to the development of a wide variety of cancers (such as sarcoma, ependymomas, soft tissue tumors, glioblastomas, and astrocytomas). Thus, the development of anti-Mdm2 therapies may restore normal p53 function in tumor cells and induce growth suppression and apoptosis. We have developed a fluorescence polarization (FP) assay to identify small molecule inhibitors that block the binding of Mdm2 to a high affinity (~4 nM) p53-derived fluorescent peptide. The change in fluorescence polarization of the ligand upon binding to a 33 Kda Thioredoxin-Mdm2 fusion protein is more than 200 mP and has an assay performance indicator value (z value) greater than 0.5 (indicating excellent response). The assay is performed in a high-throughput, 384-well, format capable of detecting single digit nanomolar inhibitors. Buffers and buffer modifiers, ligand and protein concentrations, as well as assay volumes were optimized. The assay was validated with reference Mdm2 inhibitors by dose-dependent inhibitor titrations to determine Ki values which were independently confirmed by isothermal titration calorimetry (ITC).

Cancer vaccines

451

Humoral Immune Responses to MUC1 in Women with BRCA1/BRCA2 Mutations

S. von Mensdorff-Pouilly[1], B. Hermsen[1], A.A. Verstraeten[1], F.H. Menko[2], P. Kenemans[1], R.H.M. Verheijen[1]. *[1]Vrije Universiteit medical centre (VUmc), Obstetrics and Gynaecology, Amsterdam, The Netherlands; [2]Vrije Universiteit medical centre (VUmc), Clinical Genetics, Amsterdam, The Netherlands*

MUC1 is being studied as a target antigen for the immunotherapy of cancer. Natural antibodies to MUC1 are present in the circulation of cancer patients and healthy controls. Early breast cancer patients with a natural humoral response to MUC1 have a higher probability of freedom from distant metastases and a better disease-specific survival, suggesting a possible role of MUC1 antibodies in controlling haematogenous tumour dissemination and outgrowth. The objective of the study was to evaluate humoral immune responses to MUC1 in women at hereditary high risk of breast ovarian cancer. IgG and IgM antibodies to MUC1 were measured by ELISA in serum samples obtained from 79 women with a BRCA1 (N = 67) or a BRCA2 (N = 12) mutation, 23 women with BRCA1/BRCA2 mutations in the family but themselves not mutations carriers and 125 age-matched healthy controls. History of breast cancer was present in 24 and in 4 of the BRCA1 and BRCA2 mutation carriers, respectively. MUC1 IgM antibodies levels did not differ significantly between mutation carriers, non-carriers and healthy controls. MUC1 IgG ab levels did not differ significantly between controls and non-carriers. A significant difference in MUC1 IgG ab levels was found between controls and carriers of a BRCA1/2 mutation.

Table 1

Study population (N = 227)	N	MUC1 IgG arb. U/ml Median (range)	Mann-Whitney U test two-tailed P
a. Proven carrier BRCA1or BRCA2	79	0,72 (0,39-9,13)	a/c <0.0001
b. Mutation in family, patient not carrier	23	0,84 (0,49-6,67)	b/c n.s.
c. Controls	125	0,95 (0,43-5,70)	

MUC1 ab levels did not differ significantly between mutation carriers with or without history of breast cancer. In conclusion, natural IgG ab to MUC1 ranked lower in BRCA1/BRCA2 mutation carriers than in healthy controls. Prophylactic immunotherapy with MUC1 substrates may be a strategy to reduce the risk of breast/ovarian cancer in BRCA1/BRCA2 mutation carriers, strengthening tumour immune surveillance. A possible relation between BRCA1/BRCA2 mutations and the immune system remains to be explored

452

Prolonged antigen presentation of monocyte-derived dendritic cells loaded with plga-microspheres

Y. Men[1], E. Scandella[1], S. Gillessen[1,2], B. Gander[3], M. Groettrup[1]. *[1]Laboratory Research, [2]Oncology/Hematology, Kantonsspital St. Gallen, St. Gallen, Switzerland; [3]Swiss Federal Institute Of Technology (Eth), Pharmacy, Zurich, Switzerland*

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that are specialized to capture antigens in peripheral tissues, to migrate into secondary lymphoid organs and to prime T cells. Hence they are increasingly exploited as cellular vaccines in tumor immunotherapy. Currently, in most of the ongoing DC-based vaccination studies DCs are differentiated from monocytes during 5 to 6 day cultivation in the presence of GM-CSF and IL-4, and are externally loaded with tumor-specific antigenic peptides or tumor lysates before being re-injected into the patients. However, peptide-MHC complexes have a rather short half-life, and the single peptides are restricted to their specific MHC allele, and, finally, the composition of tumor lysates contain a bulk of tumor unrelated proteins and are quite undefined. In order to obtain a sustained antigen release which is favor to a prolonged antigen presentation by DCs, in the present study we encapsulated protein antigens or MHC class I- or class II-restricted peptides into biodegradable poly(D,L-lactide-coglycolide). The yielded antigen-containing microspheres (MS) can release their content over a time period of several days to weeks. We observed that human monocyte-derived DCs (MoDCs) generated under serum free conditions were able to efficiently take up MS particles. Using MS loaded with tetanus toxoid (TT) or a synthetic peptide derived from tetanus toxin sequence 947-967 (tt30) as model antigens for MHC class II restricted presentation, we observed that MoDC loaded with MS-tt30 were able to induce a strong T cell response, which is comparable to those induced with soluble tt30. Interestingly, 10 days after MoDC taken up MS-TT, stimulation of TT-specific T cells can still be observed, while MoDCs loaded with soluble TT only induced a very weak proliferating response. Moreover, 5- to 10 fold lower amount of antigen was required for stimulating TT- or tt30-specific T cells with MS-TT. Currently we are evaluating whether the MHC class I-restricted presentation of microencapsulated antigens taken up by MoDCs exerts similar prolonged kinetics. In conclusion, microencapsulation of proteins or antigenic peptides is a potent system to deliver antigen to DCs. This could provide a considerable contribution to improve the DC-based vaccines, in terms of prolonged antigen presentation with a low antigen amount.

453

A phase I/II vaccination study of patients with minimal residual prostatic adenocarcinoma after radical prostatectomy using autologous dendritic cells pulsed with recombinant PSA (Eladem tm)

B. Barrou[1], G. Benoit[2], M. Ouldkaci[3], O. Cussenot[3], S. Agranal[3], N. Bercovici[4], M. Salcedo[4], J.L. Romet-Lemonne[4], M. Ericson[4], N. Thiounn[4]. *[1]Pitie-Salpetriere Hospital, Urology Dept., Paris, France; [2]Kremlin-Bicetre Hospital, Urology Dept., Kremlin-Bicetre, France; [3]Saint-Louis Hospital, Urology Dept., Paris, France; [4]Immuno-Designed Molecules, Paris, France*

Dendritic cells are potent antigen presenting cells activating naive T lymphocytes and initiating cellular immune responses. Twenty-four prostatectomized patients, with rising PSA values (1-10 ng/mL) as the only sign of progressive prostate cancer, were vaccinated with Eladem (autologous dendritic cells pulsed with recombinant human PSA protein) in an attempt to trigger an immune reaction against cells expressing PSA. Bone scans and pelvic-abdominal CT scans performed before the inclusion were negative for all patients. To prepare Eladem, 1010 peripheral blood mononuclear cells from a single apheresis were cultured in the presence of GM-CSF and IL-13 for 7 days, purified by elutriation and pulsed with human rPSA. Eladem was administered in 3 cycles of 3 doses over a period of 5 months. Each dose was divided into 1 ID, 4 SC and 1 IV injections. All 24 patients received the planned 9 administrations. The mean cell count injected per administration ranged from 1.5-7.2 $\times$ 10^7 cells. The total number of injected cells ranged from 1.3 $\times$ 10^8 to 6.5 $\times$ 10^8. Mean age of the patients was 66 years (range 53-80), mean time since prostatectomy 3.7 years (range 1.3-9.7) and mean PSA at baseline 3.1 ng/ml (range 1.0-7.6). The cell therapy was generally well tolerated. No SAE was observed and there were no significant laboratory findings. Four patients experienced a total of 5 adverse events considered to be reasonably related to the therapy: macular rash (3) asthenia (1) and halitosis (1) all of mild intensity. No emergent signs of autoimmunity or significant change in autoantibody status were noted. Seven patients withdrew from the study during the Month 6-12 follow-up because of progression or local disease recurrence. Circulating cancer cells (CCC) were detected by RT-PCR with PSA-specific primers in 6 patients at baseline. CCC were no longer detectable in all the 6 patients at Month 6. Eleven patients had a transient post-baseline decrease of PSA on 1 to 3 occasions ranging from 6%-39%. First decrease were observed at Month 1 in 7 patients, Month 3 in 2 patients and at Months 5 and 9 in 1 patient each. Five patients with negative results at baseline became positive for PSA-specific cytotoxic T cells. No significant correlation between PSA, CCC and cytotoxic T cells was observed. In conclusion, Eladem therapy proved to be both feasible and safe in the treatment of prostate cancer patients.