vaccine delivery at any dose. Two patients vaccinated with the lowest vaccine dose showed stable disease during more than a year. The patients with the intermediate and highest vaccine doses are still monitored with respect to clinical response. The results of CTL and T helper response assays against the individual vaccine components will be presented.

1302

Immune responses to peptide-based cancer vaccines

E. Jäger, A. Knuth, II. *Med. izinische Klinik, Krankenhaus Nordwest, Frankfurt, Germany*

Three classes of antigens recognized by cytotoxic T lymphocytes (CTL) are defined in melanoma and some other tumors: 1. Cancer-testis (CT) antigens (MAGE, BAGE, GAGE), expressed in tumors and testis, 2. Differentiation antigens (Melan A/MART-1, tyrosinase gp100/Pmel17, gp75), expressed in melanoma and melanocytes 3. Antigens defined by mutations (CDK-2/R24C, MUM-1, β-catenin). Target structures for CTL recognition are peptides of 9–10 aminoacids length. These peptides bind to MHC class I molecules and are presented at the tumor cell surface. Synthetic peptides can generate specific CTL in vitro that effectively lyse tumor cells expressing the corresponding antigen. Clinical trials using MAGE-1 and MAGE-3 peptides for immunization in HLA-A1+ patients with MAGE-expressing tumors showed objective responses (CR/PR) in some melanoma patients. We immunized HLA-A2+ melanoma patients with peptides derived from Melan A/MART-1, tyrosinase, and gp100/Pmel17. Delayed-type-hypersensitivity reactions (DTH) and peptide-specific CTL responses as well as objective tumor regressions were observed in 3/12 patients. Subsequently, the effects of systemic GM-CSF on immune reactions to peptide vaccines were assessed. Enhanced DTH reactions were observed with infiltrates of CD4+ and CD8+ T lymphocytes, CD1a+ Langerhans cells, and a strong expression of IL-2 and γIFN, suggesting the activation of CD4+ Th1 and CD8+ CTL. Objective tumor regressions were documented in 5/16 patients. The identification of further tumor associated antigens recognized by CTL and the use of adjuvants to enhance their immunogenicity will open broad perspectives for the development of polyvalent cancer vaccines to control or inhibit tumor growth in vivo and to prevent tumor escape of antigen-loss variants.

1303

Active immunization of melanoma patients with IL-2- OR IL-4-transduced allogeneic melanoma cells

F. Arienti, F. Belli, A. Mazzocchi, F. Gallino, F. Napolitano, C. Melani, M.P. Colombo, L. Rivoltini, M. Maio[1], G. Parmiani. *Istituto Nazionale Tumori, Milan; [1]Centro di Riferimento Oncologico, Aviano, Italy*

The aim of these clinical studies was to immunize stage IV melanoma patients with HLA-A2-compatible, immunogeneic human melanoma lines genetically modified to release IL-2 or IL-4 in order to elicit or increase a T cell-mediated anti-melanoma response which may affect distant melanoma lesions. These lines were characterized for transgene expression and for the presence of immunological relevant molecules before in vivo vaccination. Twelve patients were treated with IL-2 releasing line while 10 patients were treated with IL-4 gene transduced melanoma cells. The side effects of the treatment were locally mild and systemically absent. All patients were assessable for clinical response and received at least 3 vaccine administrations. Three and 2 mixed responses were clinically observed in group of patients treated with IL-2 and IL-4 transduced melanoma lines, respectively. To evaluate specific immune response, limiting dilution experiments and mixed tumor-lymphocytes cultures were performed using different HLA-A2 compatible melanoma lines, autologous line when available and peptides obtained from known melanoma antigens. This immunological monitoring was performed on peripheral blood lymphocytes obtained from each patient before and after vaccination. An increased frequency of lytic and specific lymphocyte precursors was observed in some cases. Histological and immunohistochemical analyses of biopsies obtained before and after vaccination from tumor nodules and sites of vaccine injection are in progress and will be presented.

1304

Mutant ras peptide vaccines

Gustav Gaudernack, Marianne K. Gjertsen. *Section for Immunotherapy, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway*

ras proto-oncogenes activated by point mutations within codon 12, 13 or 61 are frequently found in human tumours. Since mutant p21 ras molecules encoded by these oncogenes are specific for cancer cells, mutant p21 ras or ras-derived peptides are attractive candidates for a cancer vaccine.

We have studied T cell responses to mutant p21 ras in both healthy volunteers and in cancer patients for the purpose of developing therapeutic and prophylactic cancer vaccines. From these studies we were able to define optimal peptides for T cell stimulation that contain overlapping epitopes capable of stimulating both CD4 and CD8 T cell responses. Studies with a large number of T cell clones derived from different donors, including cancer patients demonstrated that binding of ras derived peptides to HLA class II molecules is promiscuous. Together these results indicated that immunotherapy targeted against neoplastic cells carrying ras mutations is possible.

We have now completed a pilot phase I/II clinical study, and in some of the patients we were able to elicit an immune response by vaccination with autologous ras-peptide loaded antigen presenting cells. The responding cells were both of the CD4 Th1 and CD8 phenotype and were able to kill autologous tumor cells as well as other tumor cells carrying the same ras mutation (12Gly→Val) These results indicate that ras peptide vaccination may result in the generation of a potentially beneficial immune response in cancer patients. We are presently conducting several new clinical protocols based on ras peptide vaccination of patients with different forms of cancer, either by direct intradermal injection of peptides and using recombinant human GM-CSF as adjuvant, or by intralymphatic injection of peptide pulsed dendritic cells.

1305

Oncogene activation as a prognostic marker in head and neck cancer

E. Schuuring, H. van Damme, E. Schuuring-Scholtes, V. van Buuren, M. Verheijen, J.-W. Vaandrager, R. Takes, R. Baatenburg-de Jong, P. Kluin, J. van Krieken. *Departments of Pathology and ENT, Leiden University Medical Center, Leiden, The Netherlands*

In cancer numerous chromosomal abnormalities (e.g. deletions, amplifications and translocations) are associated with the (in-)activation of tumorsuppressor and oncogenes. The detection of genetic aberrations has clinical relevance in classifying tumors or as prognostic factor. For instance, carcinomas of the head/neck region (HNSCC) frequently show amplification of EGFR and the chromosome 11q13 region, point-mutations in p53 and loss of $p16^{INK4}$. We have focused on DNA amplification of the chromosome 11q13 region that was found in 36% of HNSCC. (1) Comparison of HNSCC-patients with and without DNA amplification revealed that amplification is correlated with poor prognosis. (2) We have identified two genes *cyclin D1* and *EMS1/cortactin* that are overexpressed due to DNA amplification. For a proper understanding of the biological behavior of tumors with 11q13 amplification, we introduced these genes into cells *in vitro* to study the effect on (cell) biological properties. (3) With antibodies against the gene products, we have developed an immunohistochemical screening method that is evaluated by comparisons to southern blot and interphase FISH data. Reliable and easier detection methods will enable us to screen large series of HNSCC, to refine the classification of relevant tumors, and ultimately to design more rational therapies. All these aspects will be discussed.

1306

Predicting response in head and neck cancer: The search for the Holy Grail

Philippe A. Coucke. *Department of Radiation-Oncology, Laboratory of Radiation Biology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland*

A standard radiation therapy schedule is not the optimal treatment for all patients presenting with head and neck (H&N) cancer. To modify the treatment parameters, predictive tests are required allowing to discriminate subpopulations of patients for whom the modification of treatment parameters could be beneficial. We intend to review the current status on predictive tests especially aimed at determining proliferation status (PS),

oxygenation status (OS) and intrinsic radiosensitivity (IRS), both for tumor tissue (IRS_T) and for normal tissues (IRS_N), in H&N cancer. To date, none of these tumor and host related factors does unambiguously predict outcome if assessed prior to the initiation of radiation treatment. To do so, many more patients, with well defined patient and treatment characteristics, will have to be included in prospective studies to validate the predictive power of those tests. Moreover, resistance to irradiation being defined by a variety of factors, efforts should be devoted to the simultaneous assessment of different "predictive" parameters, independently affecting locoregional control in H&N cancer. New techniques based on molecular biology should be intensively investigated. For those who think they found the Holy Grail, large scale prospective studies, quality control and determination of variability are prerequisites that have not yet been fulfilled.

1307

Surgical treatment of regional neck nodes in the head and neck squamous carcinoma

A.S. Jones. *Department of Otolaryngology/Head and Neck Surgery, University of Liverpool, UCD Duncan Building, Liverpool L69 3GA, UK*

Purpose: Most patients with head end neck malignancy die of uncontrolled disease in the neck and 30% of patients with head and neck cancer have a neck node metastases at presentation of their primary tumour. Control of neck node disease is, perhaps, the most important aspect in the control of head and neck cancer.

Methods: The University of Liverpool head and neck data base contains some 6,000 patients and has been used to provide data for this presentation. The various methods of treating neck node metastases will be described with special reference to the surgical management of neck node metastases. Statistical analysis of the results was calculated using the SAS software.

Results: 919 patients with carcinoma of the larynx, hypopharynx, oral cavity and oropharynx are studied. Of 955 laryngeal cancers, 202 had neck node metastases at presentation. For laryngeal cancer 50% of patients were treated by radical neck dissection with a 46% 5 year survival. The corresponding figures for the other sites were, for hypopharyngeal cancer, 33% with a 42% 5 year survival, for oral cavity cancer 41% 5 year survival, for oropharyngeal cancer, 21% with a 55% 5 year survival. Full statistical details will be discussed at the presentation, including the lymph node groups and levels that are of significance in head and neck cancer. Adjuvant radical radiotherapy reduces the risk of recurrence of neck disease in a patient who has had a radical neck dissection. For the patient who has had radical neck treatment a recurrence has a very poor survival, in the region of 10%, even with salvage treatment.

Conclusion: Radical neck dissection, in spite of having been described over 100 years ago, still offers the best treatment in oncological terms for neck node metastases from head and neck cancer. Its recent combination, with post operative radical radiotherapy, has improved the results still further. In the case of head and neck malignancy, treatment of regional lymph nodes is a very worthwhile exercise offering good cure rates.

1308

A review of the fractionation trials in head and neck cancer

Søren M. Bentzen. *Department of Experimental Clinical Oncology, University Hospital of Aarhus, Denmark*

Radiotherapy (RT) is an efficient, conservative treatment in many cases of head and neck carcinoma. Further optimization of this modality may come from advances in RT dose planning and delivery, from a better understanding of the tumor biology of the disease, and/or from advances in radiation biology of normal tissues and tumors. In this lecture we look at the last of these areas. In the 1980's, several large clinical trials were initiated in Europe and the US, including trials by the EORTC and the RTOG and large trials based mainly in the UK (CHART) and in Denmark (DAHANCA 6&7). Two prototypical modifications of RT were tested: accelerated fractionation (AF) in which the overall treatment time is shortened relative to that of conventional regimens, and hyperfractionation in which the dose per fraction is reduced below 1.8 Gy. Early results from these trials are now emerging and these will be discussed in the lecture. Both AF and HF have resulted in significant therapeutic gains. However, the limits on how far AF and HF may be pushed are also becoming clear. Open questions at this time will be identified and new strategies for biologically based optimization of RT in head and neck cancer are discussed.

1309

Chemotherapy in locally advanced head and neck squamous cell carcinoma (HNSCC): Preliminary results of a meta-analysis using individual patient data of randomized trials

J.P. Pignon, L. Designé, J. Bourhis, C. Domenge, S. Guérin, M. Luboinski. *On behalf of the Meta-Analysis of Chemotherapy in Head and Neck Cancer (MACH-NC) Collaborative Group; Institut Gustave-Roussy, France*

Purpose: In spite of more than 70 randomized trials during the past 30 years, it remains controversial whether chemotherapy (CT) can improve survival for HNSCC. To test the hypothesis that CT improves moderately survival, a meta-analysis has been performed.

Methods: Randomized trials (1965–1993) comparing loco-regional treatment to same treatment plus CT were eligible. Updated individual data were collected.

Results: 76 trials (11 650 patients) were identified. In January 97, data from 51 trials (74% of the patients) were available for analysis. Among these trials, the timing of CT was: neoadjuvant in 53% of patients, concomitant in 35% and adjuvant in 12%. The type of CT was: mono-CT in 44%, poly-CT with platin in 44% and poly-CT without platin in 12%. Data on age, sex, site of tumor (oral cavity 32%, oropharynx 30%, hypopharynx 17%, larynx 15%) were available for 99% of the patients. This proportion was 98% for stages (stage III 34%, stage IV 56%) and 51% for performance status. *Overall* 5-years survival was 30%.

Conclusion: The preliminary results of this meta-analysis will be presented at the meeting. These results will be based on the above data and will also include additional data from trials currently under checking.

Supported by grants from ARC (6560), PHRC (IDF 95013) & European Commission (Biomed 961321).

1310

Non-surgical treatment in primarily resectable T3T4 larynx and hypopharynx SCC

J.L. Lefebvre. *EORTC Head and Neck Cancer Cooperative Group; Head and Neck Department, Centre Oscar Lambret, Lille, France*

For a long time, either radical surgery, S, (most often with postoperative irradiation, XRT) or definitive XRT, (with S in reserve for salvage) were the two preferred treatments for advanced but resectable larynx and hypopharynx SCC. Unfortunately no randomised trial has compared both approaches.

The appearance, in the early 80s, of active chemotherapy, CT, and the frequent correlation between chemo and radiosensitivities have reopened the discussion with a new strategy: induction CT followed, in good responders, by XRT or, in poor responders, by S. To date, two randomised trials have been published: the VA trial, in the USA, on larynx and the EORTC 24 891, in the EEC, for hypopharynx. In both trials there was no difference in survival between this experimental approach and the standard treatment (S + postop XRT) but 50% to 66% of survivors could retain their larynx. Similarly, notable advances had occurred in XRT practice: new fractionation, acceleration, both or, in a different approach, concurrent administration of XRT and CT. Clearly, there are different ways to preserve the larynx function which are to be compared. As a result, this approach must still be considered as experimental.

1311

Resection margins in melanoma surgery and the value of elective lymphnode dissection

K. T. Drzewiecki. *Department of Plastic Surgery, State University Hospital, Rigshospitalet, Copenhagen*

Results of prospective randomized clinical trials have shown that 1 cm's margin for melanomas $\leq$1 mm's in thickness and 2 cm's margins for melanomas $\leq$2 mm's thickness are oncologically safe and therefore recommended. Effectiveness of resection margins for tumours >2 mm are at present being evaluated in ongoing clinical trials. Elective lymph node dissection (ELND) for low risk melanomas are not indicated. The role of ELND in the treatment of high risk lesions remains controversial. Its benefit on long term survival having in mind morbidity of this procedure is not unanimously accepted. A novel technique of staging the disease by preoperative lymphatic mapping followed by selective lymph node biopsies gains wide acceptance. This technique can possibly better outline a subset of patients who might benefit of ELND and/or adjuvant therapy. This benefit however has to be proven in ongoing clinical trials.