mor effect was accompanied by inhibition of expression of different growth factors of the EGF family, inhibition of angiogenesis and increase in p27 expression. Moreover, when oral HYB 165 was used in combination with cytotoxic drugs, such as taxanes and platinum derivatives, a marked cooperative effect was observed resulting in sustained inhibition of tumor growth, growth factor production and angiogenesis and in a significant increase of the survival of treated mice.

Conclusions: This study is the first demonstration of the antitumor activity of an antisense oligo after oral administration. Moreover, the inhibition of expression of factors involved in the control of cell proliferation, cell cycle and angiogenesis by this novel chimeric MBO antisense PKAI represents the rationale for the future use of HYB 165, which has recently completed a phase I trial in cancer patients by i.v. route with an excellent toxicity profile.

1437 POSTER DISCUSSION

IkB-gene therapy does not enhance chemotherapy-induced apoptosis in HCC

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Hepatocellular carcinomas are resistant to systemic chemotherapy. The effect of chemotherapeutic agents is mainly mediated by apoptosis. Since there is evidence that inhibition of NFkB might dramatically increase the susceptibility of tumor cells to TNF- and chemotherapy-induced apoptosis, we investigated the Adenovirus-IkB superrepressor as an option for multimodal gene therapy for HCC.

Methods: We studied three different hepatoma cell lines: HepG2, Huh7 and a primary, early, passage HCC-cell line (Tu 5), we established from a human HCC. Apoptosis was quantified by Facs analysis. NFkB activation was investigated by gel shift experiments. For in vivo investigations xenotransplants in nude mice were transduced with Adenovirus-IkB and subsequently the mice were treated by i.p. doxorubicin (ADM) (7.5 mg/kg).

Results: In all cell lines a chemotherapy- and TNF-mediated upregulation of NFkB could be demonstrated. In cells treated simultaneously with ADM and TNF NFkB expression was significantly enhanced. Although transduction with IkB-adenovirus completely inhibited NFkB activation, there was no increase of apoptosis in cells treated with ADM and IkB. In contrast apoptosis was dramatically increased by the combination of TNF and IkB-adenovirus. Also, we could demonstrate an enhancement of apoptosis in cells treated with TNF and ADM. TNF and ADM mediated apoptosis was efficiently inhibited by dnFADD. These results could be confirmed by in vivo experiments.

Conclusion: Although ADM, as well as TNF, activate NFkB and death receptor pathways via FADD, ADM mediated apoptosis is independent of NFkB inhibition in contrast to TNF induced apoptosis.

1438 POSTER DISCUSSION

A high correlation between mutant p53 and increased expression of vascular endothelial growth factor (VEGF), combining those markers indicates poor effect by adjuvant tamoxifen in ER positive patients

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Purpose: To determine the possible association between VEGF content and p53 status, including analysis of mutation types and locations, in a series of 224 primary breast carcinomas, and the potential prognostic value of those markers.

Methods: VEGF was measured in cytosols with an ELISA, p53 status was deter-mined with cDNA-based sequensing and IHC using the antibody Pab 1801.

Results: p53 mutations by sequense-data was found in 37 (16.5%), p53 positivity by IHC was found in 39 (17.4%), the median value of VEGF was 256 pg/mg. Significant associations were seen between higher VEGF content and both mutant p53 ($p = 0.0019$), and p53 IHC positivity ($p = 0.0068$). Deletions, insertions and stop codon had significantly higher VEGF values ($p = 0.0043$) than point mutations or wt p53. Mutant p53 was correlated to a worse outcome; RFS ($p = 0.0519$), BCCS ($p = 0.0310$). VEGF was correlated to outcome in ER positive patients receiving adjuvant tamoxifen RFS ($p = 0.0471$), BCCS ($p = 0.0064$). Combining these markers was of prognostic value in all patients, RFS ($p = 0.0377$), BCCS ($p = 0.0292$), and in patients that received adjuvant tamoxifen, RFS ($p = 0.0488$), BCSS ($p = 0.0342$).

Conclusions: The results indicates that VEGF expression seems dependent on wild-type p53 loss. Combining those markers indicates poor outcome, also in ER positive patients treated with adjuvant tamoxifen.

1439 POSTER DISCUSSION

Optimisation of cervical tumour lysate loading of dendritic cells (DC) for development of a versatile and efficient anti-cervical cancer vaccine: Use of Caski cells as a model source of cervical cancer derived tumour lysate

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Rationale: Murine DC loaded with HPV 16 E7 peptide can prime powerful peptide specific CTL in vivo anti-tumour response against HPV 16 E7 bearing tumours (1). Inadequate representation of tumour antigens and haplotype restriction of synthetic peptides have limited their clinical application. However, DC loaded with tumour lysates has proved an effective alternative in treatment of melanoma (2). Nevertheless, due to paucity of cervical cancer tissue available for tumour lysate preparation, it has proved necessary to improve the efficiency of antigen loading of DC prior to clinical trial.

Aim of Study: To determine the effect of ultra-sonication and/or liposome incorporation of tumour lysate on efficiency of antigen loading of human peripheral blood derived DC, using Caski cells as a surrogate source of HPV 16 E6 & E7 antigen positive cervical cancer tissue.

Method: DC generated from human volunteers' peripheral blood derived monocytes cultured for 7 days with GM-CSF and IL-4 (3), were loaded with Caski cell lysate prepared by repeated freezing-thawing ± ultra-sonication and/or exposure to cationic liposomes DOTAP (4). Antigen loaded DC were irradiated, co-cultured with autologous PBMCs, and after two stimulations, the CTL activity was measured in a standard 51Cr release assay. HPV 16 E6 & E7 specific HLA restricted CTL activity induced was estimated using autologous BLCL targets transfected with recVac-HPV (TA-HPV) as antigen specific positive control, Wyeth-Vac as antigen negative control, and recVac-HPV (TA-HPV) as allogeneic HLA negative control.

Results: A significant HLA restricted CTL response (>10% 51Cr release at 2 E:T ratios) was demonstrated only by PBMCs primed with DC loaded with either ultra-sonicated and/or liposome exposed Caski cell lysates, at Caski cell: DC ratio as low as 1:1, indicating a need for as little as 12 mm3 of cancer tissue per 6 dose treatment regime.

Conclusion: The study supports tumour lysate as an adequate source of antigen for loading of DC for broad spectrum haplotype unrestricted priming of anti-cervical cancer specific CTL responses in a clinical trial setting.

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1440 POSTER DISCUSSION

Histamine dihydrochloride (Maxamine™), interleukin-2 (IL-2) and interferon-α (IFN-α) in multiple myeloma

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Background: Treatment with IL-2 and Maxamine has shown promise in melanoma and AML. Maxamine reverses the anergy and apoptosis of NK cells and T cells after their exposure to monocyte-derived reactive oxygen metabolites *in vitro*. Thereby Maxamine, acting via H_2 receptors on monocytes, synergizes with the activating effect of IL-2 and IFN-α on both T- and NK-cells.

Methods: Seven male patients, median age 50 years (45–63) were enrolled at a mean of 17 months (10–64) after PBSCT. Three patients, one in CR and two in PR at inclusion, were undergoing IFN-α treatment. Four other patients had relapsed and 3 had progressive disease (PD), whereas one had stable disease (SD) after salvage therapy. IL-2 (50 μg, bid, s.c.) and Maxamine (0.5 mg, bid, s.c) were self-administered by all 7 patients in repeated courses of 21 days until progression or for 6 mo. The three PR and CR patients also continued on IFN-α (9 mIU/week). **Follow up:** (from start of Maxamine therapy). The CR patient remains in CR 33+ mo at last evaluation. One PR patient is now in CR at 39+ mo. And the other