(benefit 2%, 31 trials*, 5 269 pts*) whereas the absolute benefit at 5 years was 8% for **concomitant** CT (26 trials*, 3 727 pts*). Exclusion of small trials (<80 pts) or old trials (<1980) did not modified the results. The effect of CT was not significantly different according to the rumor stage and the tumor site (larynx, hypopharynx, oropharynx, oral cavity).

Conclusion: CT led to a small but significant improvement in survival. The observed benefit depended on CT timing and was the highest with CT concomitant to radiotherapy.

**2 trials with 3-arms: 1 neoadjuvant CT, 1 concomitant CT & 1 control*

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Is there still a place for chemotherapy in patients with locally advanced head & neck squamous cell carcinoma?

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Combination chemotherapy such as cisplatin-5fluorouracil is effective in inducing a high rate of tumor responses in patients (pts) with advanced Head & Neck (H&N) cancer. In the recent decades, chemotherapy was included in organ preservation strategies or in combination with radiotherapy to improve the local control in pts with non-resectable diseases. Chemotherapy was also proposed in neoadjuvant and adjuvant settings with the aim of reducing the risk of distant metastases and improving survival. Recent results of an individual-patient based meta-analysis including about 11,000 pts and 63 controlled-trials (from 1965 to 1993) were striking. This study showed a small but significant overall reduction of the risk of death of 10±2% translating in an absolute survival benefit of only 4% at 5 years in pts receiving chemotherapy ($p<0.0001$). The benefit was observed when chemotherapy was concomitantly associated with radiotherapy (reduction of risk of death 19±3%, absolute benefit at 5-year 8%). Conversely, no benefit was observed in pts receiving chemotherapy for larynx preservation or treated in adjuvant settings. Overall, neoadjuvant chemotherapy did not significantly improve survival in pts with H&N cancer. However, a trend toward an increase of survival was observed in patients receiving cisplatin-5fluorouracil regimens. Preliminary results focused mainly on survival. More data generated from this meta-analysis on disease free survival, occurrence of second primary, and non-cancer related deaths (due to concomitant tobacco related diseases) will be required before drawing a definitive conclusion on the impact of chemotherapy in H&N cancer. However, this meta-analysis emphasizes the small benefit of current routine chemotherapies on the outcome of pts and encourage prospective clinical trials with original drugs and strategies. Th7is shows that more effective anticancer agents are urgently warranted to improve the outcome of patients with H&N cancer. From those investigational agents we will discuss recent data obtained with taxanes, antifolate analogues, and recent biological programs targeting selectively p53 in H&N cancer cells.

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Radiotherapy in head and neck cancer

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The management of Head and Neck carcinomas is nowadays based on multi-disciplinary approaches, especially in patients with bulky tumors. Their configuration varies according to host- and tumor-related factors and are aimed at improving both cure rates and quality of life. Radiation therapy constitutes one of the pillars of this therapeutic management, both for early and locally advanced disease.

In patients with *early disease*, radiotherapy is indeed as safe as surgery as regards local control and survival rates and, as a conservative treatment, it also offers a quality of life surgery is is not always able to offer, especially when radical procedures can not be avoided. The role of new techniques of high conformality radiotherapy and brachytherapy is probably determinant to reduce the incidence of severe late toxicity and offer major opportunities of radiotherapy treatments in patients with second malignancies. In stage III disease the choice in favour of radiotherapy is also guided by health economics considerations.

Larynx preservation trials have demonstrated that neo-adjuvant chemotherapy followed, in responders, by radiation therapy is as safe as strategies based on upfront surgery. Functional larynx can be kept in 30–50% of the cases treated with organ preservation programmes and a significant reduction in distant metastases is usually reported for this type of sequential application of radio-chemotherapy.

Altered fractionation has been extensively investigated over the last two decades, using regimes ranging from strong acceleration to true hyperfractionation. Most of these altered dose schedules have yielded significant improvements in loco-regional control, generally of 10 to 15% compared to those observed after conventional fractionation, with interesting trends as regards survival rates. Severe late toxicity can be observed when strong acceleration regimes are used, especially when the inter-fraction time interval is <6 hours.

Concurrent radio-chemotherapy is now considered by many centers, and not by all, as the standard approach for locally advanced disease since it has been shown to affect significantly not only the loco-regional control but also the disease-free survival, as demonstrated by recent recta-analyses. Long-term toxicity still remains to document more extensively, especially as regards dose-schedules combining cytostatic drugs and altered fractionation.

Finally the increasing role of *re-treatments* with radiotherapy for both second primaries and recurrences is currently under investigation: encouraging results are now documented both as regards tumor long-term control and functional outcome.

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Intermittent CO2 or KTP laser surgery in combined treatment of head and neck cancer

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KTP (Potassium (K+)-Titanyl-Phophate)-laser surgery in tumors of the oral cavity, the pharynx and hypopharynx allows an extended resection offering all the advantages of CO2 laser surgery with a more flexible handling during cutting with direct fiber contact to the tissue and coagulation without contact. A combined chemo-radiotherapy is started in our patients after panendoscopy with tatooing of the tumor margins and a complete staging. Mitomycin, 5 Fluoruracil and hyperfractionated radiation is performed up to 30 Gy in a first cycle. Then the CO2 or KTP-laser surgery is done in the old margins with preferation of the CO2 in the hypopharynx and the KTP laser in the oral cavity and oropharynx. Intermittend neck dissection follows this surgical procedure after a time interval of 10 to 14 days. Then the second cycle of chemoradiation completes the treatment. Bioptic controls follow these protocol after six and twelve weeks. In 20% of the patients treated no vital tumor cells could be found in the laser resected specimen any more. All the other resections were performed within healthy tissue borderlines. Tumor control in all cases including T3 stages could be performed by means of this technique with the advantage of no plastic reconstructive covering of the resection site and better functional outcome, especially for swallowing and no necessity for a tracheostomy.

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Helper-free generation of new oncotropic and oncotoxic vectors derived from MVM autonomous parvovirus

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Autonomous parvoviruses have several features that attracted attention to their potential use as vectors for cancer gene therapy. They have a strong oncotropism through targeting viral-gene and transgene expression to tumor cells, and their non-structural NS1 is responsible for their oncotoxicity. In addition, they are non pathogenic in adult animals, do not integrate into the host genome, are resistant to extreme pH and temperature conditions, are not inactivated by human complement, and, finally, seem to be associated with no or low immunogenicity. Their production was however difficult, their spontaneous titer after transfection was low (10.E + 3–10.E + 4 infectious units [iu]/ml) and stocks were contaminated by wild-type MVM that is generated through recombination between cotransfected vector and helper DNA sequences. We set up a method of concentration and purification, that allows to reach titers of 10.E + 9 iu/ml. We also generated a packaging cell line by integrating helper sequences allowing to reach spontaneous recMVM titers of up to 10. E + 7 iu per ml after three to four rounds of infection, with no concentration procedure. Although undetectable in the

initial stocks obtained by transfection, wild-type MVM still appeared during serial infections. To avoid the recombination events between vector DNA and helper sequences, responsible for these contamination with wild type virus, we removed all homology between helper and vector sequences. We based these sequence modifications on the study and sequencing of spontaneous extremely small defective virus, and on the degeneration of DNA sequences with no alteration of amino acid sequences. We thus setup a rapid and simple method that should be easily upscalable, for the production and purification of high titer vectors suitable for in vivo testing of the therapeutic efficiency of recMVM vectors against tumors. In a strategy of immunotherapy of cancer, in vitro and preliminary in vivo results have been obtained by using them to transfer the IL-2 cDNA.

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Genetically modified melanoma cells as cancer vaccines

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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; (iii) modification of other host tissues, e.g., by transfer of 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.

In the case of skin cancer, most gene therapy trials are conducted in patients with disseminated melanoma using tumor cells whose immunogenicity 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 elimination of preexisting cancer cell deposits. Based on these encouraging results, we have used IL-2-based, autologous human melanoma vaccines in a phase I trial in patients with stage IV melanoma. The vaccines' recipients did not show any overt signs of systemic toxicity and some of them developed positive delayed-type hypersensitivity (DTH) reactions to autologous melanoma cells after 2-3 vaccinations. 3/15 patients experienced a prolonged stabilization of their disease but, ultimately, all vaccine recipients succumbed to their disease.

Recent evidence indicates that cytokine-based cancer vaccines exert their protective effect in experimental animals not by a direct stimulatory effect on T cells but rather by the initiation of inflammatory events leading to the presentation of vaccine fragments by host-derived antigen-presenting cells. Assuming that this cross-priming phenomenon would also be operative in cytokine-based human cancer cell vaccines, we have recently begun to test the safety and tolerability as well as the immunological efficacy of IL-2-transfected allogeneic melanoma cells in patients with stage IV disease. Their ultimate value for therapeutic purposes remains to be determined, in a controlled fashion, in patients with less advanced melanoma.

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Anti-tumoral effects following Ad-mediated delivery of angiogenesis inhibitors

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Angiostatin is an inhibitor of angiogenesis secreted by tumors, driving metastasis into a dormant state. We have constructed an E1-deleted adenovirus that expresses the N-terminal fragment (amino-acids 1–333) of human plasminogen, including its preactivation peptide and kringles 1 to 3 (AdK3). AdK3-infected endothelial cells showed a marked, dose dependent arrest in proliferation in vitro. A single intratumoral injection of AdK3 was shown to dramatically inhibit primary tumor growth in two preestablished xenograft murine models. This inhibitory effect on tumor growth was tightly correlated with a markedly decreased vascularization within, and at the vicinity of the tumors, and a 10-fold increase in tumor cell apoptosis.

We have also assessed in mice the antitumoral effects that specifically follow the administration of AdmATF, an E1-deleted adenovirus that expresses a secretable antagonist of urokinase (uPA) which binds to its cell surface receptor (uPAR). Using different murine tumor models, we have shown that the intratumoral expression of ATF inhibits primary tumor growth and interferes with tumor cell dissemination, effects correlated with a marked inhibition of angiogenesis within and at the vicinity of the tumor mass. Finally, we have also shown that a systemic administration of the virus could protect against subsequent tumor challenge.

Angiostatic therapy using recombinant adenoviruses is plausible and efficient; nevertheless, maximal clinical benefits will require improved vectors able to sustain transgene expression.

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Modulation of antigen presenting cells-tumor cells interactions

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One of the most important finding in immunology is the discovery of peptides as the entities that bind MHC to signal self identity on the cell surface.

For the majority of neoplasias the identity of their tumor antigens remains to be determined and even for melanoma it is clear that we know only a minority of all possible antigens that could be expressed by this tumor.

Therefore, a tumor vaccine should face two different situations depending whether the antigen is known or not.

In case of known antigen the current strategy consist of loading DC with the peptide or protein or of transfecting DC with the gene coding for the antigen. Our approach is to deliver the tumor antigen directly into APC through an oral vaccination performed with attenuated bacterial vectors, such to avoiding any ex-vivo manipulation of DC. A live attenuated aroA auxotrophic mutant of Salmonella typhimurium has been used as carrier for the pCMVb vector that contains b-gal gene under the control of the immediate early promoter of Cytomegalovirus (CMV). After three courses, at 15-days interval, mice developed a specific b-gal CTL response as well as antibodies response. Mice vaccinated with the Salmonella harbouring pCMVb, but not with plasmid less carrier, showed resistance to a challenge with a highly aggressive murine fibrosarcoma transduced with the b-gal gene that behaves operationally as a tumor-associated antigen. These experiments show that Salmonella-based DNA immunization allows to specifically target antigen expression in vivo to APC, and results in induction of efficient MHC-I and II-restricted anti-tumor immune responses.

Tumor cells likely present the entire repertoire of tumor associate antigen, either known or unknown, of a certain neoplasm. To create a cellular vaccine that should favour direct, in vivo, tumor-DC interactions we transduced BALB/c-derived C-26 colon carcinoma cells with granulocyte-macrophage colony stimulating factor (GM-CSF) and CD40 ligand (CD40L) genes. DC infiltrating tumors producing GM-CSF and CD40L capture cellular antigens, likely through uptake of apoptotic bodies, and mature in situ to a stage suitable for antigen presentation to T cells. Thus, tumor cell-based vaccines engineered to favour the interaction with host DC can be considered.

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