

of these antibodies. Concerning histopathological examinations no evidence of organ damages were detected. We conclude from our data that immunization with Her-2/neu peptides successfully induced anti-tumoural immune responses, which is the basis for further development of peptide-based cancer vaccines.

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Mimotopes for high molecular weight - melanoma-associated antigen fused to albumin binding protein elicit anti-melanoma antibodies in balb/c mice

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Background: Tolerance phenomena make it difficult to elicit anti-cancer immune responses. The aim of novel cancer vaccines is to break tolerance by immunization with structures mimicking the original antigen, but being not completely identical to it, thereby stimulating low affinity B cell clones which have not been negatively selected. Appropriate mimicking structures - mimotopes - might be generated by the phage display technology.

Methods and Results: In this study we performed biopannings of phage display random peptide libraries with the anti-melanoma antibody 225.28S. It is directed against high molecular weight - melanoma-associated antigen (HMW-MAA), an antigen carried almost exclusively by melanoma and nevus cells. One selected nonapeptide mimotope was termed MelMim1 and chosen to be fused to streptococcal albumin binding protein (ABP), an immunogenic carrier molecule. The resulting fusion protein MelMim1-ABP was recognized by mAb 225.28S in ELISA and immunoblot, indicating that the fused mimotope retained its structural equivalence to the 225.28S epitope of HMW-MAA. Subsequently, groups of BALB/c mice were immunized with MelMim1-ABP, or ABP alone as a negative control. The induced humoral immune response in the MelMim1-ABP group contained antibodies against the carrier protein and against the mimotope. Importantly, the latter antibodies recognized the natural antigen HMW-MAA on 518A2 melanoma cells.

Conclusion: Our data demonstrate that peptide mimotopes fused to an immunogenic carrier protein are novel tools to induce anti-melanoma antibodies with possible functions in anti-tumor defense and are therefore candidates for the generation of epitope-specific cancer vaccines.

Cellular therapies

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Interleukin-8 (IL-8) promotes the growth of metastatic prostate cancer cells

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Prostate cancer is currently the most prevalent cancer in men. Overexpression of the chemokine IL-8 has been reported in the sera of patients with localized and metastatic cancer of the prostate. Animal models have also positively correlated the expression of IL-8 with the development of metastasis in prostate cancer. However, the mechanism by which IL-8 appears to promote disease progression remains poorly understood. We wished to determine whether IL-8 acts as a growth factor in prostate cancer cells using two prostate cell line models, the metastatic PC3 cell line and the transformed PNT1A epithelial cell line. We initially characterised IL-8 receptor (CXCR1 and CXCR2) expression on each cell line using RT-PCR, IP westerns, immunocytochemistry and flow cytometry. IP western analysis and flow cytometry illustrated a higher expression of CXCR1 than CXCR2 in both cell lines. Cell surface expression of CXCR1 was detectable in the PC3 cell line only but saponin permeabilised flow cytometry analysis of both cell lines demonstrated a high degree of intracellular receptor expression, which was later confirmed by immunocytochemistry. ELISA analysis revealed that both cell lines exhibit endogenous IL-8 secretion supporting the prevalence of receptor desensitisation. In growth assays conducted on PC3 cells, stimulation with exogenous IL-8 produced a consistent, concentration-dependent increase as assessed by cell count analysis. The response had an apparent EC50 of 1nM and a mean increase of 45% in cell number com-

pared to controls. The PNT1A cell line demonstrated negligible response to exogenous IL-8. Further studies using the PC3 cell line attempted to determine the signalling pathways that underpin the IL-8 induced proliferation of these cells. In growth assays, co-incubation with the specific pathway inhibitors, U0126, SB203580 and LY294002 established the involvement of ERK1/2, p38 MAPK and PI3K signalling cascades. Western blot analysis of the phosphorylation status of ERK1/2 and p38 by exogenous IL-8 stimulation showed activation of both pathways in the metastatic PC3 cell line. Our work to date has demonstrated the role of IL-8 as a potential growth factor in the PC3 metastatic prostate cancer cell line. Ongoing experiments are focused on using specific neutralising antibodies to CXCR1 and CXCR2 to determine which IL-8 receptor is coupled to the growth-promoting pathway.

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Analysis of the expression of 90K/Mac-2 binding protein (M2BP) in lung cancer and generation of cytotoxic T lymphocytes that recognize M2BP with an HLA-A2 restriction

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Purpose: 90K/Mac-2 binding protein (M2BP) has been reported to be highly expressed in patients with various types of cancer and to modulate the expression of surface molecules involved in immune responses on cultured cancer cells. We examine the expression of M2BP in lung cancer cells and attempt to generate M2BP-specific cytotoxic T lymphocytes (CTLs) using synthetic peptide derived from M2BP.

Methods: Eight cultured lung cancer cell lines and 28 tumor tissues from patients with lung cancer were examined for the expression of M2BP mRNA and protein. Using six peptides (9-mer or 10-mer) derived from M2BP with the HLA-A2 binding motif, we induced M2BP-specific CTLs from peripheral blood lymphocytes (PBLs) of HLA-A2-positive healthy donors by multiple stimulations of CD8-positive T lymphocytes with M2BP peptides. Antigen-specific responses of the induced CTLs were examined by the interferon-gamma production assay.

Results: Seven of the 8 (87.5%) lung cancer cell lines and 17 of the 28 (60.7%) tumor tissues were shown to express high levels of M2BP mRNA by Northern hybridization. Eleven of the 27 tissues (40.7%) were positive for M2BP expression immunohistochemically. CTLs stimulated with two M2BP-derived peptides (M2BP238-246, M2BP274-283) recognized peptide pulsed-autologous peripheral blood mononuclear cells (PBMCs) and T2 cells. These CTLs also recognized a lung cancer cell line, A549 cells with both HLA-A2 and M2BP expressions. The cytokine production by these CTLs were blocked by monoclonal antibody against HLA-A2.

Conclusions: M2BP is abundantly expressed in lung cancer and sufficiently immunogenic to elicit M2BP-specific CTLs. This molecule is expected to be useful as a target antigen in cancer immunotherapy.

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Abrogation of IRF-1 response by high-risk HPV E7 protein *in vivo*

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We have previously reported that human papillomavirus (HPV) E7 interacts with IRF-1, a key regulator of cellular immune response, and abrogates its transactivation function at the molecular level *in vitro*. To confirm our previous data, we investigated *in vivo* the E7-mediated down-regulation of IRF-1 using HPV E7-inducible cells and transgenic mice expressing HPV-18 E6/E7. When E7 was induced in the absence of tetracycline, the expression of target genes of IRF-1 (TAP-1, IFN- β , MCP-1 that are important for cellular immunity) was clearly reduced as determined by RT-PCR. In addition, the IRF-1 activity was down-regulated in E7-expressing cells into which INF- β -CAT reporter plasmid was transfected. To further investigate the E7-mediated immune regulation *in vivo*, we constructed transgenic mice expressing E6 and E7 genes of HPV-18 under the control of HPV-18 promoter (URR). From several rounds of breeding, we obtained from a transgenic line that developed a cervical dysplasia and expressed E6/E7 as determined by histological examination and RT-PCR, respectively. Subsequent RT-PCR analysis indicated that TAP-1, IFN- β , and MCP-1 genes were less

expressed in a cervical tissue derived from transgenic mouse, when compared with a cervix derived from normal mouse. From these results, we conclude that the E7 transgene expression inactivates the transactivation function of IRF-1 *in vivo*, which might be important for the elucidation of the E7-mediated immune evading mechanism that is frequently found in cervical cancer.

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Tumor necrosis alpha (TNF α) blockade as an adjunct to dose intense chemotherapy

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TNF α can be responsible for the fatigue/cachexia associated with malignancies. Docetaxel(D)when given weekly produces asthenia/fatigue in most patients(pts). We evaluated the ability of etanercept(soluble TNF receptor that neutralizes bioactive TNF α) to maintain the intensity of weekly D chemotherapy. 12 pts with refractory solid malignancies were randomized to receive D 43mg/m²/wk \times 6, every 8 wks)or D(same dose-schedule)plus etanercept(E)25mg SQ twice weekly. Escalations in the weekly doses of D in additional pts receiving E were planned in the event that dose intensity was maintained at the 43mg/m² dose. Plasma/serum samples for pharmacokinetics (PK) and unbound-TNF α and peripheral blood cells lysates for evaluation of the TNF-downstream transcription factor NF-kB and the relative expression of cytokines IL-1B,TNF α ,IFN γ and IL-6(TaqMan real time RT-PCR)were obtained. 12 pts received 111 doses of D(alone,32 doses;D/E,79 doses). Three pts, all in the D/E arm, achieved partial antitumor responses and received at least 3 cycles (6 months). During the 1st cycle, 9 doses (38%)of D were missed due to toxicity on the D arm,whereas none were missed on the D/E group. D PK parameters(AUC0-6,ng*hr/mL) were similar between days 1 and 29(717 \pm 372 and 817 \pm 470, respectively(n=12)and between single agent D(648 \pm 297,d1; 985 \pm 121,d29) and D/E (787 \pm 453,d1; 678 \pm 268,d29). Serum concentrations of unbound TNF were undetectable by bioassay in both arms. However, NF-kB relative activity (to HeLa+ cells)(9 pts) decreased on day 29(23.9 \pm 7) compared to baseline (36.9 \pm 6)in patients receiving D/E(mean % decrease, 30), and not in pts receiving D(d29, 35.2 \pm 12; baseline 32.1 \pm 12)(mean % increase, 17). Mean relative expression of IL-1B,TNF α ,IFN γ and IL-6 decreased on day 29 compared to baseline by 81,59,83 and 82%, respectively in pts receiving D/E (n=4) but increased with D alone (n=1). Three additional pts received D/E at 52mg/m²/wk D. At this dose, myelosuppression (grade 3 WBC) but not fatigue was the major factor limiting dose intensity (4/18 doses missed). In summary, the current study indicates that D PKs are similar after 4 wks of administration with or without E and that the D/E combination is well tolerated. The improved compliance in this preliminary study with this dose-intense chemotherapy regimen would suggest a role for TNF α in the associated asthenia/fatigue and offers a rationale for randomized studies focused in quality of life at conventional doses of weekly D. The cytokine and NF-kB regulated-gene expression patterns observed provide rationale for hypothesis driven explorations of this combination in man.

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A phase II trial of etanercept, a tumour necrosis factor- α inhibitor in recurrent ovarian cancer

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Purpose: There is preclinical data to support the role of TNF- α in the pathogenesis of ovarian cancer. We have initiated a phase II trial with etanercept (ENBR) in patients with recurrent ovarian cancer.

Methods: 17 patients received etanercept at a dose of 25 mg SC twice weekly. All patients were planned to receive a minimum of 3 months of treatment up to a maximum of 1 year, with disease evaluation at 3 monthly intervals. Surrogate end points for biological effect of etanercept included sE- Selectin, TNF- α , TNF-R1, MCP-1 and MMP-3 which were measured in the plasma (13 patients). A whole blood cytokine release inhibition assay was performed for IL-6 and MCP-1. The time points were pre treatment, 24hours, 7 days, 28 days and 4 weekly thereafter.

Results: Median age was 54 years (range 34-75). 15/17 patients had optimal surgery, and 2/17 had suboptimal surgery. The histological subtypes were serous (13), clear cell (1), endometrioid (2) and mixed tumour (1). Previous chemotherapy included carboplatin and/or Taxol (12 platinum sensitive and 5 resistant), 11/17 patients had second line and 7/17 had third line chemotherapy. 11/17 patients completed 3 months or more of treatment. There was no significant toxicity. Two patients achieved stabilisation of disease for 6.3 and 10 months respectively with improvements in quality of life. Median overall survival was 9.6 months. There was a significant reduction in IL-6 levels in 9/13 patients (24 hours) which was maintained until 12 weeks (6/8 patients). MCP-1 levels declined in 8/12 patients on day 1 and by 3 months was inhibited by 50% (6 patients). All other surrogate markers did not change significantly with treatment.

Conclusions: Definite biological effect is seen at 3 months of etanercept therapy. A new cohort of patients are being treated with a dose of etanercept at 25mgs thrice weekly.

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Modulation of cytokine production by SRIK-NKL, a Rare CD8+ NK cell line, by hormones: implications for carcinogenesis and treatment

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Natural killer (NK) lymphocytes are non-MHC restricted T cells involved in host defense against infection and malignancy, and can contribute to chronic inflammation via cytokine and defensin production. Tight regulation of NK function is critical, but mechanisms not fully defined. Modulation of NK activity is a potential strategy for cancer prevention and treatment. We established a unique CD8+ NK cell line (SRIK-NKL) from a patient with ALL and previously reported its constitutive cytokine profile (MIP-1 α , MIP-1 β , RANTES, GRO- α , IL-10, IL-4, TNF- α , TNF- β , IFN- γ , GM-CSF positive; IL-8, IL-6, IL-7, SCF negative). SRIK-NKL allowed us to further investigate the behavior of this unusual CD8+ subset of NK cells, specifically hormone responsiveness. Cells were incubated at 0.5x10⁶ cells/ml in RPMI 1640 5%FCS/no antibiotics in CO2 incubator at 37 degrees C for up to 24 hours with and without hormone (tamoxifen or mifepristone 0.05-0.5 mcM; progesterone, estradiol, or testosterone 0.5-5 mcM; retinoic acid 1-10 mcM; epinephrine 1 mcg/ml; substance P, VIP, somatostatin, or gastrin 1-10 mcM) and supernatants quantitated for cytokines by ELISA (R&D). RANTES was significantly inhibited to 35-59% control by mifepristone and tamoxifen; MIP-1 α was decreased by retinoic acid to 52% control; but substance P and VIP greatly increased TNF- α to 166-226% control. Thus, cytokine production by CD8+ NK cells is altered by the hormonal milieu. This may be an important regulatory mechanism to consider when designing NK-dependent biologic treatments for cancer, or during the use of hormonal agents such as tamoxifen for established disease.

Gene therapy and antisense approaches

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Phase II study of ONYX-015 in patients with hepatobiliary tumors with p53 correlative studies

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ONYX-015 is a genetically modified adenovirus, which was designed to replicate preferentially in p53 mutated cells. To test the efficacy of ONYX-015, a phase II trial of intravesical ONYX-015 was conducted in patients with hepatobiliary tumors. All patients had biopsy-proven, measurable tumors of the liver, gall bladder or bile ducts which were beyond the scope of surgical resection. Patients received intravesical injections of ONYX-015 at either 6 \times 10⁹ or 1 \times 10¹⁰ pfu/lesion up to a total dose of 3 \times 10¹⁰ pfu. The status of p53 was assessed by immunohistochemistry (IHC) or Affymetrix GeneChip microarray analysis. Studies were conducted for viral shedding and for the presence of anti-adenoviral antibodies prior to and