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

K. Koda[1], N. Nakajima[2], N. Saito[2], J. Yasutomi[2], M. Dan[3], M.E. McKnight[3], M.C. Glassy[4], K. Fukao[1]. [1]*Tsukuba University, Institute of Clinical Medicine, Tsukuba;* [2]*Department of Surgery, Chiba University School of Medicine, Chiba City, Japan;* [3]*Novopharm Biotech Inc., Scaborough, Canada;* [4]*The Rajko Medenica Research Foundation, San Diego, United States*

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

A. Maraveyas[1], L. Compton[1], R. Dunleavey[1], D. Sage[2], C. Navarette[2], D. Morton[3], A. Dalgleish[1]. [1]*St George's Hospital, Oncology, London;* [2]*NBS-South Thames Centre, Blood Transfusion Service, London;* [3]*John Wayne cancer Institute, Oncology, London, United Kingdom*

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

P. Naredi[1], J. Mattsson[2], P. Lindnér[2], K. Gehlsen[4], K. Hellstrand[3]. [1]*Umeå University Hospital, Department of Surgery, Umeå;* [2]*Sahlgrenska University Hospital, Department of Surgery, Göteborg;* [3]*Sahlgrenska University Hospital, Department of Virology, Göteborg, Sweden;* [4]*Maxim Pharmaceuticals, San Diego, United States*

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

K. Lumniczky[1], E.J. Hídvégi[1], H. Hamada[2], G. Sáfrány[1]. [1]*National Research Institute for Radiobiology and Radiohygiene, Molecular Radiobiology, Budapest, Hungary;* [2]*Cancer Chemotherapy Center, Cancer Institute, Tokyo, Japan*

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

K. Kimura[1,2], H. Nishimura[1], Y. Nimura[2], Y. Yoshikai[1]. [1]*Nagoya University School of Medicine, Laboratory of Host Defense and Germfree Life, Research Institute for Disease Mechanism and Control, Nagoya;* [2]*Nagoya University School of Medicine, First Department of Surgery, Nagoya, Japan*

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

cells. We investigated the effect of immunological gene therapy with IL-15 protein using alternative IL-15 cDNA with high translational efficiency.

Method and Results: In a malignant model using BALB/c mice and syngeneic Meth A fibrosarcoma, two expression vectors carrying murine IL-15 gene were constructed for use in tumor immunotherapy, one utilizing IL-15 cDNA with alternative exon 5 and the second utilizing IL-15 cDNA with normal exon 5. The first vector induced the production of large amount of IL-15 protein in Meth A, whereas tumor cells transfected by the second vector produced only marginal level of IL-15 protein. Although in vitro cell growth of both transfectants remained unchaged, inoculation of clones transfected with normal IL-15 cDNA resulted in progressive tumor growth, while clones transfected with alternative IL-15 cDNA led to rejection of the tumor. The clone producing high levels of IL-15 grew progressively in nude mice and anti-CD4 mAb treated mice, while the growth of the transfectants was retarded in anti-CD8 mAb or anti-asialo GM1 Ab-treated mice. Cured mice were shown to have generated immunity against a subsequent challenge with wild type of Meth A but not against Meth 1 tumor cells, another type of fibrosarcoma derived from BALB/c mice.

Conclusion: Tumor therapy based on IL-15 gene transfection was effective against Meth A tumor cells, suggesting a possible application to human neoplasms.

1447 POSTER

In vitro generation of HLA-A2 restricted cytolytic T lympho-cytes using an HLA-A2+ allogeneic SCCHN cell line for lymphocyte stimulation

T. Asai[1], K. Baba[1], T.L. Whiteside[2], W. Storkus[2]. [1]*Dokkyo university school of medicine, Otolaryngology, Mibu, Tochigi, Japan;* [2]*University of Pittsburgh, Pittsburgh Cancer Institute, Pittsburgh, United States*

Vaccines containing tumor-derived alloantigens able to elicit strong MHC class I-restricted tumor antigen-specific T cell responses in patients with HNC might be as advantageous and easier to prepare than autologous vaccines. To begin to test the hypothesis that antitumor effector T cells can be consistently generated by in vitro sensitization with antigens expressed on HNC cell lines, we established a model system, utilizing HLA-A2+ HNC cell line, PCI-13 pretreated with 1000 IU/ml of IFN-gamma, as a stimulator of allogeneic normal HLA-A2+ T lymphocytes. HLA-A2+ peripheral blood T cells obtained from leukapheresis products of 10 normal donors were sensitized by 4 cycles of co-incubation with irradiated PCI-13 cells in the presence of IL-2, IL-1b, IL-4, IL-6. In 4/10 cases CD8+ T cells lines were generated which were able to lyse PCI-13, and 2 other HLA-A2+ SCCHN targets but not HLA-A2+ non-SCCHN targets, K562 or HLA-A2- tumor targets in 4 h Cr-release assays. Lysis was blocked by anti-CD3, anti-MHC class I and anti-HLA-A2 but not MHC class II Abs. The lines were tested for the frequency of cytolytic T cell precursors (CTL-p) responsive to PCI-13 in limiting dilution assays (LDA) and by ELISPOT. The frequency of PCI-13-specific-CTL-p in the best of four CTL lines was 1.04% in LDA. ELISPOT closely approximated LDA data, with the frequency of T cells able to produce IFN-gamma in response to PCI-13 determined to be 1.4%, and this response was inhibited by anti-MHC class I Abs. The data indicate that CTL-p responsive to class I-presented HNC-associated epitopes in normal donor PBMC and can be expanded in vitro, using cytokines and repeated stimulation with the allogeneic tumor cells. Based on these results, we expect in pending experiments that using HLA-matched allogeneic tumor-derived peptides pulsed onto autologous dendritic cells, it might be possible to generate and reliably quantitate CTL-p in patients with HNC.

1448 POSTER

Modulation of human tumor associated macrophages from malignant effusions with cytokines and proteolytic enzymes

T. Mohr[1], E. Zavadova[1], E. Hauptmann[1], S. Maca[2], M. Neumann[3], H. Salzer[4], M. Micksche[1]. [1]*Institute for Tumorbiology, Cancer research, Dept. for Applied and Experimental Oncology, Vienna;* [2]*Hospital Lainz, 5th Medical Department, Vienna;* [3]*Center for Pulmonary Diseases, 1st Internal Department, Vienna;* [4]*Wilhelminenspital, Department for Gynaecology, Vienna, Austria*

Tumor associated Macrophages (TAMs) represent a major component of the lymphoreticular infiltrate of human tumors, malignant pleural effusions and malignant ascites. TAMs are functionally involved in anti-tumor defense via cytotoxic activities such as f.i. direct cellular cytotoxicity and release of cytokines. They also have the capacity to affect aspects of the biology of neoplastic tissues like vascularization, growth rate, stroma formation and dissolution. The objective of this study was to investigate the effect of various cytokines (GM-CSF, IFN-g, Il-1b and IFN-a) and a polyenzyme preparation. on the functional activity of TAMs isolated from malignant effusions of patients with ovarian, breast and lung cancer. TAMs were isolated by density centrifugation over a discontinuous Ficoll-Hypaque gradient. Peripheral blood monocyte derived macrophages (PBMMs) – serving as controls – were obtained using a combination of density centrifugation and selective adhesion followed by incubation with GM-CSF. The expression of cytokines was determined on mRNA-level via RT-PCR and on protein level via ELISAs. Biologically active TNF-a as well as cellular cytotoxicity were determined using bioassays. The activation status of TAMs differed markedly from that of PBMMs. TAMs showed a significantly lower Il-1b production and higher TGF-b production. Cellular cytotoxicity was markedly lower in TAMs when compared to PBM derived macrophages. The tested cytokines, especially GM-CSF as well as the polyenzyme-preparation were able to induce and increase the production of TNF-a and to enhance the cellular cytotoxicity. A decreased TGF-b production on mRNA and protein level was observed in TAMs treated with cytokines or the polyenzyme preparation. TAMs are one of the immune system's representatives at the host-tumor interface and reflect in some way the failure of the host to have immunologically controlled the tumor. TAMs represent a promising target to therapeutic intervention. With this study we demonstrated that it is possible to stimulate in vitro the functional activity of TAMs by treatment with cytokines or polyenzyme preparations. This might elucidate of the role of macrophages and especially TAMs in tumor defense.

1449 POSTER

Proteinases reduce metastatic dissemination and increase survival time in $C_{57}Bl_6$ mice with the Lewis lung carcinoma

Tomáš Olejár, Pavla Poučková, Marie Zadinová, Martin Wald. *Institute for Biophysics, 1st Medical Faculty, Charles University, Prague; Department of Surgery, 2nd Medical Faculty, Charles University, Prague, Czech Republic*

Purpose: Although proteases in general are considered to be prometastatic and proinvasive, the aim of presented study is to demonstrate *in vivo* an action of enzymes with different site of action, different substrate specifity and influencing directly cancer cell signalling by an other way, than tissue metalloendopeptidases.

Methods: The effect of combined proteolytic enzymes (trypsin, chymotrypsin and papain), administered by the rectal route, on the metastatic process and the time of survival in $C_{57}Bl_6$ mice with the Lewis lung carcinoma inoculated subcutaneously was investigated.

Results: In the control group, which received no enzyme treatment, 90% of animals died of the metastatic spread of cancer by day 18 after primary tumor extirpation. In Group A, which received the multi-enzyme solution from the time of primary tumor extirpation, 30% of mice died of disseminated cancer by day 25. In Group B, which was treated with the enzymes from 6 days before primary, tumor extirpation, only 10% of animals showed the metastatic process by day 15. In Group C, which received the enzymes from 24 hours after intracutaneous tumor inoculation, no metastatic dissemination was recorded. In these three groups, the enzyme treatment was carried out throughout the experiment. None of the control animals survived till the end of experiment at 100 days. The treated groups A, B and C showed survival till the end of experiment in 60%, 90% and 100% of animals, respectively.

Conclusion: In $C_{57}Bl_6$ mice with the Lewis lung carcinoma transplanted intracutaneously, administration of the enzyme mixture showed anti-metastatic effect. Although only some of the mechanisms of the enzyme effects after administration into the systemic circulation are known, our experiments have shown that these enzymes warrant further experimental studies with the prospect of being used in human medicine in integrated anti-cancer therapy, alongside surgery, actinotherapy and chemotherapy

1450 POSTER

Bioactivity of GM-CSF and IL-2 in cancer patients

P. Correale[1], D. Pozzessere[1], G. Campoggia[2], G. Fanetti[2], S. Sestini[3], G. Giorgi[4], L. Micheli[4], G. Francini[1]. [1]*Division of Oncology;* [2]*Division of Immuno-haematology;* [3]*Dpt. of Molecular Biology;* [4]*Institute of Pharmacology, Faculty of Medicine, University of Siena, Italy*

Background: GM-CSF promotes the proliferation and differentiation of professional antigen presenting cells (APC) and may synergistically interact with IL-2 in generating an efficient tumor associate antigen (TAA) specific immune-response. On these bases we designed a pilot study in chemoresistant cancer pts in order to evaluate the toxicity of the treatment with GM-CSF and IL-2 and its effects on biological and immunological parameters. The pts received 150 γ of GM-CSF sc for five days (days 1–5) followed