

One of the major immunological problems in patients following allogeneic hematopoietic stem cells (**alloHSCT**) is the delayed recovery of T lymphocytes, CD4 + T cells in particular. Post-transplant T cell immunity is mediated mainly by "ready to use" T lymphocytes derived directly from the graft, most of which are memory T cells. Unfortunately, these lymphocytes have a narrow TCR repertoire, which allows for recognizing only limited number of pathogens with which the patient has to cope. The graft contains also the small numbers of T lymphocytes CD4 + CD45RA + CD62L + CD31 + (so called **RTE lymphocytes**, recent thymic emigrants), which have a wider TCR repertoire, and thus have a greater immunoprotective potential against a variety of pathogens. While detailed reconstitution of T cells was fairly well described for the most commonly performed alloHSCT, from donor fully matched in HLA (so called **matched-HSCT** or MRD if from related and **MUD** if from unrelated donor), this aspect is still poorly understood for HSC transplantations from a donor matched only in half of the HLA antigen (haploidentical HSCT, **haplo-HSCT**). Although rarely performed, haplo-HSCT is becoming increasingly important in the absence of a donor compatible in HLA. Recently, it has become possible to carry out so-called unmodified haplo-HSCT, without prior elimination of alloreactive T lymphocytes from grafted material, which was elaborative, but prevented the occurrence of graft versus host disease (GvHD) in the recipient. In the novel approach the risk of GvHD is eliminated by the use of post-transplant cyclophosphamide (pt-Cy) which selectively kills alloreactive T lymphocytes *in vivo*. Our preliminary observations show the higher proportions of naive T lymphocytes, including RTEs and Treg lymphocytes (naive Treg: CD4 + CD25^{high} CD127⁻ CD45RA⁺), in haplo-HSCT patients compared with MUD-HSCT early after transplantation (+100 days after HSCT). This allowed us to formulate a hypothesis that the observed effect may be due to e.g. differences in post-transplant immunosuppressive regimen used in haplo-HSCT and matched-HSCT (pt-Cy vs ATG / CsA). According to well established knowledge, RTE lymphocytes are a particularly valuable cell population that, beside broad TCR repertoire, lacks alloreactivity. Moreover, RTE cells have the potential to differentiate into T memory stem cells (**Tscm**). Recently discovered Tscm lymphocytes has unique among other T cells features of "stemness", such as longevity, low level of differentiation, multipotentiality, ability of self-regeneration. This latter competence allows for rapid repopulation of Tscm even from a small initial pool of cells, which can be of great importance in T cell rebuilt process after alloHSCT. Interestingly, none of the previous studies evaluated the sensitivity (and maybe resistance) of RTEs and Tscm to commonly used immunosuppressants. We believe that the survival of such cells under immunosuppression could have a beneficial effect on the quality of immune system regeneration after HSC transplantation. Therefore, we decided to expand our preliminary research into the exploration of the immunological context that might underlay the observed effect. The primary objective of this project is a detailed early (on +100 post-HSCT) analysis of rare CD4 + T cell subpopulations (naive T lymphocytes, RTE, Tscm, naive Treg among others) in patients after haplo-HSCT vs MUD-HSCT. Specific objectives of the project are to find the potential correlation between the number of donor's RTE in the graft and proportion of RTE in the recipient after HSTC (repopulation efficiency). We are also interested in relationship between the initial number of RTEs and the appearance of stem T cells (Tscm) in patients' blood. The last step we plan detailed *ex vivo* analysis of RTEs resistance/sensitivity, their differentiation and selection in the presence of individual immunosuppressants used in GvHD prevention in patients after haplo-HSCT vs MUD-HSCT. In addition, the TCR repertoire will be evaluated in the T-cell pool to evaluate the mode of immunosuppressant action in the context of potential monoclonal T cell selection. The results obtained during the project will help to understand the immunological background of post-transplant rare T cells renewal in extremely different HLA-compatibility and in the presence of various immunosuppressive agents.