Learning the next-generation sequencing alphabet of immune reconstitution: factors determining CD8+ T-cell receptor α-chain repertoire dynamics after hematopoietic stem cell transplantation

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In this issue of *Haematologica*, Link-Rachner *et al.*[1] report their findings on CD8+ T-cell receptor-alpha (TRα) chain dynamics in patients after hematopoietic stem cell transplantation (HSCT) using next-generation sequencing (NGS) in relation to different treatment platforms. Their study aimed at unraveling the effect of post-transplant T-cell-depleting immunosuppressive therapy (namely anti-thymocyte globulin, ATG, and post-transplant cyclophosphamide, PTCy) and degree of HLA matching on the TRα diversity of naïve and memory CD8+ T-cell repertoires reconstituting the patient's peripheral blood in the first six months after transplantation. Furthermore, the authors attempted to determine the extent to which the donor's TRα repertoire influences the post-transplant repertoires in the respective patients.

The TR and its huge variability is one of the pillars of adaptive immunity. Healthy, diverse TR repertoires in normal individuals contain millions of different clones with unique TRs,[2] and provide the immune system with an arsenal of highly specific, yet also cross-reactive cells to fight off pathogens and malignant cells. Until recently, this extreme diversity limited a detailed and deep analysis of TR repertoires in healthy and pathological conditions, with most available techniques focusing on broad repertoire alterations, and extensive, cumbersome T-cell cloning required to investigate specific complementarity-determining region 3 (CDR3) variants. The advent of NGS-based high-throughput analysis of TRs has revolutionized the field of immune repertoire analysis.[3] TR NGS can now provide qualitative and quantitative information on hundreds of thousands of different T-cell clones directly from a single blood or tissue sample.

Hematopoietic stem cell transplantation is a field in which TR analysis is of extreme interest, both for medical and biological reasons. Patients undergoing allogeneic HSCT see the partial or nearly total elimination of their own hematopoietic system with radio- and/or chemotherapy followed by its replacement with that of a donor. In most cases, HSCT is the only curative therapy for the underlying disease. However, HSCT poses several risks for the patient, many of which derive from the ablation of their bone marrow and the concomitant risk of infection and pathogen reactivation. In addition, the new hematopoietic system can induce graft-*versus*-host disease (GvHD) associated with tissue and organ damage and, in some cases, death.[4] T-cell reconstitution dynamics is central to these post-transplant immune processes and represents an area of intense research in the HSCT field. High-throughput TR NGS has thus quickly attracted researchers eager to use its power to study post-HSCT T-cell clonal dynamics, its relationship to transplant-related factors, and its role in transplant complications.[5]

The study by Link-Rachner *et al.* contains a number of noteworthy aspects. Contrary to most previous reports, the researchers focus on the TRα chain. Most published TR analyses have studied the TRβ chain, probably because it is considered more diverse due to the added combinatorial potential conferred by the D segment, but also perhaps on account of the fact that TR NGS methodologies for this chain are more extended. However, both the α and the β chains contribute to TR specificity and, because of the order of the recombination events during T-cell maturation and development, one mature T cell can express two different functional α chains, both of which can pair with the cell's β chain, forming two different TR heterodimers.[6] Indeed, this happens in approximately 10% of the T cells in peripheral blood, and TRα CDR3 diversity has actually been observed to be 1.2-2.4 times greater than TRβ in T-cell subsets from a single individual.[7] Importantly, these 'dual' T cells have been associated with increased autoimmune and alloreactive capacities,[8] as well as with acute[9] and chronic[10] GvHD. Hence, analysis of TRα chains in HSCT is of special interest, and this study should encourage researchers to pay more attention to this locus in future analyses.

Furthermore, the authors designed their study in order to perform a comparison of TR dynamics between different clinical platforms. By including in their analysis patients transplanted from HLA-matched and -mismatched unrelated donors treated or not treated with ATG, haploidentical donors treated with PTCy, and related donors with no T-cell depletion (TD), the authors provide valuable insights into how these clinical platforms differ in terms of T-cell and TR dynamics during immune reconstitution at different time points in the first six months after HSCT. Despite the limited sample size, each of the groups was analyzed separately, leading to some interesting observations. First, the data suggest that TD has a stronger effect on the reconstitution of naïve than of memory T cells in terms of numbers and TRα diversity; both were significantly lower in patients treated with TD (Figure 1). Considering that diversity is deemed a hallmark of a 'healthy' T-cell repertoire,[11] this raises questions as to ways in which one could improve TR diversity in patients undergoing HSCT platforms with *in vivo* TD, as already suggested in the context of TRαβ-depleted grafts.[12]

In addition, the data from Link-Rachner *et al.* provide some intriguing new insights into the relative contribution of the donor's memory and naïve T-cell repertoires to

immune reconstitution after HLA-identical sibling HSCT in the absence of TD. The authors find that 60% and 11.5% of the TRα clonotypes in the patients' memory and naïve T cells at day 180 post transplantation could be traced back to the donors' memory repertoires, compared to only 30% and 15% to the donors' naïve repertoires, respectively (Figure 2). These data suggest that the donor's memory compartment is significantly reflected not only in the memory but also to a certain extent in the naïve CD8⁺ compartment after HLA-identical sibling HSCT. Given the high (>99%) purity of the FACS-sorted naïve and memory T-cell subsets used for the experiments, these intriguing findings are unlikely to have been impacted by sample spill-over. It should be noted that 2 of the 5 donors used for these analyses were cytomegalovirus (CMV) seropositive, while the remaining 3 donors were seronegative. In transplants performed under PTCy regimen, donor CMV seropositivity has been shown to correlate significantly with a predominance of donor CD8⁺ memory T-cell reconstitution post transplantation.[13] The data from Link-Rachner *et al.* suggest that this may hold true also for CMV seronegative donors in the non-TD setting, although a separate analysis of a larger number of seropositive and seronegative donors will be needed to verify this point.

The data also have potential practical implications. If the donor's memory repertoire plays a leading role in shaping the patient's repertoires after transplantation, the 'quality' of that donor memory repertoire might be assessed before transplant as a factor to be considered in donor selection, or as a prognostic tool for post-transplant complications. This observation becomes relevant also in

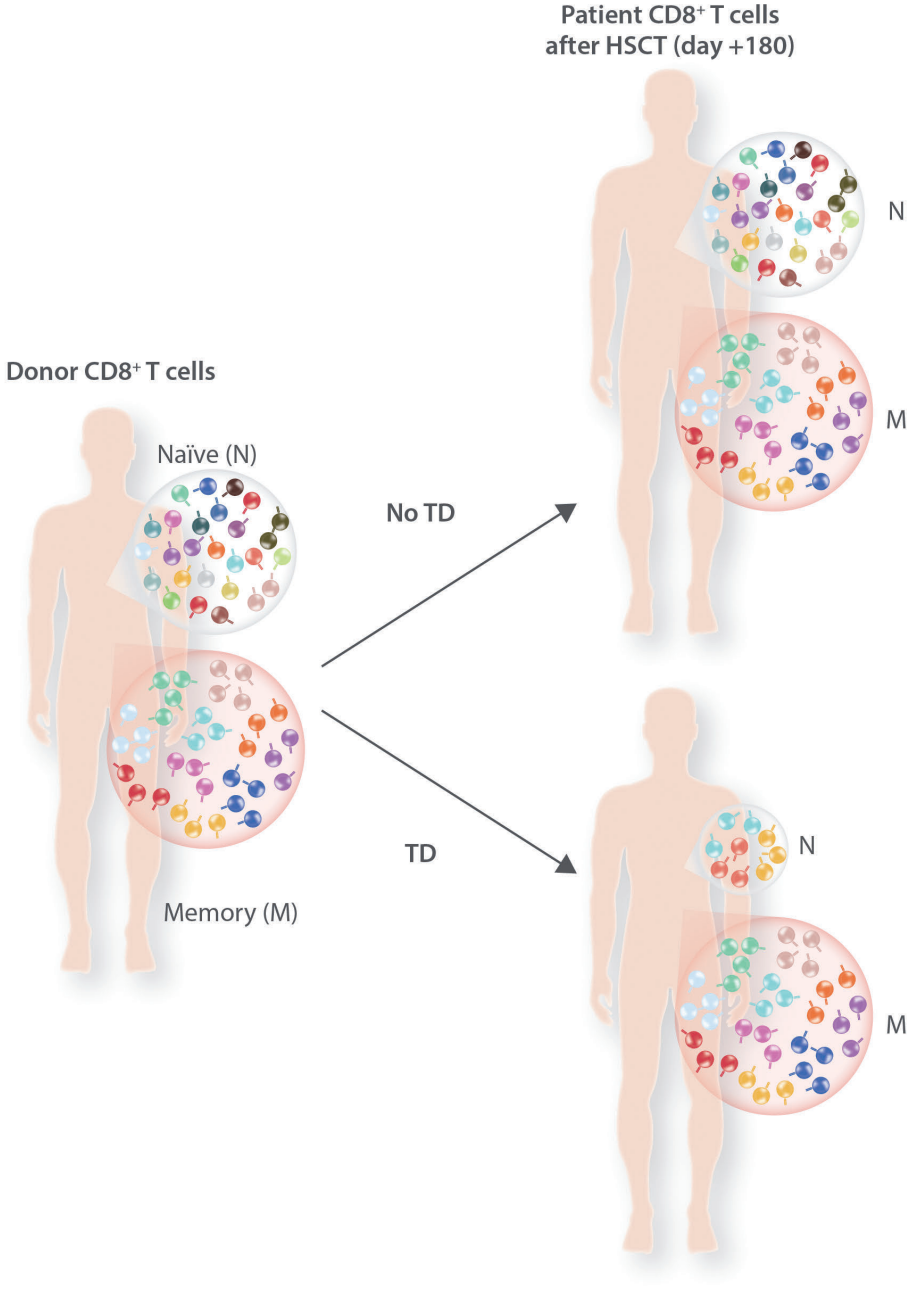

Figure 1. Impact of T-cell depletion (TD) on the size and diversity of the reconstituting naïve and memory CD8⁺ T-cell repertoires at six months post hematopoietic stem cell transplantation (HSCT). A schematic representation of the donors' (left) and the patients' naïve (N) or memory (M) CD8⁺ T-cell repertoires at day 180 post transplantation (right), in the presence (TD) or absence (No TD) of TD by either anti-thymocyte globulin or post-transplant cyclophosphamide. The size of the bubbles indicates the approximate relative size of the repertoires. Within each bubble, CD8⁺ T cells of identical T-cell receptor-α clonotypes are indicated by identical colors. A significant shrinking of both size and diversity of the naïve CD8⁺ T-cell repertoire occurs in the patient with TD compared to the patient without TD.

view of the interest in the depletion of naïve T cells from stem cell grafts in the related donor setting.[14] However, further research will be needed to understand if this occurs also on the other HSCT platforms, including cord blood transplantation,[15] and what the desired qualities of a donor repertoire are.

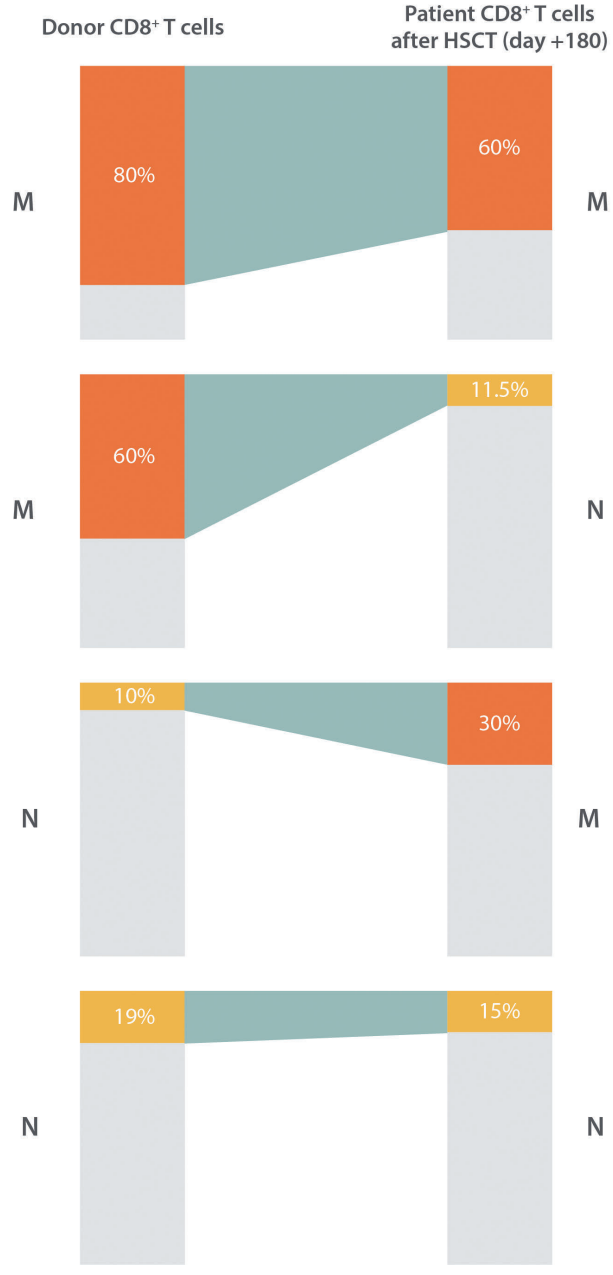

Figure 2. Relative contribution of donor memory or naïve CD8⁺ T cells to the patients' repertoires at six months post hematopoietic stem cell transplantation (HSCT). The average percentage of T-cell receptor-α clonotypes in memory (M; dark orange) or naïve (N; light orange) CD8⁺ T cells shared between 5 donors (left) and the respective patients at day 180 after HLA-identical sibling HSCT in the absence of T-cell-depleting immunosuppressive therapy. Shaded blue areas indicate shrinking or expansion of the relative percentage of shared clonotypes in the donor compared to the patient post transplantation. Non-shared clonotypes are indicated in gray. Note that an average of approximately 60% and 11.5% of the patients' memory and naïve repertoires, respectively, could be traced back to the donors' memory repertoires. In contrast, an average of only approximately 30% and 15% of the patients' memory and naïve repertoires, respectively, could be traced back to the donors' naïve repertoires. This figure uses data from Figure 2A of the study by Link-Rachner *et al.*[1]

The study by Link-Rachner *et al.* does leave some open questions that warrant further study. First, patients who relapsed were explicitly excluded from the study. However, relapse remains the main cause of treatment failure in HSCT,[16] and, similar to GvHD, a central role for T cells in the therapeutic graft-*versus*-leukemia effect has been well established.[17] Hence, NGS-based studies of T-cell repertoire characteristics that might associate with leukemia relapse after transplantation are of the foremost importance. Second, in this study, CD4⁺ T-cell reconstitution and repertoire dynamics were not analyzed, yet they are likely to play a central role in patients after HSCT, especially in clinical contexts where HLA-DPB1 mismatches are frequent (e.g. HSCT with unrelated donors).[18] Of note, the well-established permissiveness of a proportion of these HLA-DPB1 mismatches and its relationship with TR repertoire characteristics is also of interest and this is currently under investigation.[19] The delayed reconstitution of this T-cell compartment might pose methodological challenges for TR repertoire analyses, but that and its central role in the co-ordination of effective immune, as well as alloreactive responses, warrant special attention. Finally, while Link-Rachner *et al.* focus their attention on the TRα repertoire post HSCT, it would be interesting to assess whether the TRα and TRβ repertoires follow similar, complementary, or independent dynamics after different transplant settings. Parallel analysis of both chains, and potentially even attempting to determine their pairing with recent novel high-throughput approaches,[20] is likely to give an even more complete picture of TR immune reconstitution with clinical and translational relevance.

Overall, the study by Link-Rachner *et al.* illustrates how the power of NGS has revolutionized the assessment of immune repertoires and opened a broad spectrum of possibilities for unprecedented analysis of T-cell dynamics in the field of HSCT. Future studies building on this and other earlier pioneering work in this still developing field are called for to fully embrace this potential to enrich our understanding of T-cell immune reconstitution after HSCT. This knowledge should ultimately serve the objective of promoting the regeneration of a healthy TR repertoire that contributes both to the eradication of the underlying disease and to the control of transplant-related morbidities, maximizing the therapeutic potential of allogeneic HSCT.

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Mosaicism by somatic non-functional mutations: one cell lineage at a time

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Somatic mutations are abundant in most cells of our tissues.[1,2] The impact of any somatic mutation may be small and temporary if it occurs in differentiated cells without giving rise to malignant growth by unlocking their terminal differentiation. We expect a much wider and lasting impact from the same somatic mutations if they occur during earlier steps of differentiation. If they arise in a stem or progenitor cell, a whole set of cells (the lineage downstream of the progenitor) will be affected. Any such novel somatic mutation may stay with the individual for life, when different somatic mutations can accumulate in cell lineages over time.[3] The combination of somatic mutations becomes not only part of our lives, but contributes to our phenotype.[4] The variety of somatic mosaicism that will develop differs randomly among individuals and forms the basis of our healthy constitutions as well as of some medical conditions.

In this issue of the Journal, Dauber *et al.* present a technically advanced study[5] describing the molecular causes of mosaicism in 2 patients. The antigens of the Rhesus blood group system (ISBT 004) served as markers allowing the authors to take advantage of routine serology and detect affected individuals. The patients with red cell mosaicism were identified by the loss of the c antigen (RH4) in a subset of their red cells. The causes of this serological phenotype were traced to distinct precursor stages of myeloid and pluripotent stem cells, respectively.[5] As the antigens were only the markers and not the focus, this study, at the intersection of 'erythropoiesis gone wrong' and red cell antigens, has relevance beyond blood groups and offers valuable information to the hematology community.

Red cell mosaicism was documented in leukemia for ABO antigens in 1957,[6,7] and for Rh a few years later;[8] it has been documented for various blood groups many times since then.[5,9,10] During routine blood group typing worldwide, immunohematologists repeatedly encounter the incidental finding of a 'mixed field' agglutination or a discrepancy with previous results in the patient's health record. In both patients of the current study,[5] 'mixed field' agglutination was observed and prompted further investigation. Barring technical issues with the serology, such findings are infrequent, although not rare, in the absence of transfusions. Many patients could be identified with mosaicism that cannot be explained simply by recent transfusion, and these patients could then be followed up in order to evaluate the clinical implications. Possible causes are loss of heterozygosity (LOH) associated with loss of tumor suppressor gene functions,[11] or copy-neutral LOH associated with the gain of oncogenic mutations.[12,13]

Despite the discrepancy in blood group, the immediate transfusion support given to these patients is usually straightforward. Hence, further analysis is not considered necessary within the practical approach to clinical care. The clinical prognosis of LOH in hematologically asymptomatic patients is currently unknown, although the authors point out that this may now be changing. The diagnosis of red