In vitro generation of tumor antigen-specific T cells from patient and healthy donor stem cells

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Introduction: Acute myeloid leukemia remains a therapeutical challenge, as many patients relapse after chemotherapy. Allogeneic stem cell transplantation is in most of these patients the only option for cure, but carries a high risk of morbidity and mortality and a suitable donor may be lacking. Recently, advances are being made in the field of T cell immunotherapy. The classical protocol, in which peripheral blood lymphocytes (PBL) are transduced with a tumor antigen-specific T cell receptor (TCR), can generate T cells with low and possibly hazardous specificities (due to mispairing of the endogenous and introduced TCR α and β chains). Therefore, we have developed a novel protocol in which we generate tumor antigen-specific T cells from CD34+ hematopoietic stem cells. We have already succeeded in generating large numbers of tumor-specific, naive and resting T cells that only carry the introduced TCR, starting from postnatal thymus and cord blood CD34+ cells. Now we are optimizing this protocol for clinically more relevant samples, such as mobilized peripheral blood from healthy stem cell donors and from patients in remission after chemotherapy and/or other treatments, and leukapheresis samples from patients at diagnosis.

Material and methods: In our protocol, CD34+ cells were isolated from HLA-A2+ fresh patient and healthy donor samples and cultured on OP9-DL1 in the presence of SCF, FLT3L and IL-7, until T cell commitment. Subsequently, the cells were transduced with a tumor antigen-specific TCR and again co-cultured until CD4+ CD8+ double positive cells were abundantly present. At that point, agonist peptide was added, which induces maturation. Finally, cells were polyclonally expanded on feeder cells. For HLA-A2 negative samples, CD4+ CD8+ double positive cells were co-cultured with a cell line (T2 pulsed with the agonist peptide or a cell line with endogenous expression of the agonist peptide) which can present the agonist peptide to the maturing T cells.

Results: Using the above protocol, we were able to generate tumor antigen-specific T cells from 3 out of 3 healthy donor samples, 1/1 sample from a patient in remission and 2/4 samples from patients at diagnosis, who were all HLA-A2+. For most samples, multiple rounds of agonist peptide stimulation were necessary to obtain further maturation. In contrast, generation of mature T cells from CD4+ CD8+ double positive cells in postnatal thymus or cord blood co-cultures, requires only 1 round of agonist peptide stimulation.

For the HLA-A2 negative samples, we were able to generate an adequate CD4+ CD8+ double positive population from 1/1 healthy donor sample, 3/3 samples from patients in remission and 0/1 sample from a patient at diagnosis. Agonist selection using a cell line seems inefficient as CD27 is not upregulated and cells did not mature to CD4+ or CD8+ single positive mature T cells.

Conclusion: We are currently co-culturing more samples using our protocol. Furthermore, we are investigating the effect of freezing and thawing on the in vitro T cell generation process (cell numbers and efficiency). Finally, we are also working on optimizing the protocol for generation of tumor antigen-specific T cells from HLA-A2 negative patient and healthy donor samples.