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.

37

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.

38

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.

39

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.

40

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