425g Generation of Alloreactive T-Cells in-Vitro for Cellular Therapy

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Allogeneic stem cell transplantation is the only curative option for numerous patients with hematological malignancies such as chronic leukemia and non-hodgkin's lymphoma. However, Graft-versus-host disease (GVHD) caused by a subset of the T cell population, alloreactive T cells. GVHD is the major cause of mortality and limitations to the use of allogeneic stem cell transplantation. CD25 and CD69 are the most commonly used activation surface markers for the differentiation and depletion of alloreactive T cells; however, it has been reported that there is still relatively high, residual alloreactivity in cell suspensions after CD25+ and CD69+ T cell depletion. The purpose of this study is to optimize the generation of alloreactive T cells in vitro and select the optimum combination of the surface markers to represent alloreactive T cells.

In this study, supported by the National Cancer Institute, we choose eight potential surface markers that are reported to appear on the activated T cells: CD25, CD69, CD71, OX40, CD38, CD152, CD122 and HLA-DR. In order to optimize the generation of alloreactive T cells, mature dendritic cells were used to stimulate the activation of alloreactive T cells in vitro. The inclusion of these dendric cells significantly increased the expression of these alloreactive surface markers on the T cells.

A nine-parameter, flow cytometry system, BD FACSAria, was used to assess the co-expression of these surface markers on alloreactive T cells. The immuno-function of cell subsets expressing different activation surface markers was determined by a cell proliferation assay. The optimum combination of activation surface markers for alloreactive T cells was determined on the basis of expression level and functional significance.