stabilisation >6 months after surgery or radiotherapy for lesions arising during vaccination. A randomised study is needed to confirm the effect on tumor behaviour.

601

Genetically modified tumour vaccines: Transduction of IL-2 and CD80 gene

J. Bubeník, P. Rössner, J. Šímová, D. Bubeníková. *Institute of Molecular Genetics, Academy of Sciences, Prague, Czech Republic*

Purpose: The effect of therapeutic strategies based on the insertion of immunoregulatory genes into tumour cells, followed by vaccination with the resulting genetically modified tumour vaccines was evaluated in preclinical model systems.

Methods: Murine IL-2 cDNA and CD80 cDNA was used for insertion into murine plasmacytoma and 3 MC-induced sarcoma cells. The presence of the inserted genes was confirmed by hybridization of mRNA with digoxigenin-labeled probes, by CTLL assay, ELISPOT assay and the FACS cytofluorometry.

Results: Comparative studies performed with 25 IL-2 producing and 13 CD80 expressing clonal cell lines revealed that insertion of the IL-2 gene downregulates tumorigenicity more efficiently than insertion of the CD80 gene. Insertion of the CD80 gene substantially enhanced the adhesive interaction between the tumour cells and T lymphocytes. Tumour inhibitory effects of peritumorally administered vaccines were time- and dose-dependent, and efficient exclusively for small tumours. Combined vaccines expressing both IL-2 and CD80 genes were more efficient than those expressing only one of the genes. Systemic administration of irradiated cell vaccines was highly efficient after cytoreductive therapy of generalized haemoblastoses.

Conclusions: Experimental studies suggested that nongeneralized early forms of cancer, small primary tumours, minimal residual disease and micrometastases should be considered for gene therapy with IL-2 and CD80 expressing vaccines.

602

Intratumor (IT) gene transfer with recombinant adenoviral (rAd) vectors in lung cancer (LC) patients (PTS): The Institut Gustave Roussy (IGR) experience

T. Tursz, A. Le Cesne, P. Baldeyrou, E. Gautier, P. Opolon, D. Grunenwald, F. Farace, L. Zitvogel, B. Escudier, C. Schatz[1], M. Courtney[1], T. Le Chevalier. *Institut Gustave Roussy (IGR), Villejuif, 94805; [1]Transgène, Strasbourg, 67000, France*

The use of replication-defective (E1-E3 deleted) rAd vectors for the local delivery of therapeutic genes has been evaluated in LC pts at IGR since 1994. Currently, 11 pts (3 at 10^7 and 10^8, 5 at 10^9 pfu) are fully evaluable in the first phase I study of our gene therapy program testing the IT administration of a rAd containing the marker gene encoding the bacterial enzyme beta-galactosidase (β-gal). All pts received concomitant chemotherapy. Expression of β-gal was observed in 8/11 tumor biopsies (1/3 at 10^7, 2/3 at 10^8, and 5/5 at the 10^9 dose level) with a progressive increase in transgene expression based on both PCR and the number of positive tumor samples and injected sites (about 10% of infected cells at the highest dose level). All bronchoalveolar lavage samples obtained immediately after injection were positive for rAd by culture and PCR. Pts treated at the second and third dose levels had PCR-positive blood samples at day 1. Viremia (positive culture) was detected at day 1 in 2/5 pts receiving 10^9 pfu. The same 2 pts had a positive culture in sputum at day 2 or day 3. In addition, all other biological fluids were negative by culture and all but one were negative by PCR after day 12. Significant prolonged increases in anti-adenovirus type 5 antibody titers were seen in 4 pts. Sustained antibody responses to β-gal were observed in 3/4 pts treated at the highest dose level as well as strong cellular (proliferative and cytotoxic) β-gal responses in 3/4 cases studied. Major tumor regressions were seen in 7 pts. The 3 pts treated at the second dose level, all with stage IIIB disease, were deemed resectable after chemotherapy, and 2 of them are alive free of disease at 23 and 27 months after adenoviral injection. All samples taken from medical staff before and after injection of each patient were negative for wild type Ad and rAd-β-gal. This study confirms that a marker gene can be safely introduced and expressed by tumor cells using a rAd and that a single injection in humans is able to induce long-lasting cellular and humoral immunity specific to the transgene product.

603

Genetic drug activation strategies for breast cancer

K. Sikora, H. Hurst, N. Lemoine, H. Pandha. *Department of Clinical Oncology, Imperial College School of Medicine, Hammersmith Hospital, London W12, UK*

Our group has been developing a selective drug activation strategy using upstream sequences from the *c-erbB2* gene coupled to various enzymes. *ErbB2* is overexpressed in a wide range of human tumours including those of the breast, pancreas, lung and ovary. Although gene amplification may be partly responsible, in most tumours the increase in protein quantity is due to transcriptional deregulation with increased specific mRNA production.

We have developed a selective activation system using the cytosine deaminase gene from E. coli driven by the relevant upstream sequences. This chimaeric gene has been inserted into several vectors which can infect human cells both *in vitro* and *in vivo*. Selective expression of cytosine deaminase has been observed with considerably enhanced toxicity of the prodrug 5 F luorocytosine. A phase I clinical trial is now in progress for patients with nodular breast cancer.

604

Molecular biology of pancreatic cancer and implications for gene therapy

N.R. Lemoine. *ICRF Molecular Oncology Unit, Imperial College School of Medicine at Hammersmith Hospital, London W12 0NN, UK*

Our understanding of the molecular genetics of pancreatic cancer has advanced spectacularly over the last five years so that this tumour type is now one of best characterised of all malignancies. A small proportion of cases result from inherited predisposition due to germline transmission of a mutated CDKN2 or BRCA2 gene while patients with familial pancreatitis due to a mutated cationic trypsinogen gene have a greatly increased risk of developing pancreatic cancer. The majority of cases are sporadic and are characterised at the molecular level by several key genetic abnormalities. The most frequent of these is point mutation of the dominant oncogene KRAS, a lesion which occurs as an early, and possibly initiating event in tumorigenesis. Inactivating mutations of the tumour suppressor genes TP53, CDKN2 and SMAD4 are also frequently observed and this constellation of genetic defects sets pancreatic cancer apart from other types of cancer, a feature which could have important implications for molecular diagnosis.

Genetic intervention for cancer prevention and therapy is becoming a clinical reality and several approaches are being pursued for pancreatic cancer. As well as tumour suppressor gene replacement and oncogene blockade, strategies with a potential bystander effect are showing promise. These include genetic prodrug activation therapy using selective expression of suicide genes and genetic immunomodulation with cytokines and tumour-associated antigens.

605

Surgical treatment of pancreatic cancer – Recent progress

H.G. Beger. *Department of General Surgery, University of Ulm, Germany*

Pancreatic cancer is the fourth commonest fatal tumor disease. Over the past years a continuing increase in yearly incidences was registered; the prognosis of pancreatic cancer is unfavourable in most pts. More than 80% of pts. have a stage III or stage IV tumor at the time of diagnosis. Between 1982 and 1993 471 pts. with pancreatic cancer were treated in the Department of General Surgery, University of Ulm. The pts.' mean age was 62 years, ranging from 29 to 90 years. In 284 pts. (68%) the tumor was in an advanced stage with metastatic spread to lymph nodes or adjacent organs (stage III or IV). Only in 44/471 pts. the tumor could be resected at stage I. In our patient collective the resection rate was around 35% (145/416), the conventional Whipple operation was applied in 60% of cases. 23 pts. underwent a pylorus-preserving duodenopancreatectomy. Our data confirmed the results published by Klingenbijl et al., that there is no difference in survival between the conventional and the pylorus-preserving Whipple operation. It can be assumed, therefore, that the less extensive resection with preservation of the pylorus may mean a better quality of life for pts. without shortening the survival time. Due to the far advanced tumor stage 40% of pts. (190/471) could only be treated by a bypass operation. Regardless of the therapeutic option the mean survival times were 15.4 months for stage I, 9.6 months for stage II, 8 months for stage III and 5 months for stage IV tumors. The mean survival time of 102 pts. with the conventional Whipple resection was 11.3 months, in the pylorus-preserving