple tumors.

584 ORAL

Induction of anti-tumor immunity in vivo using cytokines and an agonistic antibody against 4-1BB

L. Borges[1], R.E. Miller[2], J. Jones[2], D.H. Lynch[2]. [1] *Immunex Corp., Molecular Biology, Seattle, USA;* [2] *Immunex Corp., Oncology, Seattle, USA*

Several studies have shown that tumor-specific T cells can be isolated from cancer patients and that these cells are capable of recognizing and killing autologous tumor cells in vitro. Despite the presence of tumor-reactive T cells, tumors frequently grow and metastasize, indicating that anti-tumor immune responses are suppressed or not strong enough to eliminate cancer cells in vivo. In an effort to boost anti-tumor immunity, we treated tumor-bearing mice with cytokines or agonistic antibodies that act at the level of antigen-presenting cells and immune effector cells. To generate and