the need for a high radiation dose. On the other hand reducing the volume of the excision specimen and separate removal of primary tumor and lymph node metastases will lead to much improved cosmetic results, as shown in a recent major trial of the EORTC. In this trial 5569 patients were randomized to investigate the curative potential of a boost dose of radiotherapy, as part of the breast conserving treatment. The precise localization of the tumor bed by the surgeon will help the radiation oncologist in a much more accurate delivery of the radiation boost dose, therefore optimizing the local control rate in patients with early breast cancer.

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New approach to staging the axilla by endoscopic axillary lymphadenectomy (EAL) in early breast cancer

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Staging of axillary lymphnodes remains the essential factor of prognosis in breast cancer. Actually some teams are very reluctant to do it because of the high morbidity in conventional surgical adenectomy. Not the most for its early complications: lymphorrhea, lymphocele but for its delayed complications lymphoedema, shoulder stiffness and arm swelling. To avoid all those problems a lot of solutions were invented: axillary lymphadenectomy limited to the picking of the only "sentinel" lymphnode. None of those methods is fully satisfying. Morbidity is still important in functional lymphadenectomy: picking of the "sentinel" node seems not to be enough. We propose a new way of adenectomy in the axilla: liposuccion of the axillary fat and endoscopic picking of all the remaining lymphnodes. Fat aspiration leeds to an anatomical and conservative dissection of the axilla preserving the vessels, the nerves and permits an electif and complete removal of all the lymphnodes by this way adenectomy fulfills his prognostic and therapeutic aim.

Early morbidity is very low and transitory, delayed morbidity is near to zero without any lymphoedema. It seems as if liposuccion and EAL (Endoscopic Axillary Lymphadenectomy) will be the technique of lymphadenectomy in the next millenium for early breast cancer.

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Quality control aspects in surgery

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For a long time surgery was the keystone of any treatment of breast cancer. Last years, one has the impression that surgery is not that important any more since outcome seems to be defined by stage of the disease at time of diagnosis and the use of adjuvant treatment. The evolution to more breast conservative treatments has put a lot more strain on the surgeon and the radiotherapist, since not only locoregional control and survival, but also cosmesis is considered an important endpoint.

Most of the patients are primarily treated outside the frame of clinical trials, so decision making and selection is based on individual experience, believe of the surgeon or patients' preferences. The surgical procedure itself – even within the frame of prospective trials, where surgery is considered 'standard'- is submitted to a large range of variation as has been detected by an EORTC pilot study on that matter. Pathology reports not only reflect the way surgery has been performed but also the thoroughness of the pathologist. On these uncontrolled bases, adjuvant treatment regimens are tailored and investigated in prospective trials.

Better education and special training in breast disease are necessary. A computer assisted decision making process and accurate documentation of the surgical procedure and pathology may lead to a better understanding of how decisions are taken and build up a more accurate data base to analyse process and outcome in a prospective way.

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Mechanisms of vaccination with cytokine gene-transduced cells

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Purpose: Gene therapy is certainly a powerful tool. Yet it is also a seductive concept. This, coupled with the difficulty of establishing of appropriate controls has lead and is continuing to lead to over-optimistic conclusions. There is thus an urgent need for a tenable definition of its potential.

Methods: A transplantable, aggressive and metastasizing mammary carcinoma (TSA) that spontaneously arose in a BALB/c mouse and mammary tumors arising in *neu* transgenic BALB/c mice were used as a model to explore the potential of vaccination with cytokine gene engineered cells in: a) inducing a protective immunity in normal mice; b) curing incipient spontaneous metastases; c) curing small and large solid tumors; d) preventing tumor development in transgenic mice.

Results: The cytokine released by engineered tumor cells: a) influences the characteristics and efficacy of the local reaction; b) leads to the induction of a memory reaction towards parental tumor cells skewed toward a TH1 (IL-2, IFN-γ, IFN-α, IL-12), a TH2 (IL-4) or a mixed (IL-10) response; c) elicits (IL-2, IFN-γ and IL 12) a systemic reaction that protects against incipient metastases (IL-2, IFN-γ, IL-12), but hampers (IL-12) established tumors marginally only; d) protects (IL-12) against the development of spontaneous tumors.

Conclusion: Manipulation of the antitumor response through vaccination with cytokine-engineered cells is a real prospect. Selection of the inducing cytokine makes it possible to shape the features of a primary inflammatory reaction and the ensuing antitumor memory. These findings are leading towards a more distinct evaluation of the potential of cytokine-gene engineered cells in protecting cancer patients with minimal residual disease, or those expected to have a recurrence after a long disease-free interval, and in subjects with high risk of cancer.

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Gene therapy for melanoma

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Gene therapy approaches for the successful combat of cancer include several conceptually different strategies: (i) enhancement of the tumor's immunogenicity; (ii) modification of the host immune system, e.g., by transducing tumor-infiltrating lymphocytes with TNF or IL-2 genes or by expressing tumor antigen genes in professional antigen-presenting cells; (iii) modification of other host tissues, e.g., by transfer of cytotoxic drug resistance genes into hemopoietic progenitor cells; (iv) introduction of corrective genes (e.g., wild-type p53) into tumors; (v) transfer of enzymes for prodrug therapy, e.g., introduction of the viral thymidine kinase gene into tumor cells which then become sensitive to ganciclovir.

In the case of melanoma, most gene therapy trials are conducted with melanoma cells the immunogenicity of which has been augmented by transfection with genes encoding cytokines (e.g., IL-2, IL-7, GM-CSF) and/or costimulatory molecules (e.g., CD80).

We and others have shown (i) that highly tumorigenic mouse melanoma cell lines lose their tumorigenicity upon transfection with IL-2, (ii) that mice injected with IL-2-transduced melanoma cells are protected when challenged with wild-type tumor cells, and (iii) that administration of IL-2-transfected melanoma cells into mice can induce the elimination of preexisting cancer cell deposits. Based on these encouraging results, we have tested the safety and immunostimulatory potential of an IL-2-based, autologous melanoma vaccine in patients with stage IV disease. Results obtained are promising and provide the basis for a phase II study aiming at evaluating the therapeutic efficacy of such vaccines.

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Vaccination with GM-CSF transduced melanoma cells: A promising treatment

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GM-CSF is the most effective cytokine in vaccines designed to generate an anti-tumor effect and induce long-term tumor specific memory in animal models (Dranoff et al, PNAS 1993; 90: 3538–43). We have explored this approach in patients with advanced, metastatic melanoma using autologous tumor cells transduced with huGM-CSF and the MGF-S retroviral vector. 28 patients have been randomised to 5 or 50 × 10^6 cells (secreting 40–800 ng GM-CSF/10^6cells/24 hr) q 3 wk × 3. The vaccinations are safe and well tolerated. The local and immunological effects are greater at the higher dose. A lymphocytic infiltrate at the vaccine site at d3 changes at d8 to one in which eosinophils, Langerhans cells and a vasculitis predominate. Conversion of the DTH response occurs in all patients. Increases in the number of precursor CTLs have been seen in blood and in distant metastases. We have seen stabilisation of previously progressive disease for longer than 6 months without further intervention in 5 patients, and in a further 3 again

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 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.

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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 (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.

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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 luorocytosine. 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 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