

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

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

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**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 CTL 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.

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

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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 ( $\beta$ -gal). All pts received concomitant chemotherapy. Expression of  $\beta$ -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  $\beta$ -gal were observed in 3/4 pts treated at the highest dose level as well as strong cellular (proliferative and cytotoxic)  $\beta$ -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- $\beta$ -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.

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### Genetic drug activation strategies for breast cancer

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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 fluorocytosine. A phase I clinical trial is now in progress for patients with nodular breast cancer.

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### Molecular biology of pancreatic cancer and implications for gene therapy

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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.

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### Surgical treatment of pancreatic cancer – Recent progress

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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 Klingenberg 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

Whipple group it was 12.1 months. The 5-year survival rate after tumor resection was 9%.

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### Radiation oncology in pancreatic cancer

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Radiation therapy has been utilized in the care of patients with pancreatic cancer in two clinical settings: 1. as "definitive therapy" for unresectable or locally advanced non-metastatic disease and 2. as adjuvant or neoadjuvant therapy in resectable tumors. This presentation will review past and current data assessing the role of radiation therapy in conjunction with 5-fluorouracil based chemotherapy in these two clinical settings. Recent efforts combining irradiation with taxanes and gemcitabine will also be discussed.

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### Developments in systemic therapy of pancreatic cancer

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Adenocarcinoma of the pancreas is a profound therapeutic challenge. Only a small fraction (about 3%) of patients with this disease will survive more than two years. In general, pancreatic cancer (PC) is considered to be a chemoresistant disease. It has been difficult to identify chemotherapy agents that have substantial efficacy as evidenced by objective tumor regressions or prolonged survival. For technical reasons localized PC is difficult to measure using 3-dimensional imaging; thus, novel surrogate endpoints must be explored such as reduction in circulating tumor associated antigens such as CA 19-9 or in improved quality of life or symptoms. The latter approach was used to define efficacy and gain FDA approval for gemcitabine (2'-deoxy-2'-difluorocytidine) in the U.S. This interesting compound is a prodrug which undergoes intracellular metabolism to active phosphorylated moieties. The accumulation of these metabolites is dose and dose-rate dependent; clinical studies evaluating this are in progress. Other new approaches for systemic therapy of PC are based on the molecular, biochemical and structural findings in PC. Identification of tumor antigen phenotype has spawned trials of radioimmunoconjugates and tumor vaccines. A molecular hallmark of PC, a mutated Ras oncogene, has encouraged the development of new drugs that inhibit farnesyl transferase activity, an important enzyme in Ras activation. Another approach involves matrix metalloproteinase enzymes which can degrade tissue extra-cellular matrix interfering with tumor invasion, neovascularization, and metastases.

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### Imaging processing for evaluation and reduction of geometrical uncertainties in prostate irradiation

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The three major sources of geometrical uncertainties in radiotherapy of prostate cancer are: target volume delineation, organ motion, and patient set-up. Using image processing techniques, these uncertainties have been quantified in three dimensions and reduced. Usually the whole prostate is the target, making target volume delineation relatively easy compared to other tumour sites. All errors introduced at this stage are purely systematic, i.e., they influence all treatment fractions. In a pilot study, automatic image registration was used to include MR in the treatment planning process. It was found in an inter-observer study with three observers using CT and MR of 18 patients that the systematic differences between the target volumes delineated in CT and MR are significantly larger than the inter-observer variation. For example, the average target drawn on MRI has a 30% smaller volume compared to CT (a systematic difference of about 8 mm at the posterior aspect). Prostate motion has studied using image registration of repeat CT scans. An advantage of the this approach is that the motion of the prostate can be directly related to the volume of other organs such as the rectum or the bladder. In this study of 11 patients in 4 scans, the largest motion was a rotation around a left-right axis near the apex with a standard deviation of about 4 degrees. The prostate motion was found to be strongly correlated to rectal volume differences. In our clinical protocol, patients instructions to have empty therefore now re-scanned if the rectal volume

exceeds 150 cm<sup>3</sup>. In this way, large systematic errors in the prostate position (introduced by the planning CT) are eliminated. Finally, patient set-up has been studied using electronic portal imaging. In our institute, the set-up deviations are 2 mm standard deviation (for each axis) both for day-to-day variations and for systematic deviations. Without any correction, the vector length of the systematic set-up error exceeds 5 mm for 30% of the patient population. Using off-line image analysis and a simple decision protocol, virtually all systematic set-up errors exceeding 5 mm have been eliminated with relatively low workload: about one third of the fractions is imaged, while on average less than one correction per patient is performed. Using newly developed image processing tools, the set-up error has been quantified in 3-D, i.e., including all rotations. It was shown that the magnitude of the rotations is less than 1 degree standard deviation for all three directions. The combined effect in 3-D of the three geometrical uncertainties will be shown on clinical data on a video.

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### Advances in conformal therapy

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During the last two decades major changes have taken place in radiation oncology: the "imaging revolution" of the 70ies/80ies is being followed in the 90ies by the "3 D treatment planning revolution" which means 3 D assessment of anatomy and optimization of dose distribution for target and critical organs using specific tools for estimating tumor control and normal tissue complication probabilities.

**Conformation Radiotherapy (CR)**, which converts 3 D treatment planning into clinical radiotherapy, is the adaptation of the volume treated by radiation to the target volume, as precisely as possible, with maximum sparing of normal tissue. Although this aim has been pursued for decades, a high degree of conformation has become only possible within wide clinical use by recent developments in medical linear accelerators (multileaf collimators) and computer technology including networking. CR in clinical practice means at present multiple static fields (4-6), each shaped according to the individual target. Acute and long term adverse side effects are dramatically reduced by this technique. On the other hand, radiation dose escalation has become possible, resulting in a higher degree of (local) tumor control with acceptable radiation related morbidity. The most common tumor sites treated at present by CR are prostate, gynecology, liver, lung, base of the skull, brain, with very promising clinical data reported so far.

CR represents one of the most important tools for improving the therapeutic ratio in present and future radiotherapy.

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### The European quality assurance program in radiotherapy

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The concept of Quality Assurance (QA) has recently broadened from its restricted definition of technical maintenance of equipments and treatment delivery toward a comprehensive approach of all activities in the radiotherapy department, from the moment the patient enters it until the moment he leaves, and even later during the follow-up. The comprehensive approach is favoured because it is recognised that partial organisation of some key steps in the radiotherapy process is not sufficient to guarantee the patient (and society) that each individual will receive appropriate optimal care for his/her disease. Also, besides the important technological aspects of radiotherapy, more emphasis has been recently put on QA of treatment indications, of treatment protocols, of treatment reporting and of systematic registration of side-effects and complications.

A comprehensive QA system reduces the likelihood of errors and accidents, it ensures continuing quality improvement, it increases efficiency, it raises staff morale and introduces a cultural change, it reduces the chance for litigation and it is a management tool.

The structure of such a system has been extensively discussed in the report "QA in radiotherapy" (R&O, 35: 61-73, 1995). The practical implementation of the principles, however, needs a specific methodology, which constitutes the project on which the ESTRO commission for QA has been working for the past 2 years. The report on this methodology will be discussed.