p43/EMAP-II expression and apoptosis of tumour infiltrating lymphocytes in CRC ($p = 0.04$ by active caspase 3; $p = 0.02$ by cleaved PARP).
Conclusions: P43/EMAP-II expression is associated with hypoxia and high lymphocyte counts in colorectal cancer. Furthermore P43/EMAP-II expression is associated with apoptosis of tumour infiltrating lymphocytes.

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538 POSTER

Immunotherapy of melanoma: construction and characterization of DNA vaccines encoding mk2–23 SCFV antigen

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Anti-idiotype MK2–23 monoclonal antibody (anti-id Mk2–23mAb), structurally mimics the high molecular weight (HMW)-melanoma-associated antigen (MAA). HMW-MAA antigen is expressed in a vast majority of melanoma lesions with limited inter- and intra-lesion heterogeneity, and negligible expression in normal tissues. Because HMW-MAA is expressed on the surface of malignant cells, it represents a logical target for anti-idiotipic immunotherapy. Indeed, melanoma patients immunized with MK2–23 mAb developed anti-HMW-MAA antibodies, which associated with regression of metastases and survival prolongation in a few patients. In order to fulfill regulatory requirements for larger clinical investigations, we explored the feasibility of replacing MK2–23 antibody immunization with naked scFv DNA vaccines.
To generate scFv MK2–23 DNA expression vectors, the variables heavy (VH) and light (VL) chains of MK2–23 hybridoma were cloned in a pVAC plasmid, which allows the anchorage of the expressed antigen to the surface of mammalian cells. pVAC plasmids encoding MK2–23scFV antigen were assembled in two orientations, expressing either the VH or the VL chain at the amino terminus of the transgene product, linked together by a 10-amino acid linker (Ln). In vitro transfections of 293 cells with both (VH Ln VL) and (VL Ln VH) MK2–23 pVAC plasmids, demonstrated that the MK2–23 scFv antigen, expressed in both configurations was equally recognized by anti-HMW-MAA mAb (Cell-ELISA and FACS analysis). Next, we demonstrate that both intramuscular and gene-gun immunizations of Balb/c mice with scFv MK2–23 plasmids induced the production of antibodies against MK2–23 as well as HMW-MAA antigens.
Notably, while multiple gene-gun immunizations were required to elicit a strong immunoresponse, a single intramuscular injection of scFv MK2–23 DNA was sufficient to generate significant levels of circulating anti-HMW-MAA antibody.
The information gained from this study may be relevant for developing novel clinical vaccines for the treatment of malignant melanoma.

539 POSTER

Cell kinetic effects of chemotherapy (CT)+pegfilgrastim in circulating progenitor cells (CPCs) of breast cancer patients

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Background: Several clinical trials have been designed to evaluate the clinical effects of pegfilgrastim in dose-dense CT regimens both in lymphomas and in breast cancer. From a biological standpoint, one of the concerns of these studies is the possible cytokinetic sensitization of the CD34+ cell subsets by pegfilgrastim administration that could be detrimental when the following course of CT is started. To date, no detailed information are available on the proportion of CPCs that proliferate, remain quiescent or undergo apoptosis after in vivo treatment with CT+pegfigrastim.
Material and methods: On the basis of our previous experiences with filgrastim, we have evaluated the impact of Docetaxel (80 mg/sqm, day 1)+Epirubicin (75 mg/sqm, day 1), followed by a single dose per cycle of pegfilgrastim (6 mg s.c. on day +1) on the actual proportion of CPCs undergoing G0/G1, S and G2/M phases of the cell cycle or showing apoptotic features. The CT schedule is applied in metastatic and locally advanced breast cancer pts and it is planned every 14 days for up to 4–6 courses. Using multiparameter flow cytometry (FCM), Annexin V expression was quantitated at a single cell level and correlated with cell cycle phases (DNA content profile) in CD34+/38+ CPCs.
Results: Peripheral blood (PB) samples from 9 pts at their first course were studied. 7 days following pegfilgrastim the % of CD34+/38+ CPCs in S-phase was 12.5±5 while 4.8%±3 of this cell subset showed apoptotic features. One week later, these values were 7.9%±5 and 9.8%±3, respectively.
Conclusions: Our study is ongoing and these results show that: 1) Docetaxel/Epirubicin at standard dosages followed by pegfilgrastim exerts stimulatory effects on cell cycle status of PB-derived CD34+/38+ hematopoietic progenitors, protecting them at the same time from apoptosis; 2) this effect is particularly evident 7 days after pegfilgrastim administration and tends to decrease on one week later. These findings could be useful when dose-dense CT programs are supported with pegfilgrastim as well as when this cytokine is tested for the mobilizing capacity of CPCs for autografting.

540 POSTER

Action of immunotherapy with Interleukin-2 on innate immunity cells in peripheral blood and in tumoral tissue of pancreatic adenocarcinoma patients

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Background: Recent evidences indicate a crucial role of innate immunity cells, like Natural Killer (NK) cells and eosinophils, in host anticancer defence. Cancer patients with high levels of NK cells and eosinophils in peripheral blood show a lower incidence of metastases and a better prognosis. Recombinant Interleukin-2 (rIL-2) immunotherapy is known to stimulate the innate immunity cells, that in pancreatic cancer patients are usually reduced and furthermore impaired by surgical operation. The purpose of this study is to evaluate the toxicity of preoperative high dose and postoperative low dose rIL-2 treatment, as well as the biological effects on innate immunity both in peripheral blood and in cancer tissue, in patients with resectable pancreatic adenocarcinoma.
Material and methods: Thirteen patients (8 males, 5 females, mean age = 65 years) received rIL-2 immunotherapy consisting in a preoperative subcutaneous administration of 12 millions IU/day for 3 consecutive days and two postoperative cycles (on 30thand 60th days) of 3 millions IU/day for 6 consecutive days. We evaluated absolute number of NK cells and eosinophils before rIL-2 administration, on 1st, 7th, 14thand 30th postoperative days and after each postoperative cycle. 11 patients underwent pancreaticoduodenectomy, 1 splenopancreatectomy and 1 total pancreatectomy. We also analized eosinophil and NK cell tumoral infiltration in pancreatic surgical specimens.
Results: Toxicity profile was moderate. In the whole early postoperative period we observed a significant increase of both NK cells and eosinophils, comparing to basal values ($p < 0.05$ in each sample). In the late postoperative period (from 30th day) innate cells count didn't further significantly improve. The histopathological and immunohistochimical analysis didn't find out any significant intratumoral infiltration of NK cells neither of eosinophils.
Conclusions: This work demonstrates that preoperative high doses rIL-2 administration is able to counteract surgery-induced deficiency of NK cells and eosinophils in peripheral blood in the early postoperative period, even if it can't overcome local mechanisms of immune tumor escape in cancer tissue. Considering the important role of innate immunity in anticancer defence, its immunotherapy induced amplification may improve the control of minimal residual disease and metastatic cells spreading in the perioperative period.

541 POSTER

Hybrid-primed lymphocytes and hybrid vaccination prevent tumor growth of Lewis Lung Carcinoma in mice

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Dendritic cell (DC)–tumor cell hybrids are currently being evaluated as a novel anti-tumor vaccination strategy. We here explored in an animal model whether administration of DCs fused with poorly immunogenic carcinoma cells could elicit an anti-tumor response.
Fusion of C57BL/6 mice bone marrow derived DCs with Lewis Lung Carcinoma (LLC1) cells resulted in around 50% fusion efficiency. Hybrid cells (HC) were used to explore three potential tumor-therapy strategies: protective immunization, vaccination and adoptive cellular therapy.