Detection of specific T-cells in the peripheral blood and in the bone marrow of patients with early breast cancer recognizing epitopes from Tumor Antigens

Eleni Galani

Background

Immunotherapy
Immunotherapeutic approaches to the management and treatment of malignant disease are showing promise. Such strategies have been tested largely in melanoma and renal cancer, but their application to other carcinomas, including breast and ovarian, is now under investigation. Although it is not totally clear which components of the immune response are crucial for tumor rejection, the adaptive cellular compartment, which includes both cytotoxic CD8+ cells (CTL) and helper CD4+ cells, is thought to play a major role. Antigen presentation by dendritic cells is also likely to be important.
Feuerer et al. recently suggested that the memory T-cells derived from the bone marrow of patients with breast cancer have therapeutic potential in vivo and could become the basis for effective immunotherapy for breast cancer (1). Patients' bone marrow and peripheral blood contained memory CD8+ T-cells that recognize epitopes from specific tumor-associated antigens (2). However, these memory T-cells in the periphery were anergic, whereas those in the bone marrow were functional (1). Tetramers were used to identify the tumor specific CD 8 T-cells, in both the peripheral blood and in the bone marrow (1,2).

Tetramers
Over the last five years, antigen-specific T-cells have been identified by using tetrameric peptide-MHC-class I complexes carrying the specific class I peptide epitopes (tetramers). Using HLA A02*01 tetramers carrying epitopes from either the MUC1 or HER-2/neu antigen, Feuerer and colleagues were able to detect in the periphery and bone marrow of breast cancer patients specific CD8 T-cells that were not present in normal individuals (1,2).
In this project we attempted to confirm and expand these important observations by focusing on the tumor-associated antigens MUC1 and c-erbB2/HER2 and by using tetramers to a novel tumor-associated antigen that is localized to the nucleus.

The MUC1 epithelial mucin
Expression of the MUC1 surface mucin is up regulated in most breast, ovarian and pancreatic carcinomas, and because of differences in glycosylation the cancer associated mucin is antigenically distinct from the mucin expressed by normal epithelial cells (3,4). MUC1 also contains peptide epitopes, that can bind to MHC class I molecules from A02*01; it was a tetramer carrying one such epitope that detected reactive T-cells in PBL and bone marrow of breast cancer patients (1,2,5)

The c-erbB2/HER2 antigen
HER-2/neu (also known as c-erbB-2) oncogene encodes a 185 KDa transmembrane tyrosine kinase receptor, which is a member of the epidermal growth factor receptor family. Its amplification is one of the most common genetic alternations associated with human breast cancer, and it is associated with more aggressive clinicopathologic features and poor prognosis (6). Monoclonal antibodies directed against HER/2 neu have been developed and used in clinical practice.

Aim
The aim of the project was to determine whether tumor-specific T-cells reactive with MUC1 or c-erbB2/HER2 peptides and a novel, nuclear tumor-associated antigen could be detected in peripheral blood of early breast cancer patients, as a preliminary to isolating T-cells from the bone marrow for evaluation of functionality.

Detection of tumor antigen-reactive T cells in PBL of breast cancer patients (HLA A 0201)
According to the published data, the percentage of $CD8^+$ T-cells binding the MUC 1 peptide tetramers in bone marrow and peripheral blood is higher in breast cancer patients than in normal, healthy donors. Moreover, reactive T-cells were not found in the bone marrow of healthy donors, although they were found in the bone marrow of breast cancer patients. A pilot study of patients with breast cancer using tetramers carrying MUC1 and c-erbB2/HER peptides was performed to confirm and expand these published results. In addition, the binding of tetramers carrying peptides from a novel, nuclear protein were also analyzed in this series of peripheral blood samples from patients and healthy donors. These preliminary experiments were necessary before it was possible to proceed to obtain bone marrow samples.

After informed written consent was obtained, 20 ml of blood was collected from patients with breast cancer prior to their primary surgery. The heparinized blood was immediately subjected to Ficoll gradient centrifugation, and the cells in the interphase layer were collected.
HLA A2 patients were selected through staining with the anti-HLA A*0201 antibody BB7.2 FITC, and the samples were analyzed by flow cytometry. Tetramers of the HLA A*0201 allele carrying specific peptides were used to detect the presence of memory T-cells (without in vitro stimulation) in the HLA A2 positive samples. Peripheral blood T-cells were incubated for 30 minutes at room temperature with phyto erythrin (PE) conjugated tetramers. Fifteen minutes after the addition of the tetramer, an anti-CD8 monoclonal antibody conjugated to PC 5 was also added. Samples were washed twice and analyzed using the flow cytometer. The binding of the tetramers to HLA A*0201 healthy donors was also analyzed. A sample from an A2 negative non-breast cancer patient was used each time as an internal negative control.
Of sixty patients with non-metastatic breast cancer whose blood was tested with the A2 antibody, twenty-nine were shown to be HLA A*0102 positive. These and nine healthy A2 positive donors have now been entered into this ongoing study. Our results to date confirm the published data for MUC 1 (7), but T-cells reactive with c-erbB2/HER2 were not detected, even though 18% of the patients were HER2 positive by immunohistochemistry. A novel finding was that in 30% of the patients, CD8+T cells could be detected that bind to a tetramer carrying a peptide from the novel, nuclear tumor-associated antigen.

Conclusions
Our study confirmed the data with MUC 1 peptide and identified a peptide from a nuclear antigen, which was recognized in breast cancer patients and not in healthy donors. The number of HLA A*0201 breast cancer patients and healthy donors needs to be increased to confirm these findings. The LREC has given ethical approval for the extraction of samples of bone marrow from patients undergoing surgery for breast cancer, after their informed consent has been obtained. Once the binding of the tetramers has finally been established using peripheral blood, the presence of CD8 cells in patients' bone marrow can be investigated

Acknowledgements
I would like to express my sincere gratitude to ESMO for providing the financial assistance that enabled me to acquire important skills during the course of this work. I would like to thank all the members of the Cancer Research UK, Breast Cancer Biology Group, for their warm hospitality and friendly support. I would like to thank Prof. Joyce Taylor-Papadimitriou and Dr. David Miles for their guidance. And I would like to express my gratitude to Prof. Meletios Dimopoulos for his significant assistance.

References

1. Feurer, M., Beckhove, P., Bai, L., Solomayer, E-F., Bastert, G., Diel, I.J., Pedain, C., Oberniedermayr, M., Schirrmacher, V., Umansky, V. (2001) Therapy of human tumours in NOD/SCID mice with patient derived reactivated memory T-cells from bone marrow. **Nature Med.** 7(4): 452-458.

2. Feurer, M., Rocha, M., Bai, L., Umansky, V., Solomayer, E-F., Bastert, G., Diel, I.J., Schirrmacher, V. (2001) Enrichment of memory T-cells and other profound immunological changes in the bone marrow from untreated breast cancer patients. **Int. J. Cancer** 92: 96-105.

3. Gendler, S.J., Lancaster, C.A., Taylor-Papadimitriou, J., Duhig, T., Peat, N., Burchell, J., Pemberton, L., Lalani, E.-N. and Wilson, D. (1990) Molecular cloning and expression of the human tumor-associated polymorphic epithelial mucin. **J. Biol. Chem.** 265, 15286-15293.

4 Miles, D.W. and Taylor-Papadimitriou, J. (1999) Therapeutic aspects of polymorphic epithelial mucin in adenocarcinoma. **Pharmacol Ther** 82: 97-106.

5 Heukamp, L C, van der Burg, S.H., Drijfhout, JW. Melief, C.J.M., Offringa R. Taylor-Papadimitriou, J., (2001) Identification of three non-VNTR MUC1-derived HLA-A*0201-restricted T cell epitopes that induce protective anti-tumor immunity in HLA-A2/K^b-transgenic mice. **Int. J. Cancer.** 91: 385-392.

6 Fisk, B., Blevins, T.L., Wharton, J.T., Ioannides, C.G. (1995) Identification of an immunodominant peptide of Her-2/neu protooncogene recognised by ovarian tumour specific cytotoxic T lmphocyte lines. **J. Exp. Med.** 181: 2109-2117.

7. Brossart, P., Heinrich S.K., Stuhler, G., Behnke, L., Reichardt L.V., Stevanovic, S., Muhm, A., Rammensee, H.G., Kanz, L., Brugger, W. (1999) Identification of HLA-A2-Restricted T-Cell Epitopes Derived From the MUC1 Tumour Antigen for Broadly Applicable Vaccine Therapies. **Blood.** 93:4309-4317.