abovementioned findings and may facilitate a more limited and focused node dissection, avoiding dissection of unnecessary areas.

In conclusion, new knowledge in this field is challenging the standard lymph node staging procedures, i.e. obturator node dissection, as the sentinel nodes are often found farther away from the primary tumor site. To avoid extensive lymph node dissections with their concomitant morbidity, the novel techniques described will be useful, but further refinements will be necessary and are doubtlessly underway.

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Intra-arterial cisplatin and concomitant radiation for inoperable head and neck cancer

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Purpose: To determine the feasibility and effectiveness of intra-arterial cisplatin with concomitant radiotherapy (RADPLAT) for locally advanced, inoperable stage IV, squamous cell carcinoma of the head and neck.

Patients and Methods: From April 1997 to December 1999, eighty-five patients with locally advanced head and neck carcinoma were treated with radiotherapy (70 Gy, 7 weeks, 35 fractions) and concomitant supra-selective intra-arterial cisplatin (150 mg/m2, day 1, 8, 15, 22) and systemic sodium thio-sulfate rescue (RADPLAT). Main inclusion criteria were: inoperable squamous cell carcinoma of the head and neck or cancer requiring total glossectomy, any N, M0, Karnovsky performance status at least 60%, no prior surgery, radio- or chemotherapy. The median age was 50 year (40–69). Tumor characteristics: 75 patients had a T4 tumor, 10 T3, (3–10 cm), 61 had N+ disease, (1–10 cm).

Results: All patients received the scheduled treatment. Complete remission was achieved in 90%. At 40 months: Disease free survival, Locoregional Control and Local Control were 50%, 62% and 68% respectively. No treatment interruptions or dose limitations resulted from acute toxicity. One patient had a treatment-related death. Seventeen percent had grade IV (CTC) hematological toxicity, no other grade IV side-effects were seen. Grade III acute toxicity (RTOG): mucositis in 43%, upper GI in 60%, hearing loss in 10%.

Conclusion: The RADPLAT treatment schedule is feasible with excellent response rates and organ preservation. Based on the results of this study, a multicenter phase III trial comparing radiotherapy and concomitant systemic cisplatin versus RADPLAT is ongoing.

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Postoperative Combined Radiation and Chemotherapy Improves Disease-Free Survival (DFS) and Overall Survival (OS) in Resected Adenocarcinoma of the Stomach and G.E. Junction. Results of Intergroup Study INT-0116 (SWOG 9008)

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The cure rate for patients with resected gastric cancer is 5% - 40%. INT-0116 was designed to evaluate post-operative adjuvant chemoradiation in resected gastric cancer.

Study Design: Patients with stages Ib through IV M0 adenocarcinoma of the stomach or gastroesophageal junction who had undergone gastric resection with curative intent were randomized to postoperative follow up or chemoradiation. The treatment consisted of one cycle of 5-FU (425 mg/m*)/Leucovorin (LV) (20 mg/m*) in a daily x5 regimen followed by 4,500 cGy (180 cGy/day) given with 5-FU/LV (400 mg/m* and 20 mg/m*) on days 1 through 4, and on the last 3 days of radiation. One month after completion of radiation, two cycles of daily x5 5-FU/LV (425 mg/m* and 20 mg/m*) were given at monthly intervals.

Results: Between 8/1/91 and 7/15/98, 603 patients were accrued to this study, 47 (8%) of which were ineligible. Nodal metastases were present in 85% of cases. The combined modality regimen in this program was tolerable. There were 3 (1%) toxic deaths. Grade 3 and grade 4 toxicity occurred in 41% and 32% of cases, respectively. The gr. 3 toxicities were: hematologic (54%), GI (33%), infection (6%), neurologic (4%). OS and DFS analyses were based on intention to treat. With 3.3 years of median follow up, 3-year DFS is 49% for treatment and 32% for observation (p=0.001); 3-year OS is 52% for treatment and 41% for observation (p=0.03).

These results demonstrate a 44% improvement in relapse-free survival (hazard ratio of 1.44), and a 28% improvement in survival with median survival of 27 months in the observation arm vs. 42 months in the treatment arm (hazard ratio 1.28). Postoperative chemoradiation may now be considered a standard of care for high-risk R0 resected locally advanced adenocarcinoma of the stomach and GE junction.

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Radiochemotherapy is more effective than dose escalation in locally advanced head and neck cancer: results of a german multicentre randomized trial

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Purpose: Is accelerated chemoradiation more effective than accelerated radiotherapy alone?

Methods: Between March 1995 and May 1999, 384 patients with locally advanced H&N cancer were recruited for this multicentre study from 10 German centers. Male/female ratio was 82%/18%, mean age was 55 years. The oropharynx (60.4%) and hypopharynx (32.3%) were the predominant tumour sites, oral cavity tumours accounted for only 7.3% of all tumours. 5.5% and 94.5% of all tumours were stage III and IV, respectively. All patients with stage III- and IV-disease lacking evidence of distant metastases qualified for the treatment. Three target voumes (TV) were defined as follows: 1. Macroscopic tumour and lymph nodes 2. High-risk regions for lymphatic spread 3. Low-risk areas of lymphatic spread. The overall treatment time in both study arms was 6 weeks (40 days). The fractionation in study arm A was 14 Gy/2 Gy q.d. and b.i.d. 1.4Gy to a total dose of 77.6 Gy. Mitomycin C on days 5 and 36 @ 10mg/m* and 350 mg/m* 5-FU as bolus plus a 120 hrs. continuous infusion of 600 mg/m* 5-FU were additionally applied.

Results: The median follow-up was 30 months for all patients. The absolute values of locoregional failures in arm A vs. B were 49.7% vs. 37.6% (p=0.03). The total no. of metastases did not differ with 30.6% (arm A) vs. 34.9% (arm B). Actuarial locoregional control values were 46.4% (arm A) vs. 57.0% (arm B) @ 2 years (p=0.03). The hazard ratio (HR) was 0.72 (CI: 0.53-0.98). The overalll survival rates were 39.1 (arm A) vs. 49.4% (arm B) @ 2 years (p=0.05). The HR was 0.80 (CI: 0.62-1.04). None of seven parameters tested for acute grade 3 and 4 morbidity were statistically different in both treatment arms. Of 12 parameters tested for late grade 3- and 4 morbidity, only dysphagia (p=0.01) turned out to be pronounced in treatment arm A.

Conclusions: These results give evidence that accelerated radiotherapy of 70.6 Gy plus MMC/5-FU is superior to 77.6 Gy of accelerated fractionation alone in terms of locoregional control and overall survival at equitoxic levels of acute and late radiation morbidity.

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Concurrent chemo/radiotherapy in cervical cancer. What don't we know?

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Radical radiation therapy had been the accepted standard of care for advanced cervical cancer. In February 1999 the U.S. NCI issued a rare Clinical Announcement: "..five randomized phase III trials show an overall survival advantage for cisplatin-based therapy given concurrently with radiation therapy." "..strong consideration should be given to the incorporation of concurrent cisplatin-based chemotherapy with radiation therapy in women who require radiation therapy for treatment of cervical cancer."

The trials compared various doses and schedules of concurrent cisplatin-containing chemotherapy to pelvic irradiation alone, extended field irradiation or radiation and hydroxyurea. A similar reduction in the relative risk of death was observed. Absolute survival benefits ranged between 9 and 18%. These and other relevant studies including the two large randomised Canadian studies showing no detectable benefit will be reviewed.

The data suggest that the optimal regimens of concurrent chemoradiation are ill defined and may not be the "standard" of weekly cisplatin. A large study using oral 5-FU and Mitomycin has demonstrated benefit, as did one using Epirubicin. One study did not show benefit with concurrent infusional 5-FU when added to optimized radiation.

The Canadian study of pelvic irradiation with or without concurrent weekly cisplatin did not show survival benefit. The relative risk of death with concurrent therapy was 0.91. A number of possible explanations may

account for the different conclusions amongst this and other positive studies: 1) no benefit exists when weekly cisplatin is added to "optimal" RT; 2) this is a negative study by chance; 3) concurrent chemotherapy with radiation benefits only some subsets of patients; 4) given the negative association between anemia and outcomes a fall in hemoglobin related to the use of cisplatin could negate any incremental benefits of its use; 5) statistically the results are not incompatible; 6) the Canadian study represents a false negative result.

An analysis of these possible explanations will address the issue of the validity of concurrent cisplatin/radiation as the new standard of care. Probably, some incremental benefit is obtained with concurrent chemotherapy; benefit may be smaller than currently appreciated if chemotherapy is added to "optimal" radiation therapy; optimal concurrent chemotherapy remains to be defined.

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Natural T cell response against HPV16 and development of optimal peptide based vaccine

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Background: In our mouse models effective preventive vaccines against tumor outgrowth include HPV16 synthetic peptides of various lengths in IFA. The immune system is apparently capable of excising the exact MHC class I-binding peptides from exogenously offered proteins and long peptides. Furthermore, we have shown that tumor-specific T helper cells play an important role in the immune defense against solid tumors. On the basis of this we favor long peptides or proteins, thus offering all potential CD4 and CD8 T cell-epitopes, for future anticancer vaccination trials.

Methods and Results: We have now explored how optimal CD8+ CTL induction can be achieved in C57BL/6 mice, utilising molecularly defined triggers of dendritic cell (DC) activation or GM-CSF admixed with a long HPV16 E7 peptide containing a protective CTL epitope. The vaccinating potency of the long (32-mer) peptide was superior to that of the exact MHC class I binding 9-mer and optimal CTL induction was achieved with peptide formulated with MPL (detoxified LPS) or CpG. These adjuvants trigger DC via toll like receptor (TLR) 4 and 9, respectively.

Stimulation of human PBL with long overlapping (32–35 mer) peptides of HPV 16 E6/E7 allowed induction of primary HPV16 E6/E7-specific T-cell responses as well as visualisation of memory T-cell responses in a minority of HPV16 positive patients. In the course of these studies the first three HPV16 E6/E7 epitopes presented by HLA class II were identified.

Conclusions: All potential MHC class I and II epitopes processed from long (30–35 mer) peptides appear to be presented to host CD8+ and CD4+ T cells. Long peptide-based vaccines are thus independent of the use of exact T cell epitopes and can be administered to subjects independent of their HLA-type. These vaccines can be markedly potentiated by molecularly defined triggers of DC activation. On the basis of this we have started a phase I/II peptide vaccination trial with 12 peptides (32-mers) covering the entire length of HPV16 E6 and E7 in patients with cervical cancer or VIN III lesions.

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Monitoring CTL responses in melanoma patients vaccinated with MAGE antigenic peptides

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Because of their strict tumor specificity, MAGE antigens are good candidates for the development of cancer vaccines. About 150 patients, mostly with metastatic melanoma, have so far been enrolled in several small studies designed to assess the toxicity, the tumor regression and the CTL responses produced by immunization with MAGE antigen delivered in the form of peptides, proteins or recombinant viruses. Several vaccination studies with MAGE-3 or MAGE-1 peptides have been performed. Most schedules included 3 to 4 subcutaneous and intradermal vaccinations at 4-week intervals. Some general features have emerged: no significant toxicity was observed; tumor regressions were observed in about 20% of melanoma patients, whereas complete or partial clinical responses were observed in 10%; regressions were observed more often in patients with non-visceral stages of melanoma than with visceral stages; some regressions proceeded very slowly; cutaneous regressions were observed in the absence of significant inflammation.

Until recently, we failed to detect anti-Mage CTL responses even in patients who displayed clinical responses, suggesting that no massive CTL responses had occurred. Recently, we used in vitro stimulation with peptides of groups of about 105 CD8 T cells followed by tetramer analysis of the responder lymphocytes after 14 days. In one patient, who showed a partial response of a very large melanoma metastasis after vaccination with the Mage 3.A1 peptide, the frequency of anti-Mage 3.A1 CTL-precursors in the blood raised from less than 3. 10-7 of CD8 before vaccination to about 3. 10-5. Analysis of the T cell receptor sequence indicated that the response was monoclonal. The responder lymphocytes were CCR7- RO+ or CCR7- RA+, indicating that they belonged to the effector memory cells or fully differentiated effector cells. These results demonstrate that peptide vaccination with Mage peptide in the absence of adjuvant can at least sometimes induce a CTL response. Similar results have been observed in other vaccinated patients suggesting that tumor regression can be initiated in patients by a very low frequency of anti-Mage CTL.

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Identification of targets for immunotherapy of lymphomas

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Even though the clonal idiotypes of the B-cell lymphoma-associated immunoglobulins have been successfully employed in clinical vaccine trials, the need for custom-made individual vaccine production has compromised their widespread use. In contrast, cancer-testis antigens (CTA) are expressed in a variety of human cancers, but not in normal tissues, except for testis, and represent promising targets for immuno- and genetherapeutic approaches. Since little is known about their composite expression in non-Hodgkin's lymphomas (NHL), we investigated the expression of 8 CT genes (MAGE-3, MAGE-4, CT-7, HOM-MEL-40/SSX-2, SSX-1, SSX-4, HOM-tES-14/SCP-1, and HOM-TES-85) in 54 NHL specimens. CLL expressed only HOM-TES-14/SCP-1 (1/7 positive), but no other CTA. 10/10 follicular lymphomas were negative for all CT genes tested. The most frequent expression of CTA was observed in the centroblastic subtype of diffus large B-cell lymphomas: 4/14 cases expressed SSX-1 and HOM-TES-14/SCP-1, respectively, and HOM-MEL-40/SSX-2, HOM-TES-85 and CT-7 were expressed in 1/14 cases each. SSX-1, SSX-4, HOM-TES-14/SCP-1, and CT-7 each were expressed in 1/8 immunoblastic lymphomas, while CT-7 was the only CTA found to be expressed in 1/8 Burkitt's lymphomas, and SSX-1 the only one in 1/7 lymphoblastic lymphomas. We conclude that the expression of most CTA in NHL is rare, and that the identification of additional CT genes with frequent expression in NHL is badly needed. Of the cancer testis antigens identified to date, only SSX-1 and SCP-1 are expressed in diffuse large B-cell lymphomas of the centroblastic subtype at a frequency sufficient to justify their use in NHL vaccine trials. Supported by Kompetenznetz Maligne Lymphome of the BMBF.

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Vaccines targeting key molecules in carcinogenesis

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Many tumor antigens, including melanoma differentiation antigens and many of the so called cancer testis antigens, do not seem to be required for maintaining the malignant phenotype of the cancer cell. Consequently, under the selective pressure resulting from immune surveillance following active immunisation against such targets, antigen loss variants emerge that are no longer vulnerable to immune effector cells such as CTLs. To avoid this, we have focused on antigens that are formed as a consequence of genetic alterations occurring in key molecules during carcinogenesis. Mutations in oncogenes and tumor supressor genes are selected for during the carcinogenic process, and the resulting aberrant proteins give rise to tumor specific antigens. The emergence of antigen loss variants in this case are therefore less likely to take place. Following this strategy we have identified new antigens resulting from mutations in ras oncogenes, transforming growth factor beta receptor II (TGFbetaRII), Bax and Caspase 5. These mutations occur in a high proportion of patients with distinct forms of cancer. Most cancer vaccines tested up to now have a narrow field of application, since most tumor antigens are expressed only by subgroups of tumors. Another interesting target is therefore the reverse transcriptase catalytic