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Efficient *in vitro* expression of human reverse transcriptase (hTERT) in dendritic cells of lung cancer patients using RNA electrotransfection

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Patients with advanced lung cancer have a poor prognosis after standard radiochemotherapy. An innovative treatment strategy is the targeting of tumor associated antigens with cellular effector cells. The telomerase catalytic subunit (hTERT) is an attractive target of cytotoxic T cells as it is highly expressed in both NSCLC and SCLC cells.

To activate tumor antigen specific CD4+ helper and CD8+ cytotoxic T lymphocyte responses genetically modified dendritic cells (DC) are increasingly used. In this study, we used electro-transfection of DCs with hTERT mRNA that enables an HLA independent whole antigen approach potentially targeting a wide range of hTERT epitopes. Immature, i.e. non-proliferating human DCs were prepared from peripheral blood monocytes in serum-free growth medium, GM-CSF and IL-4. Subsequently the DCs were electroporated in transfection buffer with mRNA and matured in a cytokine cocktail consisting of IL-1beta, IL-6, TNF-alpha and PGE_2.

To establish and optimize mRNA electroporation conditions the DCs of a healthy individual were transfected with green fluorescent protein (GFP) mRNA. The percentage of electro-transfected DCs was 35% determined by selecting GFP expressing cells using flow cytometry. Importantly the RNA electrotransfected DCs retained their typical morphological and immunophenotypical characteristics, expressing high levels of HLA-DR and no lineage markers. CD83 as an indicator of maturation was expressed in 44%. The costimulatory molecules CD80 and CD86 were expressed in 73% and 97%. Having established electroporation parameters we transfected monocyte derived DCs with hTERT mRNA in 2 lung cancer patients. Verification of transfection efficiency was performed by analyzing the induction of telomerase activity with the TRAP assay. Twenty-four hours after electrotransfection the measured activity in the DCs was equivalent to HL60 cells that biologically express high levels of hTERT. Non-transfected DCs did not show any telomerase activity. These data show that strong hTERT expression can be achieved in DCs of lung cancer patients using mRNA electrotransfection. They provide the basis for further preclinical and clinical studies.