Cytokines/immunobiology/ immunotherapy

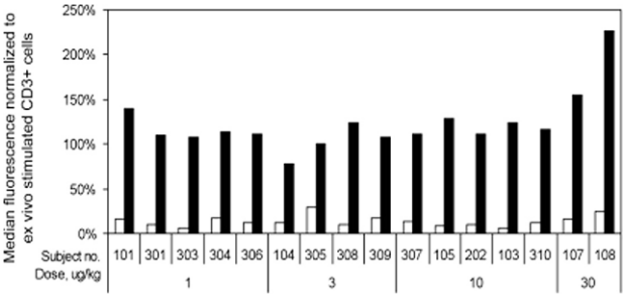
Poster presentations (Mon, 31 Oct)
Cytokines/immunobiology/immunotherapy

531 POSTER

Phase 1 dose-escalation study of intravenous recombinant human Interleukin-21 (IL-21) in patients with metastatic melanoma: preliminary results of tolerability and effect on immunologic biomarkers

G.A. McArthur[1], J. Cebon[2], B.K. Skrumsager[3], K. Skak[3], I.D. Davis[2]. [1]*Peter MacCallum Cancer Centre, East Melbourne, Australia;* [2]*Austin Health, Ludwig Austin Oncology Unit, Melbourne, Australia;* [3]*4Novo Nordisk A/S, Copenhagen, Denmark*

Background: Interleukin-21 (IL-21), a pleiotropic class I cytokine, has significant anti-tumor activity in several preclinical tumor models including metastatic melanoma.
Material and methods: In an ongoing phase 1 dose-escalation study, IL-21 is administered intravenously to patients with AJCC stage IV metastatic melanoma using two different dose regimens. At each dose level in the planned dose range of 1 μg/kg to 1 mg/kg, cohorts of two patients are being treated with IL-21 either thrice weekly for 6 weeks ('3×/week') or with 3 cycles of daily dosing for 5 days followed by 9 days of rest ('5+9'). The objectives are to estimate the maximum tolerated dose of the two dose regimens, to estimate the minimum biologically effective dose, and to optimize the dose regimen for future studies.
Results: Seven cohorts of two patients each have been enrolled in the 3x/week regimen at dose levels of 1, 3 10, and 30 μg/kg or the 5+9 regimen at dose levels of 1, 3 and 10 μg/kg. No dose limiting toxicity (DLT) has been reported so far. All patients experienced one or more adverse events, but no drug-related serious adverse events have been observed among the 11 completed patients. By RECIST criteria, one patient at each dose level of 1, 3 and 10 μg/kg had stable disease at the week 8 assessment. Dose- and dosing regimen-related effects on pharmacodynamic and immunologic biomarkers have been observed. Preliminary analyses have shown that increased levels of sCD25, phosphorylated STAT3 (pSTAT3), and perforin/granzyme B mRNA after IL-21 treatment. The figure shows the relative levels of pSTAT3 in $CD3^+$ lymphocytes from 16 patients isolated before and 15 minutes after dosing. The data are expressed as percent of the pSTAT3 level in $CD3^+$ lymphocytes from patient blood stimulated ex vivo with 10 ng/ml IL-21.



Conclusions: This ongoing study has shown that IL-21 administered in either of two dose regimens at doses up to 30 and 10 μg/kg respectively has been well-tolerated. Preliminary evidence of biological activity has been observed, even at doses as low as 1 μg/kg.

532 POSTER

IL-1 RA expression in ascites of advanced ovarian cancer patients influences the overall survival

A. Mustea[1], D. Koensgen[1], C. Pirvulescu[1], E.I. Braicu[1], P.M. Sun[1], D. Sofroni[2], W. Lichtenegger[1], J. Sehouli[1]. [1]*Charite, Medical University Berlin, Gynaecology and Obstetrics, Berlin, Germany;* [2]*Oncological Institute, Gynaecology and Obstetrics, Chisinau, Moldova*

Background: Interleukin-1β (IL-1β) and IL-1 RA are known to be critically involved in ovarian carcinogenesis. There are limited data on expression of IL-1β and RA in ascites in patients with ovarian cancer. The aim of this study was to determine whether expression of IL-1β and RA in ascites may influence the prognostic of ovarian cancer.
Methods: In a prospective study from 2001 to 2003, 33 patients with primary ovarian cancer and 20 with recurrence and 50 women with benign gynaecological diseases as a control group were enrolled. IL-1β and RA levels in ascites were analysed with ELISA technique.
Results: The median age of the patients was 55.6 years (range 19–80) in the ovarian cancer group and 40 years (range 15–89) in control group. The median follow-up period was 26 (0–42) months. The concentrations of IL-1β and IL-1 RA in ascites were significantly increased in patients with ovarian cancer in comparison to control group, for both cytokines ($p < 0.0001$). The IL-1β level in ascites correlated significantly with the histopatological grading ($p = 0.038$). IL-1 RA level in ascites was correlated with FIGO stage ($p = 0.049$).
Using Kaplan-Meier method and long-rank test was showed that patients with low level of IL-1 RA in ascites had a significant longer overall survival (34.6 vs. 17 months, $p = 0.01$) and progression free survival (24.6 vs.12.8 months, $p = 0.008$) in comparison with patients with high level of IL-1 RA in ascites. Application of multivariate Cox regression analysis showed IL-1 RA expression in ascites to be an independent prognostic factor for overall survival ($p = 0.04$).
Conclusions: The data suggest that lower expression of IL-1 RA in ascites correlated significant with improved clinical outcome in patients with ovarian cancer. IL-1 RA may have important roles in the growth and development of ovarian cancer and shows a prognostic relevance.

533 POSTER

Autologous large multivalent immunogen vaccine for the treatment of stage IV malignant melanoma and stage IV renal cell carcinoma

A. Dudek[1], V. Math[1], M. Mescher[2], J. Miller[1]. [1]*University of Minnesota, Medicine, Minneapolis, USA;* [2]*University of Minnesota, Laboratory Medicine and Pathology, Minneapolis, USA*

Renal cell carcinoma is the sixth leading cause of cancer death with a survival rate of 11% for stage IV cancer. More than a million skin cancer cases occur each year globally with a 5 year survival rate of 7–9% and 10 year survival rate of 3–6% for stage IV melanoma. These survival statistics have remained essentially unchanged for several decades. Although immune stimulation with high doses of interleukin-2 has demonstrated efficacy in the treatment of malignant melanoma or renal cell carcinoma, it can be applied only to limited groups of patients. Vaccination has also been explored as a means to boost antitumor T cell responses, which results in the specific killing of malignant cells. We have developed a novel approach of vaccine-induced augmentation of tumor-specific cytotoxic T lymphocyte (CTL) responses using (5 m diameter) latex or silica beads.
Methods: 61 patients with diagnosis of malignant melanoma or renal cell carcinoma were randomized to the following treatment arms: large multivalent immunogen (LMI) vaccine alone, Cyclophosphamide 300 mg/m^2 and LMI vaccine, and Cyclophosphamide 300 mg/m^2, LMI vaccine and subcutaneous IL-2 at 1.75×10^6 IU/m^2 for 1 week starting on day 5 after LMI vaccine.
Results: No grade 4 toxicities (by NCI CTC v3.0) were observed in either arm. For patients with malignant melanoma: median and 12 months survival were 8.78 months (95%CI: 7 months, NA) and 46.1% (95%CI: 23.4%, 68.8%), respectively, and median time to disease progression was 2.76 months (95%CI: 1.88, 6.25). For renal cell carcinoma patients: at median follow-up of 12 (range 0.4–30.4) months overall survival was 75.7% (95%CI: 59.9, 91.4%) and time to disease progression was 12.2 months (95%CI: 6.41 months, NA). One patient with melanoma and one patient with renal cell carcinoma have documented partial response (by RECIST criteria).
Conclusion: LMI vaccine has activity in malignant melanoma as documented by clinical responses and has activity in renal cell carcinoma as measured by prolonged time to disease progression. Phase II study of allogeneic vaccine is now in progress for patients with malignant melanoma to confirm feasibility of LMI strategy in this setting. Phase II study of autologous vaccine in patients with renal cell carcinoma is now in preparation to confirm prolonged time to disease progression observed in this group of patients.

534 POSTER

Association of Electrochemotherapy and CpG DNA: toward a new vaccination approach for cancers with cutaneous localizations

S. Roux[1], C. Bernat[2], B. Al Sakere[2], P. Opolon[2], L. Zitvogel[1], A.F. Carpentier[3], L.M. Mir[2], C. Robert[1]. [1]*Institut Gustave Roussy, Immunology unit ERM0208, Villejuif, France;* [2]*Institut Gustave Roussy, UMR 8121 CNRS, Villejuif, France;* [3]*Hôpital de la Pitié-Salpêtrière, INSERM U495, Paris, France*

Introduction: electrochemotherapy (ECT) is a new therapeutic approach for tumor reduction, effective on cutaneous and subcutaneous cancers. Its local efficacy and the absence of systemic side-effect have been

demonstrated in mice and humans. Oligodeoxynucleotides containing unmethyled CpG motifs (CpG ODN), bind to the Toll-Like Receptor 9 (TLR9) and are powerful immunostimulating agents. We investigated whether tumor antigens released after ECT could efficiently prime the immune system and induce a systemic antitumor response when associated with CpG ODN.
Materials and methods: in a first set of experiments we analysed by immunohistochemistry the nature of the cellular recruitment induced after ECT and the expression of TLR9 mRNA by quantitative RT-PCR in tumors. In a second set of experiments, we investigated the effectiveness of the association ECT-CpG-ODN in two subcutaneous mouse tumor models: a fibrosarcoma (LPB) and a melanoma (B16F10). We studied both local and systemic anti-tumoral effects of this association using a model in which two tumors were inoculated but only one was treated. The specific immune response was further studied in the subcutaneous B16OVA tumor model.
Results: ECT induced the recruitment of CD11c and Mac1 positive cells expressing TLR9 in LPB tumors up to 72 hours after ECT. Our results showed a strong local efficacy of the ECT-CpG-ODN as well as antitumor effects on the controlateral non treated tumors in the two models. In nude mice, no effect was observed on tumors, suggesting a mechanism mediated by T lymphocytes. Moreover, the combination of ECT and CpG-ODN induced a 3-fold increase of specific anti-OVA CD8 lymphocytes in the tumor-draining lymph node, compared to ECT alone.
Conclusion: the combination of ECT, allowing tumor destruction, together with a suitable immunostimulating adjuvant could be a new strategy to treat patients with subcutaneous tumor localizations.

535 POSTER

Biological, histological and clinical impact of preoperative IL-2 administration in radically operable gastric cancer patients

F. Romano, M. Piacentini, M. Conti, A. Scaini, M. Scotti, L. Degrate, R. Caprotti, F. Uggeri. *San Gerardo Hospital – II UNiversity of MIlan, Department of Surgery, Monza, Italy*

Background: Surgery induced lymphocytopenia and this decrease in host defences, related to IL-2 endogenous embalance during postoperative period could promote the proliferation of possible micrometastases and the implantation of surgically disseminated tumour cells. Moreover Tumor infiltrating lymphocytes (TILs), activated by endogenous IL-2 release, is linked to prognosis in cancer patients The aim of this randomized study is to assess the biological (peripheral blood cells count, related to the grade of immunosuppression), histological (TILs) and clinical (overall and disease free survival) impact of preoperative low doses administration of IL-2 in patients with radically operable gastric cancer.
Materials and methods: This prospectic study enrolled 89 consecutive patients with histologically proven gastric adenocarcinoma who underwent radical surgery from October 1999 to December 2003 (M/F 49/40; mean age 67; range 42–82). Patient were randomized to be treated with surgery alone as controls (45 patients) or surgery plus preoperative treatment with recombinant human IL-2 (44 patients). We considered the total lymphocyte count and lymphocyte subset (CD4, CD4/CD8) during the preoperative period, before IL-2 administration, and on the 14^{th} and 50^{th} day, peritumoral stromal (fibrosis) reaction, neutrophils, lymphocytes and eosinophils infiltration in tumor histology, and morbidity disease free and overall survival were evaluated.
Results: Two groups were well-matched for type of surgery and extent of disease. All the patients underwent radical surgery plus D2 lymphadenectomy. At baseline, there were no significant differences in total lymphocyte and lymphocyte subsets between groups. The control group showed a significant decrease of total lymphocytes, CD4 cells, and CD4/CD8 ratio at the 14^{th} postoperative day relative to the baseline value. In the control group 65% of patients had a decreased of CD4 under 500 cells/mmc. Instead it has been observed in IL-2 group a significant increase over the control group values of total lymphocytes and CD4 cells (14^{th} total lymphocytes and CD4: IL-2 vs control $p < 0.05$). Moreover in this group only 15% patients had CD4 under 500 cells/mmc. This difference, in CD4 count, is significant even at the 50^{th} postoperative day ($p = 0.006$). IL-2 group showed lower postoperative complications (4/44 vs 13/45; $p < 0.05$), and higher lymphocyte/eosinophil infiltration into the tumor ($p < 0.0002$). Median follow up was 36 months (range 12–72) and median overall and disease-free survivals were longer, even if not significantly, in the IL-2 group than in the control arm ($p = 0.07$ and $p = 0.06$ respectively).
Conclusion: This randomized study would suggest that a preoperative immunotherapy with IL-2 is a well tolerated treatment able to prevent surgery induced lymphocytopenia. IL-2 seems to neutralise the immuno-suppression induced by operation and so to stimulate the host reaction against tumour tissue (lymphocytes/eosinophils infiltration). Furthermore IL-2 seems to have an impact on clinical course reducing morbidity of surgery and ameliorating overall and disease free survival

536 POSTER

Mechanisms of transcriptional upregulation of DR5 by chemotherapeutic drugs and sensitization to TRAIL-mediated apoptosis

S. Baritaki[1], M. Neshat[2], S. Huerta-Yepez[2], T. Sakai[3], D. Spandidos[1], B. Bonavida[2]. [1]*University of Crete, Clinical Virology, Heraklion, Greece;* [2]*University of California, Microbiology, Immunology and Molecular Genetics, Los Angeles, USA;* [3]*Prefectural University of Medicine, Molecular-Targeting Cancer Prevention, Kyoto, Japan*

TRAIL, a member of the TNF family, has been shown to kill sensitive tumor cells with minimal toxicity to normal tissues and is a new candidate for immunotherapy in the treatment of drug-refractory tumor cells. However, many drug-resistant tumor cells are also resistant to TRAIL and such tumors require sensitization to reverse TRAIL resistance. We, and others, have reported that several sensitizing agents (ex. CDDP, ADR, chemical inhibitors, etc.) in combination with TRAIL result in significant synergistic apoptosis, however the mechanisms by which this sensitization is achieved still remain unclear. Based on the observed upregulation of Death Receptor 5 (DR5) expression, induced by the sensitizing agents (*Ng et al., Prostate, 53: 286, 2002; Huerta-Yepez et al., Oncogene, 23: 4993, 2004*), we hypothesized that many of those drugs may, directly or indirectly, interfere with a repressor factor of the DR5 transcription.
Examination of the DR5 promoter revealed the presence of one binding site for the transcription repressor Yin Yang 1 (YY1), suggesting that YY1 may negatively regulate DR5 transcription. This hypothesis was tested by examining a luciferase reporter system (pDR5 wild type) and plasmids in which the YY1-binding site was either deleted (pDR5/-605), and/or mutated (pDR5-YY1 mutant).
Using the PC-3 prostate (androgen independent) tumor cell line as a model system, we showed that PC-3 transfected with pDR5 wild type resulted in basal luciferase activity, whereas treatment with CDDP or ADR significantly augmented luciferase activity. PC-3 cells transfected with pDR5/-605 or pDR5-YY1 also resulted in significant potentiation of the basal luciferase activity. Inhibition of YY1 by siRNA revealed increased sensitization of tumor cells to TRAIL-mediated apoptosis. Reduced YY1 DNA binding properties and downregulation of the NF-kB promoter activity were also shown to be triggered by drug treatment.
These findings indicate that YY1 negatively regulates DR5 transcription inducing tumor cells' resistance to TRAIL. They also support the hypothesis that drugs-induced upregulation of DR5 expression is mediated via inhibition of the transcription repressor YY1. On a clinical aspect, the above findings suggest that tumor cells overexpressing YY1 will be resistant to TRAIL-mediated apoptosis. Therefore, inhibition of YY1 may be clinically useful in the therapeutic application of TRAIL in resistant tumor cells.

537 POSTER

P43/EMAP-II expression in colorectal cancer is associated with hypoxia, enhanced lymphocyte infiltration and apoptosis

M. Youssef[1], P. Symonds[1], I.O. Ellis[2], J.C. Murray[1]. [1]*University of Nottingham, WDDC, Nottingham, United Kingdom;* [2]*University of Nottingham, Pathology, Nottingham, United Kingdom*

Aims: P43/Endothelial monocyte-activating polypeptide II (p43/EMAP II) is a proinflammatory cytosine and a chemoattractant for mononuclear phagocytes and polymorphonuclear leukocytes, found in culture supernatants of many tumour cell lines. We recently demonstrated that p43/EMAP-II induces apoptosis in mitogen-stimulated lymphocytes, and suggested that it may be a constituent of a novel immune evasion mechanism employed by tumour cells [1]. Furthermore p43/EMAP-II release is enhanced by hypoxia [2]. Our study has examined the association between p43/EMAP-II expression and hypoxia in colorectal cancer (CRC), and also the association between p43/EMAP-II and lymphocyte apoptosis.
Methods: Formalin-fixed, paraffin-embedded archival tissue samples from a well-characterised population of 72 patients diagnosed with colorectal tumours were used in immuno-histochemical studies. Antibodies against p43/EMAP-II, carbonic anhydrase (CA IX) as a surrogate marker of hypoxia, and CD3 to identify tumour-infiltrating lymphocytes (TIL) were used. Areas of p43/EMAP-II and CA IX staining were quantified using computer-aided image analysis. Antibodies against active Caspase-3 and PARP were used to identify apoptosis in TIL.
Results: P43/EMAP-II expression was correlated with CA IX expression in CRC. Patients with high p43/EMAP-II expression seemed to do better than those with low, and the reverse was true for CA IX. There was also a positive correlation between p43/EMAP-II and the lymphocyte counts in CRC ($p = 0.03$), as well as between CA IX and lymphocyte counts ($p = 0.02$). The presence of CD3+ cells was a good prognostic indicator in terms of overall survival. There was a significant association between