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

subunit of the human telomerase complex (hTERT). This enzyme is involved in immortalisation of cancer cells, and is expressed in high levels in 80-90% of all human tumors. Overexpression is partly due to gene amplification in the tumor cells. The gene is turned off in most normal human tissues, and hTERT is therefore an attractive target for immunotherapy, where hTERT may represent a "universal" tumor associated antigen. Based on these new, tumor specific and tumor associated antigens, candidate cancer vaccines have been tested in clinical trials involving patients with pancreas, colorectal and lung carcinomas and melanomas. The majority of these trials have been small phase I/II protocols primarily focused on safety aspects, protocol optimisation and immunological endpoints. By using and intradermal route of injection and the use of GM-CSF as an adjuvant 50-90% of patients demonstrate immune responses to the vaccines, depending on the antigen and patient group. In some trials a significant association between increased survival and an immune response was observed, indicating that such vaccines may have a clinical potential.

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Treatment of melanoma by adoptive transfer of CTL

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The two different approaches in the treatment of melanoma by immunotherapy are active immunotherapy which is represented by vaccination and passive immunotherapy with cellular therapy and mainly Tumor Infiltrating Lymphocytes (TIL). These ones used in melanoma stage IV (AJCC) induce a response rate of about 35% with a short duration of clinical response. Two main points are related to the efficiency of TIL: firstly the tumor burden and secondly the injection of specific T cytotoxic lymphocytes. Concerning the first point, it appears that the TIL would be more efficiency with a low tumor burden. Concerning specific cytotoxic T lymphocytes of melanoma antigens, they are considered as crucial in the anti-tumor response. However, until now a direct correlation between specific T cytotoxic activity and clinical response has never been demonstrated. In this context, the aim of our study was to demonstrate the efficiency of TIL used as adjuvant therapy in melanoma stage III after lymph node resection and to determine the percentage of specific TIL injected and the correlation with the clinical response.

88 eight patients have been included in an adjuvant randomized open study after positive lymph node resection. One arm (n = 44) received IL2 alone during 2 months, 6 weeks after the surgery and the other arm (n = 44) TIL and IL2 in the same condition. Moreover, among the TIL arm, the percentage of specific cytotoxic T lymphocytes has been determined in 26 patients by studying the intra-cytoplasmic production of gamma interferon by TIL incubated with autologous melanoma cell line. With a median follow up of 3 years, this study shows a significant decrease of relapse (73% without relapse in TIL arm versus 44% in IL2 arm $p = 0.019$) and an increase of survival in patients treated with TIL with only one invaded lymph node ($p = 0.026$). The percentage of specific TIL injected varied between 0, 3% and 10%. The injection of specific TIL was significantly associated with a decrease of relapse and an increase of survival ($p = 0.02$).

In conclusion, this study demonstrates for the first time, a clinical benefit of TIL used as adjuvant therapy in melanoma stage III with a correlation between relapse and overall survival and the injection of specific cytotoxic T cells.

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Genetic alterations in intraductal and invasive breast cancer

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Breast cancer develops by transformation of normal epithelial cells into carcinoma in situ, followed by progression to invasive breast cancer, which can than acquire the potential to metastasize. There are several types of breast cancer, and consequently several distinct genetic pathways can be identified. Lobular carcinoma in situ and invasive lobular carcinoma are characterized by inactivation of the E-cadherin gene; well differentiated ductal carcinoma in in situ (DCIS) is characterized by frequent inactivation of an unidentified tumor suppressor gene on chromosome 16q; p53 mutation and HER2 gene amplification are involved in the development of poorly differentiated DCIS and invasive carcinoma.

Several studies have revealed that the spectrum of genetic alterations in the in situ tumors is comparable to that of the invasive carcinomas. An important question remains which genetic alterations are involved in the progression from carcinoma in situ to invasive breast cancer. We have compared the genetic alterations in both the in situ and invasive component of the same tumor. Of 12 invasive breast carcinomas with a relatively large in situ component, we have microdissected both components, isolated DNA and performed comparative genomic hybridization (CGH). In some tumors, we have found a few distinct differences between otherwise identical genome profiles of both components, suggesting that the number of genetic alterations involved in breast cancer progression is limited. In one tumor, we found high-level amplification of a region on 8q in the invasive component only. This region was further analyzed by fluorescence in situ hybridization (FISH). We believe that amplification of a region on chromosome 8q is involved in the progression of carcinoma in situ to invasive breast carcinoma.

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Genetic alterations in putative precursor lesions to breast cancer

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The multistep model of carcinogenesis in the breast suggests a transition from normal epithelium to invasive carcinoma via non-atypical and atypical hyperplasia and in situ carcinoma. The introduction of mammographic screening has led to the increased detection of 'borderline lesions' and this has highlighted deficiencies in our understanding of these lesions.

Atypical Ductal Hyperplasia (ADH): ADH is a controversial lesion, which shares some but not all features of DCIS. Despite clear diagnostic criteria agreement even amongst experienced breast pathologists has been low. Lakhani (1995) demonstrated that LOH identified at loci on 16q and 17p in invasive carcinoma is also present in ADH with a similar frequency. This indicates that ADH is a neoplastic proliferation. There is support for this view in the literature. The studies demonstrate that within the limits of current investigations, there is no significant difference between ADH and DCIS. ADH as currently defined may represent a small focus of DCIS rather than a separate entity.

Hyperplasia of Usual Type (HUT): Retrospective studies indicate that this lesion has a relative risk of 2 for the subsequent development of invasive carcinoma. O'Connell et. al. have demonstrated LOH at many different loci in HUT with frequencies ranging from 0-15%. These figures are similar to those of Lakhani et. al. (1996) - range 0% at 13q to 13% at 17q. These frequencies are lower than in DCIS and ADH (range 25-55%). At least a proportion of non-atypical hyperplasias are also clonal, neoplastic proliferations.

Apocrine Hyperplasia: Apocrine papillary hyperplasia is considered to be a benign lesion despite a similar architecture to low grade DCIS. Jones et al have investigated genetic alterations in 10 benign apocrine hyperplasia and compared these to apocrine ductal carcinoma in-situ (DCIS) and invasive apocrine carcinomas of the breast using comparative genomic hybridisation (CGH). All lesions exhibited DNA copy number changes. The average number of alterations in apocrine hyperplasia was 4.1 compared to 10.2 in apocrine DCIS and 14.8 in invasive carcinoma. The changes show considerable overlap with those identified in in-situ and invasive apocrine carcinoma. The data suggest that apocrine hyperplasia may be a precursor of apocrine carcinoma.

Normal tissues: LOH identified in invasive carcinoma is also present in morphological normal lobules. The frequency and significance remains unknown

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Molecular biology of malignant lymphoma: implications for diagnosis

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Malignant lymphomas consist of Hodgkin lymphomas (HL) and Non-Hodgkin lymphomas (NHL; 90% of B- and 10% of T-cell origin). Normal and neoplastic B- and T-cells express unique B-cell receptors (BCR) and T-cell receptors (TCR), respectively. These receptors are shaped after gene rearrangements in precursor B- and T-cells. Thereafter BCR genes undergo additional modifications, hypermutation and class switching. Recent studies suggested that all gene alterations are mediated by DNA breaks and repair; therefore they also can lead to chromosomal translocation. B-cells undergo these alterations on the functional and non-functional allele. Many investigators focused on the functional BCR allele. Apart from providing useful clonality assays, these studies also led to the concept that B-NHL can be distinguished in pregerminal center, germinal center and postgerminal center lymphomas. A clinically relevant implication is that chronic lymphocytic leukemia (CLL), formerly thought to be one disease, consists