except for testis. The genes of the MAGE family are all located on the q terminal region of the X chromosome. The putative proteins produced by these genes present almost identical hydrophobicity patterns, suggesting that they exert the same function, but this function remains unknown. Gene MAGE-4 carries at least eight alternative first exons preceded by different promoters. The MAGE gene family may therefore ensure that the same function is placed under the control of nineteen different promoters, allowing for very specific spatial and temporal regulation. Gene MAGE-3 codes for a second antigen presented by HLA-A1. The relevant antigenic peptide is encoded by the MAGE-3 sequence that is homologous to the MAGE-1 sequence that also codes for an antigen presented by HLA-A1. Recently, another peptide that is encoded by MAGE-3 and binds to HLA-A2 has been found to be recognized by CTL. Two additional genes that code for tumor antigens and are expressed only in tumors and in testis have been isolated. These genes, named BAGE and GAGE, are unrelated to each other and to the MAGE family. MAGE, BAGE and GAGE are expressed in a significant proportion of tumors of different histological types, such as melanomas, head and neck carcinomas, non small cell lung carcinomas and bladder tumors. They are not expressed in certain types of tumors such as leukemias. Genes coding for differentiation products, such as tyrosinase and Melan A in melanomas, also code for antigens recognized by autologous CTL.

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RECOGNITION OF TUMOR ANTIGENIC PEPTIDES BY CD8 POSITIVE T CELLS

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While earlier attempts to define human tumor-associated antigens have relied on the use of monoclonal antibodies, current studies are focused on the identification of potential rejection tumor antigens that are recognized by $CD8^+$ cytolytic T lymphocytes (CTL). Through their antigen-specific receptors (TCR), $D8^+$ T cells recognize a ligand composed of a short peptide (generally 8–10 amino acids) bound to a class I molecule of the major histocompatibility complex (MHC). Exogenously added synthetic peptides corresponding to intracellularly produced antigenic peptides can bind to cell surface-associated "empty" MHC class I molecules, resulting in the formation of complexes that mimic the natural CTL ligands. Studies using single amino acid substituted peptide derivatives indicate that some specific CTL from single individuals may use widely different TCR for recognition of the same tumor peptide, whereas a much more restricted TCR segment usage has been observed in CTL responses to other tumor peptides. The clinical significance of these findings will be discussed.

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ACTIVE IMMUNIZATION OF MELANOMA PATIENTS WITH IL-2-TRANSFECTED ALLOGENEIC MELANOMA CELLS. A PHASE I–II STUDY

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The aim of this clinical study was to immunize stage IV melanoma patients with a HLA-A2-compatible, immunogenic human melanoma line (Me14932) genetically modified to release IL-2 in order to elicit or increase a T cell-mediated anti-melanoma response which may affect distant melanoma lesions. Me14932/IL-2 cells produce an average of 2282 pg/mL/10^5 cells/24 hrs and the level of cytokine produced after lethal irradiation was more than 50% of the IL-2 released by the non-irradiated cells for a period of at least 3-wks. Patients were injected s.c. at days 1, 13, 26, 55 with 5×10^7 or 15×10^7 irradiated melanoma cells each time. Five patients showed progression of disease. Two patients treated with 5×10^7 melanoma cells and one treated with 15×10^7 cells showed simultaneous evidence of partially regressed lesions and persisting unchanged metastases. To evaluate the specific cytolytic T-cell response induced by vaccination, limiting dilution analysis and MLTC utilizing different HLA-A2 melanoma lines and peptides were performed with lymphocytes obtained before and after immunization. Increased frequencies of lytic and specific lymphocytic precursors were observed. In two cases, biopsies of tumor nodules taken prior and after vaccination revealed a mild increase of CD4+ and CD8+ cells in samples obtained after immunization.

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IMMUNOTHERAPY WITH AUTOLOGOUS, IRRADIATED MELANOMA CELLS TRANSDUCED WITH THE GM-CSF GENE

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Irradiated melanoma cells, that are transduced with the GM-CSF gene, produce GM-CSF for about one week. The local production of high levels of GM-CSF could attract and stimulate antigen presenting cells, such as dendritic cells, which can present the melanoma associated antigens to cytotoxic T cells (CTLs). In a murine model vaccination with irradiated, GM-CSF transduced B16 melanoma cells protected against challenge with wild type melanoma cells. Based on these data, we are conducting a phase I trial in melanoma patients with advanced disease. Autologous melanoma cells are cultured and transduced with the GM-CSF gene. Subsequently, the cells are irradiated and used for three subcutaneous vaccinations at intervals of three weeks. At distant sites irradiated, nontransduced cells were injected to study any reaction against the original melanoma cells. Local erythema, swelling and itching are the symptoms at the vaccination sites. After the 2nd and 3rd vaccinations the area of redness around the vaccination increases. Lymphocytes infiltration is seen in biopsies of vaccination sites. No systemic toxicities were observed. CTL precursor frequencies are being analysed to measure any enhancement of immune response against autologous melanoma cells.

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NEWCASTLE DISEASE VIRUS INFECTED INTACT AUTOLOGOUS TUMOR CELL VACCINE FOR ADJUVANT ACTIVE SPECIFIC IMMUNOTHERAPY OF RESECTED COLORECTAL CARCINOMA

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An active specific immunization (ASI) procedure with two types of autologous tumor cell vaccines (ATV) is tested for adjuvant immunotherapy of resected colorectal carcinoma to provide preliminary information on local immunological skin responses, side effects and two year survival rates. For vaccine preparation, the tumor derived freshly isolated and cryopreserved cells were thawed, purified by Percoll density centrifugation and depleted of tumor infiltrating lymphocytes by immunomagnetic-beads. Atter inactivation by 200 Gy, the cells of this ATV were either infected by Newcastle Disease Virus (NDV) or they were admixed with *Bacillus Calmette Guérin* (BCG) organisms. Vaccination was performed in the arm beginning 6–8 weeks after operation, 3 times at two week intervals. Of 57 patients that received ASI, 48 were treated by virus infected autologous tumor cell vaccine (ATV-NDV) and 9 with the BCG admixed vaccine (ATV/BCG). The mean value of delayed hypersensitivity skin reactions (DTH) from ATV-NDV treated patients was 18 mm for the first vaccination and 26 and 29 mm for the following ones. While application of ATV-NDV was associated with only mild side effects, the ATV/BCG vaccine lead to long lasting ulcers and to more serious side-effects. The 2 year survival rates obtained with ATV-NDV was 97.9% while the survival rate with ATV/BCG was 66.7% ($P < 0.01$). The mean survival of 661 patients from a historical control was 73.8%.

Thus, the type and quality of the tumor vaccine for ASI-treatment appears to be important. The findings with ATV-NDV necessitate corroboration in a prospective randomised controlled study.

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ORGAN CONSERVATION IN BLADDER CANCER

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The use of radical radiotherapy for localised muscle-invasive bladder cancer carries the potential disadvantages compared to cystectomy of local tumour relapse, the formation of new tumours of the bladder urothelium, and the possibility of late radiation side effects on the bladder. The benefits of this approach include physiological micturition and the

preservation of sexual potency. Previous trials have not demonstrated inferior survival in patients with T3 bladder cancer managed by radical radiotherapy reserving salvage cystectomy for those who recur. However, these trials have not been large. We have recently re-analysed the Institute of Urology trial which registered 189 patients between 1965 and 1976 to determine long-term results. The 5 year survival in 91 patients randomised to radical radiotherapy was 27.9% (19.1–37.8%). Whereas for 98 patients randomised to radiotherapy + cystectomy, the 5 year survival was 39.8% (30.3–49.8%). Since this trial, there have been advances in both radiotherapy and surgery. Conformal radiotherapy offers the potential for reducing radiation side-effects. Additionally, we have completed a study of accelerated fractionation treated to doses between 57.6 and 64 Gy in 32 fractions over 26 days. Eighty five patients were treated in this study and of 70 who had check cystoscopy at 3–6 months, a pathological complete response was seen in 80% with 2 further patients demonstrating carcinoma *in situ* only. We conclude that a trial is required based on modern staging and treatment techniques to compare radiotherapy and cystectomy for localised muscle invasive bladder cancer.

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BLADDER CONSERVATION USING COMBINED EXTERNAL IRRADIATION AND IRIDIUM 192 IMPLANTATION

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For selected bladder carcinomas we consider iridium 192 implantation as the treatment of choice. Selection criteria that we use are:

1. Stages T1G3, T2 and T3a.
2. Solitary tumours.
3. Diameter not exceeding 5 cm.
4. No previous tumour elsewhere in the bladder.

From 1987 to 1994 63 patients have been treated. Results can be summarized as follows:

—Incidence of bladder recurrence at 5 years is 36%.

—About one half of the bladder relapses consist of secondary tumours.

—Isolated bladder relapses can be salvaged in the majority of the patients, many times with conservation of the bladder.

—Incidence of distant metastases at 5 years is 33%.

—In about one half of the cases distant metastases are combined with a bladder recurrence.

—Distant metastases are the major cause of death.

—Acute toxicity is limited and mainly related to the surgical procedure.

—Late toxicity is very limited.

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NEOADJUVANT CHEMOTHERAPY AND ITS ROLE IN BLADDER CONSERVATION

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Radiation therapy (RT) has been employed in the treatment of bladder cancer since the early 1900's, but its role in the curative management of bladder cancer is still controversial. Effects of RT are limited by cellular RT resistance, tolerance of normal tissues and the risk of systemic dissemination. Chemotherapy is active systemically, but few agents or combinations equal the antitumour activity of RT. The study of combined chemotherapy and RT is confounded by factors such as: specific tumour type, type of normal tissue involved, drugs selected, drug dosage and scheduling, RT dose, RT fractionation and overall treatment time and sequence of administration of each modality. Strategies for combining the two modalities include the use of drugs and RT concurrently, neoadjuvant chemotherapy or adjuvant chemotherapy. The first two approaches have been investigated in bladder cancer as CT delivered prior to or with RT also has the potential to improve local control beyond that achieved with RT alone. Current experience using concurrent and neoadjuvant chemotherapy in invasive bladder cancer will be reviewed with emphasis on the bladder conservation aspect of such management.

1003

CHEMOTHERAPY AND RADIATION THERAPY WITH SELECTIVE ORGAN-SPARING TREATMENT OF INVASIVE BLADDER CANCER

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Several recent (1–4) reports supply new overall survival data for transurethral surgery and chemoradiotherapy as good as any reported cystectomy series which supports its safe and selective use for clinical stage T2–T3a patients. One likely important key to the improved success is the early identification of incomplete responders to chemoradiotherapy by prompt re-cystoscopy. This allows for prompt cystectomy before local regrowth (and a second chance for metastases) occurs.

Series	Pts	5 yr Overall Survival	5 yr Survival with Bladder Preservation
Mass Gen Hosp (4)	53	48%	38%
U of Erlangen (1)	197	45%	36%
RTOG (4 yr data, 3)	42	54%	45%

This multimodality and selective (by initial response) approach may, for stage T2–T3a patients, now become a standard treatment option in the US. Patients with tumor-associated hydronephrosis are not good candidates for bladder sparing.

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RESECTION OF UNRESECTABLE HEPATIC METASTASES FROM COLORECTAL CANCER WITH NEOADJUVANT CHRONOTHERAPY

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In this study, we report a 6-year experience of the management of patients (pts) with hepatic metastases initially considered as unresectable and subsequently submitted to a resection following an efficient chemotherapy. From Apr. 1988 to Dec. 1994, 53 out of 330 pts (16%) with liver metastases initially considered unresectable underwent hepatic resection with a curative intent. All pts have been treated by intravenous chronomodulated chemotherapy combining 5 Fluorouracil, Folinic acid and Oxaliplatinum using an ambulatory programmable-in-time pump. Initial unresectability assessed by the same surgical team was related to large (n = 10), multinodular (n = 22), centrally located tumors (n = 8) or to the presence of extrahepatic disease (n = 13) with peritoneum (n = 6), epiploon (n = 3), lungs (n = 4). Pts received 3 to 29 courses of chemotherapy (mean = 9) for 2 to 21 months (mean = 7).

Results: An objective reduction of tumour size was observed following chemotherapy in all pts subsequently submitted to liver resection. A significant reduction of tumor markers was also demonstrated for CEA and CA 19-9. Mean follow up is currently 24 months for the whole group (range 4–68). Patient survival rate is 55% at 3 years similar to that of pts submitted to liver resection as a first treatment. Survival rate is higher in those 40 pts with technically unresectable lesions (large or multinodular or centrally-located) than in those 13 with extrahepatic disease, 58% vs 39% respectively.

Conclusion: A second-step resection may be achieved in some unresectable pts with the help of an efficient chemotherapy. The benefit in survival seems comparable to that obtained with liver resection as a first intent. This therapeutic strategy involves a multimodality approach including repeat hepatectomy and extrahepatic surgery.

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NOVEL METHOD FOR DETECTING LIVER METASTASES

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All patients undergoing curative surgery for colorectal cancer should have an accurate staging of their liver prior to treatment. However, conventional radiological methods which rely on the differences of density of neoplastic tissue and normal liver have limits in their resolution rarely detecting lesions less than 1 cm in diameter. An alternative approach is