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A NEW ERA IN RADIOTHERAPY
S.A. Leibel, G.J. Kutcher, R. Mohan, G.S Mageras, M.J. Zelefsky, C.C. Ling, Z. Fuks
Department of Radiation Oncology. Memorial Sloan-Kettering Cancer Center, NY 10021, U.S.A.
The search for new radiation treatment techniques to improve local tumor control continues to represent a major challenge in the management of localized human cancer. Before CT scans became available for planning radiation treatments, tumor target volumes and the boundaries between tumor and normal organs were poorly defined. To reduce the risk of local relapse, radiation target volumes classically included a wide "safety" margin of surrounding normal tissue. Restrictions imposed by normal tissue tolerance often limited the radiation doses administered to less than optimal levels. CT-assisted treatment planning improved the ability to define tumor target volumes for radiotherapy. However, the limitations imposed by computer technology have confined the use of CT information in conventional 2D treatment planning to one or at most a few CT slices at or near the central axis of the radiation beam, whereas treatment planning at levels beyond the central axis is not based on detailed CT anatomical information.

The introduction of three-dimensional conformal radiation therapy (3D-CRT) has heralded a new era in radiotherapy. Sophisticated computer-aided techniques are used to plan and deliver prescribed radiation doses, conforming the desired dose distribution to the entire 3D configuration of the tumor. The use of 3D imaging technology for treatment planning has reduced the risk of underdosing parts of the tumor. In addition, the effective exclusion of normal tissues from the volume carried to high radiation dose levels has allowed an escalation in tumor dose to levels beyond those feasible with conventional 2D radiotherapy. The aim is to improve local tumor control, as failure to control the primary tumors is associated with increased rates of both metastatic disease and mortality.

Because of the increasing complexity of 3D treatment planning and the need to evaluate large numbers of competing plans, a critical development for realizing the full potential of 3D-CRT is the introduction of computer-aided optimization of treatment planning. In addition, the extraordinary increase in the complexity of treatment delivery requires the introduction of immobilization devices, computer-controlled treatment delivery machines with automated multileaf collimators for beam shaping, on-line verification systems with a potential for feedback control loops to automatically correct set-up errors during treatment, and patient safety devices. Dosimetric methods are being developed to compensate for the inaccuracy in 3D treatment caused by organ motion. Many of these components have been implemented at the Memorial Sloan-Kettering Cancer Center.

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MINIMAL ACCESS SURGERY IN THORACIC ONCOLOGY
I. Besznyák
Department of Surgery, National Cancer Institute 1122 Budapest, Hungary
Minimal access surgery—primarily in the field of laparoscopic cholecystectomy—has rapidly become spread worldwide and is already one of the fundamental components of the surgical arsenal. Its dissemination in surgical oncology has been much slower, which especially refers to thoracic oncology.

Based on his own experience and literature data the author outlines the indications of the so-called video-assisted thoracic surgery (VATS), the advantages, disadvantages and results of the method in the field of intrathoracal tumours. Although the development of this method is hard to predict as yet, in the author's opinion in-depth and careful investigations are needed for the precise assessment of its value. The author suggests that VATS will probably not occupy such a role in thoracic surgical oncology as it already fulfils in general surgery.

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LAPAROSCOPY AND LAPAROSCOPIC ULTRASONOGRAPHY IN THE ASSESSMENT OF UPPER GASTROINTESTINAL CANCER
M.A. Cuesta, P.J. Borgstein, S. Meijer
Imaging of abdominal organs has greatly improved with the introduction of ultrasonography and computed tomography. Despite the advantages these techniques offer, the surgeon dealing with gastrointestinal malignancies is often faced with a wide discrepancy between pre and peroperative staging. Laparoscopy performed before a planned laparotomy will exclude these metastases. As a result of this more accurate staging an unnecessary laparotomy may be avoided. For less obvious hepatic and pancreatic lesions, it is now possible to perform a laparoscopic ultrasonography introducing transducers of either 5 or 7.5 Megahertzs through cannulas of 15 or 10 mm. This technique has been used in the preoperative assessment of 93 patients with "curable" gastrointestinal tumors.

Results: Using laparoscopy and laparoscopic ultrasonography we obtained important information which included a change in the planned preoperative surgical treatment in 44 of the 93 patients (48%). This information was obtained by: (a) visualisation of liver or peritoneal metastases by laparoscopy in 27 of the 44 patients, (b) the use of ultrasonogaphy for the study of less obvious liver lesions in another seven, and (c) by the combination of data obtained by visualisation and ultrasonography in another ten patients.

Conclusion: Laparoscopy and laparoscopic ultrasonography is a reliable technique in the assessment of patients with upper gastro-intestinal cancer. The combination of visualisation of the whole abdominal cavity and ultrasonography for less obvious liver lesions included a change in the planned preoperative surgical treatment in almost half of he patients. This change implies avoidance of an unnecessary laparotomy permitting a more adequate and less invasive palliative treatment.

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MINIMAL ACCESS SURGERY IN ONCOLOGY
D. Dargent
The gynaecologists were the first to use minimal access surgery. This kind of surgery was, during years, limited to benign conditions. We were the first, in December 86, to use it in the management of cancer while performing a laparoscopic assessment of the pelvic lymph nodes for a woman affected by an infiltrative cervical cancer. We have now an experience of more than 400 cases. Pretherapeutic assessment of the retroperitoneal lymph nodes remains the best of the good indications of laparoscopy in the field of gynecologic oncology.

Laparoscopy enables us to assess either the pelvic or the aortic retroperitoneal lymph nodes, using either the direct retroperitoneal approach or the transumbilical transperitoneal one. Many data clearly established that such an assessment is as reliable as the classical surgical assessment, while avoiding all the drawbacks of the open surgery. Knowledge of the lymphnodal status enables us to take at the good time (before radical surgery or radiotherapy) the good decision: in the good cases (no risk factor, no lymphnodal involvement) the treatment can be exclusively surgical and the surgery can be performed using the vaginal route: in the bad cases (risk factors and/or lymphnodal involvement) the treatment has to be multimodal using combinations (chemotherapy, radiotherapy, surgery) whose choice depends on the extend of the disease as it has been defined by the laparoscopy.

The chosen example is representative regarding the role of minimal access surgery in oncology. The laparoscopic debulking surgery has been advocated by some surgeons in thoracic, abdominal and pelvic surgery. This practice appears as hazardous except, maybe, in the frame of neoadjuvant chemotherapy protocols during which a laparoscopic debulking of the residual masses can be undertaken. However the actual place of laparoscopic surgery is not here. Laparoscopy's first role is selecting the cases for an appropriate treatment.

Minimal access surgery is a cost effective tool to use in order to improve the cost effectiveness of the treatment of pelvic, abdominal and thoracic cancer.

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MINIMAL ACCESS SURGERY IN UROLOGIC-ONCOLOGY—NEW AND APPROVED TECHNIQUES
P. Alken
Department of Urology, Klinikum Mannheim, University of Heidelberg, 68167 Mannheim, Germany
Minimal access surgery in urologic oncology has along tradition: organ preserving transurethral resection or laser ablation of bladder tumors and transurethral resection of prostate carcinoma eventually combined with laser therapy. Endoscopic techniques are used to treat urothelial tumors of the upper urinary tract. To these approved techniques minimal invasive laparoscopic lymphnode extirpation in cases of prostatic and bladder cancer have been added just as laparoscopic nephrectomy in cases of small renal cell carcinomas or transitional cell carcinomas of the upper urinary tract. New techniques like laser irradiation of the whole bladder in case of transitional cell carcinomas have been perfected. Even

less invasive techniques like focused ultrasound therapy for renal tumors, bladder tumors and prostatic carcinomas are rapidly evolving.

These new techniques in urologic oncology and other noninvasive techniques that have been developed in the last years have a tremendous impact on urology and will eventually change this medical speciality.

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REJECTION OF CYTOKINE GENE TRANSFECTED MOUSE TUMORS: THERAPEUTICAL IMPLICATIONS

Th. Blankenstein
Max-Delbrück-Center for Molecular Medicine, 13122 Berlin, Germany

Cytokines provided locally at the tumor site may initiate an effective anti-tumor immune response which leads to rejection of a tumor which otherwise grows progressively. Experimentally, this can be tested by gene transfer into cultured tumor cells followed by the analysis of the tumorigenicity of such genetically engineered cells. This approach allows to analyse the function of a given cytokine *in vivo* and to elucidate the therapeutical value of genetically engineered tumor cells as vaccines. Our experience includes experiments with about ten cytokines and the results can be summarized as follows: (1) some cytokines possess anti-tumor activity in this system, others do not; (2) a local and continuous cytokine supply seems to be essential for tumor rejection; (3) the tumor cell derived cytokines act in a dose-dependent manner and in the absence of systemic toxicity; (4) the immunological effector mechanisms induced by different cytokines are partly cytokine-specific, partly redundant (and usually involve T cell dependent and independent mechanisms; (5) tumor rejection and mechanism thereof may be different with different tumor cell lines transfected with the same cytokine gene.

Cytokine gene modified tumor cells as vaccines are currently tested in first clinical trials. However, critical parameters such as vaccine potency of cytokine gene transfected tumor cells, optimal level of cytokine expression, reasons for varying vaccine effects in different tumor models, influence of irradiation of vaccine cells on their efficacy and attempts to improve vaccine efficacy (e.g. by coexpression of cytokines and T cell costimulatory molecules as B7) have to be further addressed in experimental tumor models.

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PRECLINICAL MODEL FOR GENE THERAPY: ROLE OF THE HOST

M. P. Colombo[1], G. Forni[2], A. Stoppacciaro[3]
[1]Istituto Nazionale Tumori, Milan
[2]CNR Centre of Immunogenetics and Oncology, Turin
[3]Department of Biopathology, University of Rome, Italy

Three different experimental systems based on cytokine gene transduction can provide evidence that (I) systemic immunity does not always follow tumor regression; (II) a cytokine combination that efficiently induces systemic immunity does not induce CTL activity and does not exert therapeutic effects; (III) a different cytokine combination induces both CTL and protection in Winn assay without inhibiting tumor take and outgrowth.

(I) C-26/G-CSF regression is coupled with infiltration of leukocytes releasing secondary cytokines and depends on $CD8^+$T cells, regressor mice however, remain susceptible to a challenge with C-26 cells.

(II) TSA/IL-4 more efficiently than TSA/IL-2 induce protection against a challenge with live TSA cells. To transfer IL-4 mediated systemic immunity, both lymphocytes and serum from immune mice are needed. TSA/IL-4 cells when used as vaccine to cure TSA bearing mice were without effect, whereas TSA/IL-2 were moderately effective.

(III) C-26/IL-12 cells showed delayed tumor onset that was NK dependent. Immunocytochemical characterisation of leukocytes infiltrating C-26/IL-12 tumors showed few infiltrating T cells in non-depleted mice but abundant infiltration by $CD8^+$ T cells in tumors from mice depleted of $CD4^+$ T cells and CD4 depletion allowed tumor regression in about 30% of mice. This is not due to a CD4-mediated suppression since mice primed with C-26/IL-12 cells possessed lytic lymphocytes and $CD8^+$ T cell which mediated protection against C-26/IL-12 in a Winn assay.

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GENE AND PEPTIDE THERAPY OF TUMOR METASTASES IN MURINE MODELS

L. Eisenbach, O. Mandelboim, A. Porgador, D. Plaksin, M. Fridkin

Highly metastatic clones of malignant murine tumors are characterized by low immunogenicity and reduced MHC Class I expression. Metastatic lesions of human tumors are similarly impaired in HLA expression. Gene modification using plasmid and retroviral vectors carrying cDNAs for MHC Class I, γIFN, IL-2 or IL-6 increases immunogenicity of lung carcinoma (3LL) and melanoma (B16) cells. Vaccines based on irradiated gene modified cells and their combinations were used in diseased animals and achieved certain cure rates. CTL recognize peptide sequences of defined length presented in the groove of MHC class I. TAA peptides presented by H-$2K^b$ were purified from 3LL carcinoma and proven to be mutants of a peptide from the gap junction protein connexin 37 and normal peptides of an aberrantly expressed β globin gene. Structural aspects and therapeutic efficacy of peptide vaccines will be discussed.

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COMBINATION GENE THERAPIES FOR THE TREATMENT OF MALIGNANT MELANOMA *IN VIVO*

R. Vilea, Heung Chong, R. M. Diaz[1], I. Hart[1], S. Castleden, A. Tuszynski
Imperial Cancer Research Fund Laboratory of Cancer Gene Therapy
[1]Richard Dimbleby Cancer Research Laboratory, Rayne Institute, St Thomas Hospital, Lambeth Palace Road, London SE1 7EH, U.K.

For *in vivo* gene delivery, we have used the murine tyrosinase promoter to restrict expression of genes to melanoma cells. These genes are aimed either at enhancing the immunogenicity of tumour cells (cytokines and members of the B7 family of genes) or at killing tumour cells directly (HSV*tk*). Recently, we have observed, and characterised, the generation of an anti tumour immune response following *in vivo* killing of established tumour deposits with HSV*tk*, suggesting that both approaches can be combined to improve the efficacy of *in vivo* gene therapy. Data will be presented on the development of novel double expression vectors in which the HSV*tk* gene is co-expressed with a series of immunomodulatory genes to augment this anti-tumour immunity. Our data demonstrate that protocols aimed at enhancing tumour cell immunogenicity *in vivo* are most likely to be successful by the co-expression of more than just a single therapeutic gene within the tumour cells.

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MUCINS AS MARKERS OF CELL DIFFERENTIATION AND NEOPLASTIC TRANSFORMATION

F. X. Real
Institut Municipal d'Investigació Mèdica, 08003-Barcelona, Spain

Mucins are synthesized by glandular epithelia and are the major components of mucus. The cloning of cDNAs encoding human apomucins facilitates the analysis of their structural complexity and heterogeneity. Until now, 8 independent genes (MUC1-MUC7) have been identified which encode Ser/Thr-rich proteins with repetitive domains.

Using these cDNAs, as well as anti-apomucin antibodies raised against a variety of immunogens (i.e. native and deglycosylated mucins, synthetic peptides, fusion proteins), a considerable amount of information has been obtained regarding the pattern of expression of each gene in tissues. Each mucin gene has a distinct normal tissue distribution. Thus, MUC1 and MUC5B are expressed in a wide variety of normal epithelia, whereas MUC2 is mainly expressed in the intestine, MUC5AC in the respiratory tract and in the stomach, and MUC6 in the antrum. Multiple mucin genes can be expressed in a given tissue and at the single cell level, although in certain tissues a high degree of specialization is observed: in the stomach MUC5AC is present in the superficial epithelium whereas MUC6 is present in antral glands. In the stomach, apomucin expression correlates with Lewis antigen expression, although it is not clear whether the primary amino acid sequence of mucins contains instructive signals for glycosylation.

Altered expression of mucin genes in pathologic states has now been demonstrated, in particular in cancer tissues. In colonic and gastric cancers, loss of expression of MUC2 and MUC5AC takes place, respectively. In contrast, MUC2, MUC4 and MUC5AC are aberrantly expressed in pancreas cancer tissues. In benign proliferative lesions of the colon and the pancreas, changes in the expression of mucin genes have also been demonstrated. Preliminary data suggest that the pattern of mucin gene expression in cancer tissues may be related to the biological behaviour, although more work is necessary in this area.