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

PR patient relapsed after 24 mo. The three patients with PD at inclusion progressed, whereas the SD patient remained stable throughout the study. No dose reductions were necessary. IL-2 induced fever, aches, fatigue and inflammation at injection sites. Maxamine injections induced short-lasting symptoms from vasodilatation, such as headache, flush, mild hypotension and tachycardia.

Conclusion: Maxamine given as an adjunct to immunotherapy in myeloma patients after PBSCT is safe and feasible. Future studies will focus on non-progressive patients.

1441 POSTER

Human monoclonal antibody immunotargeting therapy for colon cancer

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Purpose: A human monoclonal antibody (HuMAb) SK1 recognizes a glycoprotein that is expressed on the majority of colon cancer tissues. We previously demonstrated that the antibody strongly inhibits the cancer cell invasion *in vitro* and accumulates efficiently to cancer tissues *in vivo*. The current study was performed to evaluate the safety and the pharmacokinetics of escalating dose of a HuMAb SKI in patients with advanced colon cancers.

Patients and Methods: HuMAb SK1 was administered intravenously at 2, 4, 10 mg once in two weeks, totally 3 times to three consecutive groups of three patients with recurrent colon cancer who had been extensively pretreated.

Results: Among nine patients treated, slight fever that subsided without medication was seen in one patient. There were no tumors that showed complete response (CR) or partial response (PR) to the therapy. However, in 6 out of 9 patients, the rate of rise of serum CEA level reduced significantly during 4 weeks following treatment ($p = 0.042$), and the similar tendency lasted for the next 4 weeks ($p = 0.049$). In 4 patients, serum titer of anti-idiotypic IgG antibody to SK-1 continued to increase during at least 8 weeks following the treatment.

Conclusion: HuMAb SK-1 can be safely administered. This natural antibody not only possesses a direct cytostatic activity against colon carcinoma, but may induce carcinoma-related, anti-idiotypic antibody responses.

1442 POSTER

3-Fold increase in survival for stage IV melanoma patients treated with MCV allogeneic vaccine: Confirmation of previous phase II data

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Treatment of metastatic melanoma with chemotherapy and immunotherapy has not significantly improved the overall survival, although some responders on IL-2 based regimes have had a long term survival of some years. The only significant claim of increased survival has been associated with the PMCV vaccine developed by Morton and colleagues at the JWCI. In previous phase II studies they have claimed 2 year survivals of between 40–60% in Stage IV patients depending on the extent of surgery. Prior to commencement of a multi-centre randomised study we independently assessed the effect of this vaccine on patients with Stage IV melanoma. From August 1994 to August 1997, 33 patients with Stage IV melanoma, 17 female and 16 male, 13 stage IV M1a & 20 M1b, performance status = 0 were treated in a single institution phase II study in the U.K. with PMCV & BCG. The protocol stipulated extensive surgical excision prior to entry to render patients, if possible, to NED (no evidence of disease) status. Twenty-five surgical episodes were recorded for these patients to conform to the eligibility criteria. A further 60 surgical episodes have been recorded to date in patients continuing on vaccine treatment. Clinical responses, by WHO criteria, were recorded in only 3 patients (1CR & 2PR, all in soft tissue). With a median follow -up of 3 years, the survival of these patients is 110 weeks (CI 95% 72–145). This is significantly greater than historical controls from our and other U.K. institutions (median 7–11 months).

Our 2 year survival rate approaches 50% and is similar to that published by Morton et al for stage IV melanoma from the USA treated with PMCV.

1443 POSTER

Histamine dihydrochloride (Maxamine TM) potentiates the effect of interleukin-2 (IL-2) and interferon-alpha-2b (IFN-alpha-2b) in the treatment of solid tumors

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Purpose: Monocytes and macrophages can prevent activation of T cells and NK cells by release of reactive oxygen metabolites. Maxamine inhibits the release of reactive oxygen metabolites. When T cells and NK cells were exposed to phagocytes in vitro, the combination of Maxamine and IL-2 increased activated viable NK cells 12-fold and activated viable T cells > 60-fold. Maxamine has been tested in advanced melanoma and renal cell carcinoma patients as an adjuvant to IL-2 and IFN-alpha-2b.

Methods: Maxamine (1 mg, s.c., bid) was given in combination with IL-2 (4.8–18 MIU/m^2/day) and IFN-alpha-2b (3–5 MIU/day) to patients with advanced melanoma (20 patients) or renal cell carcinoma (3 patients).

Results: Maxamine caused the expected flushing but did not augment side effects associated with IL-2 and IFN-alpha-2b, and the treatment could be administered for at least one year. Mean survival for the 20 patients with advanced melanoma exceeded 15 months, and responses were observed in 2/3 patients with renal cell carcinoma.

Conclusion: The results from phase I/II studies indicate a survival benefit when Maxamine is given as an adjuvant to IL-2- and IFN-alpha-2b- based biotherapy. As a consequence two randomized phase III studies in advanced melanoma and a phase II study in advanced renal cell carcinoma are ongoing in the U.S., Europe and Australia.

1445 POSTER

Treatment of brain tumors with autologous cancer cell vaccines and radiotherapy

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Purpose: To improve the potential life expectancy of glioma patients, we have studied the combined therapeutic effect of autologous, cytokine producing cancer cell vaccines and local radiotherapy in experimental murine gliomas.

Methods: Murine gliomas were established by intracranial transplantation of glioma 261 (Gl261) cells. Autologous cancer cell vaccines were produced by transduction of in vitro growing Gl261 cells with adenoviral vectors encoding various murine cytokines (IL-2, IL-4, IL-6, IL-7, IL-12, GM-CSF, TNFa, LIF, LT). Tumor bearing mice were subcutaneously vaccinated with cytokine producing irradiated GL261 cells. In addition, vaccination therapy was combined with local radiotherapy of tumors.

Results: About 20–40% of glioma bearing mice were efficiently cured by vaccines producing either IL-2, IL-4, IL-12 or GM-CSF. The therapeutic effect of these vaccines depended on the cytokine level produced by transduced cells. The combination of vaccination and radiotherapy substantially improved survival rates: about 70–100% of tumor bearing mice were cured. The vaccination therapy induced the specific activation of cytotoxic T lymphocytes against Gl261 tumor cells as measured by cell-mediated citotoxicity assay and immunohistochemistry.

Conclusion: The combination of vaccination therapy with local radiotherapy of tumor might be efficiently used to improve survival rates of glioma bearing patients.

1446 POSTER

Immunogene therapy for murine fibrosarcoma using IL-15 gene with high translation efficiency

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Purpose: Numerous lines of evidence suggest that genetically modified tumor cells expressing cytokines can abrogate the ability of tumors to grow. IL-15 is a novel MW 15,000 cytokine that shares many of biological activities of IL-2 including induction and the proliferation of NK cells and T and B