Cancer Cell International

Poster presentation

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The European Searchable Tumour Cell and Databank, ESTDAB, as a tool for research in cancer immunology

L Müller[1], D Schadendorf[2], S Stevanovic[3], A Knights[1], E Badalian[1], L Wedel[1] and G Pawelec*[1]

Address: [1]University of Tübingen Medical School, Center for Medical Research, Waldhörnlestr. 22, D-72072 Tübingen, Germany, [2]Skin Cancer Unit, German Cancer Research Center, Theodor Kutzer Ufer 1, D-68135 Mannheim, Germany and [3]Department of Immunology, Institut for Cell Biology, Auf der Morgenstelle 15, D-72076 Tübingen, Germany

Email: G Pawelec* - graham.pawelec@uni-tuebingen.de

* Corresponding author

from Association for Immunotherapy of Cancer: Cancer Immunotherapy – 2nd Annual Meeting
Mainz, Germany, 6–7 May 2004

Published: 1 July 2004

Received: 28 April 2004

Cancer Cell International 2004, **4**(Suppl 1):S40

This article is available from: http://www.cancerci.com/content/4/S1/S40

Collections of tumour cell lines continue to represent valuable resources in cancer research. However, cell banks rarely provide detailed immunological characterisation of the lines that they offer. To redress this omission, ESTDAB was founded as an EU infrastructures project, (see http://www.medizin.uni-tuebingen.de/estdab). It now provides the world's largest collection of melanoma cell lines, and is the only cell bank to offer the researcher the ability to seek cells with combinations of immunologically-relevant characteristics (go to http://www.ebi.ac.uk/ipd/estdab/index.html). These include HLA class I and II high resolution typing, HLA expression patterns, tumour antigen expression, cytokine secretion, adhesion molecule expression, apoptosis resistance, surface glycosylation patterns and other parameters. These lines are available to bona fide investigators for research purposes. Examples of studies being performed using this resource are elution of HLA class I- and II-bound peptides to identify novel melanoma-associated antigens, assessment of mechanisms of MHC antigen loss, defects in antigen processing pathways, secretion of suppressive factors, influence of the culture environment and presence of other cell types on cancer cell characteristics, and activity of glycosyltransferases creating tumour-type glycan structures. Together with the EU project OISTER (Outcome and Impact of Specific Treatment in European Research on Melanoma, coordinated by D. Schadendorf), we are continuously collecting new cell lines generated from patients entered into immunotherapy trials. The appropriateness of these lines for monitoring purposes is being established. Here, we report studies on four melanoma cell lines which were chosen for propagation and elution of HLA-bound peptides by "acid-stripping" from the surface of viable cells. FACS analysis of HLA expression before and after this procedure confirmed that HLA class I expression decreased after peptide elution. The peptide content of the eluted samples was established by mass spectrometric analysis, and the eluates screened for immunogenicity *in vitro* on T cells of normal donors selected for sharing at least one HLA-I allele with the melanoma cell line. T cells were cultured in the presence of different cytokines and titrated amounts of eluate, using either autologous antigen-presenting cells, APC (eluate-pulsed irradiated PBMC, dendritic cells generated from the same donor, or HLA-matched B-cells). After several rounds of restimulation, T cell lines recognising eluate-pulsed autologous APC were obtained. Investigation of their exact antigenic specificity is now in progress. For studies on MHC class II-bound peptides, monoclonal antibodies were used to isolate HLA-DR, DQ and DP molecules from lysates of at least 10^{10} tumor cells. From these, HLA class II-bound peptides were eluted at low pH and fractionated by HPLC. After screening T cell reactivity, positive fractions were sequenced by MALDI-TOF in order to determine potential tumor-specific antigens and identify related T cell epitopes. Potentially immunogenic candidates thus far identified include an HLA-DR-restricted sequence derived from a possibly mutated stress protein (grp78) NVMRI-

INEPTAAAIA. Several non-mutated peptides from over-expressed molecules did not show evidence of immunogenicity *in vitro*. The availability of the ESTDAB collection will facilitate immunological investigation of *in vitro* human melanoma models.

Acknowledgements

Supported by DFG Pa 361/5-1, 5-2, The Wrangell Foundation, ESTDAB (EU contract QLRI-CT-2001-01325) and OISTER (EU contract QLG1-CT-2002-00668).