activate dendritic cells in vivo, we used Flt3L, IFN-a and CD40L. To activate T cells, we used IL-2, IL-15 and an agonistic antibody against 4-1BB, a member of the TNF receptor family. Mice were inoculated intradermally with tumor cells and treated with cytokines and the anti-4-1BB antibody either alone or in combination. Treatment of mice for 10-20 days with Flt3L inhibited tumor growth and significantly increased the number of tumor rejections. Flt3L induced both innate (NK cell-mediated) and adaptive (T cell-mediated) immune responses. Combining Flt3L with IL-2 or IL-15 did not result in any beneficial effects, but combining Flt3L with IFN-a or CD40L significantly improved anti-tumor immune responses. Neither IFN-a nor CD40L alone had any significant effects on tumor growth. The beneficial effect of combining Flt3L with CD40L correlates with an overall increase in the number of dendritic cells, but not T, B or NK cells. Re-challenge of the Flt3L+CD40L-treated mice with tumor cells resulted in complete blockage of tumor growth, indicating that these animals had developed long-term anti-tumor immunity. Significant improvements in the anti-tumor immune response were also observed in mice treated with the anti-4-1BB antibody. Two to four bolus injections of this antibody markedly boosted anti-tumor immunity. Depletion of CD8 T cells, but not NK or CD4 T cells, completely eliminated the anti-tumor immune response induced by 4-1BB. Combining Flt3L and anti-4-1BB resulted in enhanced anti-tumor immunity compared to treatments with either reagent alone. Our results show that a strong anti-tumor immune response can be generated in mice treated with cytokines (Flt3L, IFN-a, and CD40L) or an agonistic antibody against 4-1BB. This approach can potentially be used to treat tumors for which no specific antigens are known or to boost anti-tumor immunity in vaccination protocols.

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Models of active specific immuno therapy of human malignancy bone metastases

P. Correale[1], G. Cusi[2], A. Scardino[3], F.A. Lemonnier[4], L. Micheli[1], M. Sabatino[1], D. Pozzessere[1], R. Petrioli[1], G. Giorgi[1], G. Francini[1].
[1] University of Siena, Oncopharmacology Center, Siena, Italy; [2] University of Siena, Department of Molecular Biology, Siena, Italy; [3] INSERM, Unitè 418, Villejeuif, Cedex, France; [4] Institut Pasteur, Department SIDA-Retrovirus, Paris, France

The occurrence of bone metastases is a very common and detrimental event associated with human cancer. Epithelial malignancies give rise to bone metastases when they acquire the ability to produce the parathyroid hormone related protein (PTH-rP), a secreted protein, which is considered a critical factor for tumor cell survival and growth in bone tissue. Since PTH-rP in the adult life is mainly produced by several very common cancer cell lineages, such as lung, prostate and breast carcinomas, we have investigated whether it could be used as a TAA target for the active specific immunotherapy of tumors giving bone metastases. To generate a PTH-rP specific CTL response in vitro we have utilized a previously described protocol employing low dose IL-2 and autologous dendritic cells, pulsed with PTH-rP peptides having HLA-A2.1 binding motifs or infected with influenza virosomes containing PTH-rP plasmid genes (GC90V). This protocol allowed the generation of multiple PTH-rP peptide specific CTL lines from PBMC of normal HLA-A2.1+ donors or TIL derived from a HLA-A2.1+ patient with prostate carcinoma. These CTL lines recognized three different PTH-rP peptides and killed HLA-A2.1+ prostate and breast carcinoma cells that produced large amounts of PTH-rP. Intranasal administration of GC90V to BALBc mice resulted in a significant CTL immune response to PTH-rP without occurrence of side effects or autoimmunity as evaluated in post mortem study. Similar results were also obtained in HLA-A2.1 expressing HHD transgenic mice immunized with the three PTH-rP derived epitope peptides. These results demonstrate that PTH-rP is a tumor antigen, that an anti-tumor PTH-rP CTL response may be induced in vitro and in in vivo and that PTH-rP peptides and GC90V may be potential candidate for use in immunotherapy against prostate cancer and bone metastases from the most common epithelial malignancies. (Partially supported by National Council of research, CNR, Italy).

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Therapy of HPV16-induced carcinomas with IL-2 gene modified and dendritic cell (DC)-based tumour vaccines

J. Bubeník[1], V. Vonka[2], J. Šímová[1], M. Šmahel[2], M. Indrová[1], R. Mikyšková[1], L. Mendoza[1]. *[1] Institute of Molecular Genetics, Prague; [2] Institute of Haematology and Blood Transfusion, Prague, Czech Republic*

Purpose: To examine local and systemic effects of vaccines against HPV16-associated carcinomas.

Methods: Vaccination with IL-2 gene-modified and DC-based tumour vaccines; surgically induced minimal residual tumour disease (SMRTD).

Results: To examine local and systemic effects of dendritic cell (DC)-based vaccines in a mouse model resembling human papilloma virus (HPV) type 16-associated carcinomas, murine kidney cells were malignantly converted by *in vitro* co-transfection of activated H-*ras*, HPV16 E6/E7, and neomycin resistance gene DNA. The resulting MK16 neoplastic cells grew s.c. in syngeneic mice and metastasized to lungs and lymph nodes (Šmahel, Sobotková, Bubeník et al., Brit. J. Cancer, in press). Immunization with HPV16 E6/E7 and activated H-ras plasmid DNA could specifically inhibit growth of the HPV16-induced tumours in the immunized mice. Priming of the proliferative anti-MK16 responses was efficient when DC and DC lines were pulsed with lysates of MK16 cells or HPV16 E7 (aa 49–57 RAHYNIVTF) synthetic peptide and co-cultivated *in vitro* with syngeneic spleen cells. Local pretreatment with DC could substantially inhibit growth of a subsequent inoculum of the MK16 cells. MK16 tumours were injected peritumorally with irradiated and MK16 lysate-pulsed DC; significant differences were found between MK16 tumour growth and survival of mice in the DC vaccine-treated and control groups (Indrová, Bubeník, Šimová et al., Int. J. Mol. Med., in press). Immunotherapy of the MK16 carcinoma transplanted in syngeneic mice was accomplished using genetically modified, IL-2-producing tumour vaccines. Both, small MK16 tumours, 2–4 mm in diameter, and MK16 SMRTD could be completely cured with IL-2 gene-modified tumour vaccines.

Conclusion: Taken collectively, the results indicate that the activating, co-stimulatory, and mitogenic signals delivered by HPV16 oncoprotein-pulsed DC as well as insertion of the IL-2 gene should be considered for the construction of vaccines for treatment of primary human HPV16-associated carcinomas and SMRTD.

Psychosocial and economical aspects of cancer

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The guessing game: waiting for the results of diagnostic investigations for symptoms of breast disease

K. Poole. *Institute of Cancer Research, Macmillan Practice Development Unit, Centre for Cancer & Palliative Care Studies, London, United Kingdom*

Purpose: Over recent years there has been increasing concern with the psychological impact of undergoing diagnostic investigations for breast abnormalities. UK NHS Executive guidelines have stipulated that "minimising" delay in the diagnostic process is critical to reducing patient anxiety. This paper presents the results of a multi-method research study that assesses patient distress during the peri-diagnostic interval, and explores the ways in which women reached their own diagnoses during this time.

Methods: Participants (n=98) completed the State Trait Anxiety Inventory immediately following investigations at the out-patient clinic. That same evening and for the following two days, patients completed diaries including the Profile of Mood States and Daily Coping Scale, and repeated the state anxiety assessment upon return to clinic for results. A subset of 20 women were interviewed about their experience and these data were combined with interview data (n=20) from a concurrent project.

Results: 75% of the cohort recorded levels of anxiety, confusion, uncertainty and depression equivalent to those reported for psychiatric out-patients. This distress was sustained throughout the waiting period, and emotion-focused strategies dominated coping efforts. Interviewees explained how they were able to guess their diagnosis from various "cues" which could be categorised according to type: temporal, interpersonal, procedural and spatial. Inferences appeared to be underpinned by an urgent need to reduce uncertainty and enhance prediction.

Conclusion: Service structure does impact upon patient's psychological distress, and further research is critical to the development of an evidence-based service for all women undergoing diagnostic investigations.

*This work was completed when the author was a doctoral student at School of Nursing Studies, University of Wales College of Medicine, Heath Park, Cardiff, South Wales.