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Thymus and immune reconstitution after allogeneic hematopoietic stem cell transplantation in humans: never say never again

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Abstract

Assessment of the host immune status is becoming a key issue in allogeneic hematopoietic stem cell transplantation (allo-HSCT). In the long-term follow-up of these patients, severe post-transplant infections, relapse or secondary malignancies may be directly related to persistent immune defects. In allo-HSCT, T-cell differentiation of donor progenitors within the recipient thymus is required to generate naive recent T-cell emigrants (RTE). These cells account for a durable T-cell reconstitution, generating a diverse T-cell receptor (TCR) repertoire and robust response to infections. It is now possible to quantify the production of RTE by measuring thymic T-cell receptor excision circles or 'TREC' which are small circular DNA produced during the recombination of the genomic segments encoding the TCR alpha chain. Here we discuss the role of thymic function in allo-HSCT. The pre-transplant recipient thymic function correlates with clinical outcome in terms of survival and occurrence of severe infections. Post-transplant, TREC analysis showed that the thymus is a sensitive target to the allogeneic acute graft-versus-host disease (GvHD) reaction but is also prone to recovery in young adult patients. In all, thymus is a key player for the quality of immune reconstitution and clinical outcome after allo-HSCT. Thymic tissue is plastic and it is a future challenge to halt or reverse thymic GVHD therapeutically by acting at the level of T-cell progenitors generation, thymic homing and/or epithelial thymic tissue preservation.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is widely used in the treatment of hematological malignancies as an immunotherapy effective through graft-versus-leukemia (GvL) reaction. This curative allogeneic response can have severe drawbacks, such as the frequent and severe graft-versus-host disease (GvHD). Another main concern is the profound and long-lasting immunodeficiency consecutive to this procedure, especially now with the development of innovative strategies such as umbilical cord blood transplantation (CBT) or transplants from haploidentical family donors (Haplo-HSCT). A potent immune reconstitution (IR) is essential to limit the infection risk and disease relapse. Reconstitution of the different lymphocyte populations (B, T, NK, NKT) and antigen presenting cells of myeloid origin (monocytes, macrophages and dendritic cells) should be considered not only quantitatively but also foremost qualitatively in terms of functional subsets. As the general parameters of IR in allo-HSCT have been recently reviewed extensively in adults and children (1–3), we aim to focus here on some aspects of T-cell reconstitution, especially the role played by the thymus in allo-HSCT, which appears to be pivotal for long-term reconstitution and could be the target of therapeutic improvements.

Naive/memory T-cells homeostasis after allo-HSCT: why the thymus is a key player?

Donor lymphoid progenitors undergo both a process of differentiation and maturation in the peculiar surroundings of lymphopenia and allogeneicity in the host. T-cell reconstitution takes place progressively under the so-called 'thymic-dependent' pathway, in addition to the expansion of mature T-cells from the transplant (Figure 1). Indeed, the recovery of T-cell counts during the first 6 months after transplant

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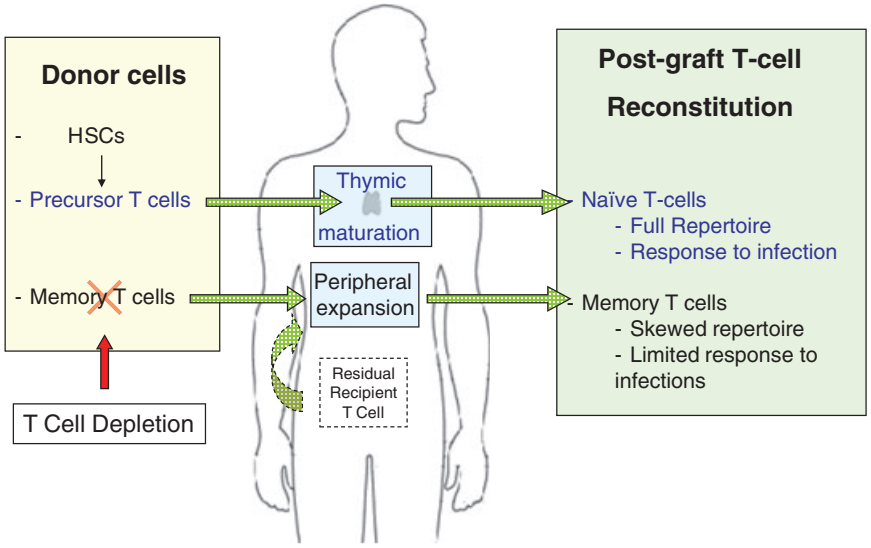

Figure 1 T-cell reconstitution after allogeneic hematopoietic stem cell transplantation (allo-HSCT) proceeds through two different pathways: - Homeostatic peripheral expansion of mature T-cells from the transplant or residual recipient T-cells. - *De novo* generation of recent T-cell emigrants from donor lymphoid progenitors. The respective importance of these two pathways are function of the transplant source (bone marrow *vs* hematopoietic stem cells (HSC) from cord blood or peripheral blood), manipulation (T-cell depletion or not) and recipient conditioning (myeloablative *vs* non-myeloablative).

relies mainly on the cytokine-driven [interleukin (IL)-15, IL-2, IL-7] peripheral expansion of mature T-cells. These mature T-cells originate either from the donor in the case of a non-T-cell depleted (TCD) bone marrow (BM) transplant or from host T-cells having survived the conditioning regimen in the case of a T-cell depleted transplant (4). They respond quickly to previously encountered pathogens, are easier to trigger, faster to respond and enter tissues more readily than naive T-cells. They are frequently directed toward periodically reactivated herpes viruses [cytomegalovirus (CMV) or Epstein Barr virus (EBV)] which they help keep under control. This could explain the fewer viral infections recorded after blood compared to marrow HSCT (5).

In the long term, broad immune responses need the reconstitution of a naive T-cell repertoire able to respond to a broad range of pathogens encountered by the host and to tumor antigens. Reconstitution of this compartment is a long-time ongoing process which implies a functional recipient thymus to recapitulate a complete T-cell ontogeny. Naive T-cells also seem more dependent than memory T-cells upon recognition of self-major histocompatibility complex (MHC)-peptide complexes for their survival in the periphery. Therefore, MHC mismatches may be considered detrimental for IR in many aspects, including impaired thymic selection but also homeostasis of the naive T-cell compartment.

Naive and memory lymphocyte populations are mainly assessed by surface phenotyping; however, classical markers such CD45RO for memory T-cells and CD45RA or CD62L for naive T-cells are not always reliable. Naive T-cells may undergo expansion without phenotypic changes and have a long lifespan (6). In addition, memory CD45RO+ T-cells may revert to a naive CD45RA+ phenotype, especially in the case of persistent herpes virus infections (7). Therefore, other markers should be added to definitely assess naive T-cells and the different categories of memory T-cells. CCR7, a molecule involved in the homing of T-cells to lymph nodes, CD27 and CD28, are the most useful markers for naive *vs* memory T-cell phenotyping.

T-cell diversity can be directly evaluated by 'immunoscope' or 'spectratyping' based on the size diversity analysis of the CDR3 β-chain region as an index of the diversity of the whole αβ-T-cell population. T-cell diversity is almost exclusively accounted for by the naive population. Indeed, in healthy adults, memory T-cells, which account for approximately 1/3 of the total T-cells, contribute less than 1% of the αβ-T-cell diversity (8). A practical consequence for HSCT is that evaluation of the T-cell repertoire diversity reflects the extent of the naive T-cell compartment. Overall, early after HSCT (within 6 months after graft), many abnormalities of the T-cell repertoire occur and are difficult to correlate with the clinical status of the patient (9). Conversely, late after graft (after 1 year at least) and in a still ongoing process even 2–3 years post-transplant, it is possible to correlate repertoire diversity with the occurrence of GvHD, severe infectious complications, relapse or hematopoietic chimerism in the case of TCD (9–11). T-cell repertoire reconstitution is delayed in cases of T-cell depletion or in CD34+ purified grafts and is improved in cases of full donor hematopoiesis. Techniques of T-cell receptor (TCR) β-chain sequencing have also clearly separated T-cell clones mediating GvHD and GvL as a proof of principle to monitor GvHD-causing clones in HSCT recipients (12).

Finally, as we stated above, a '*bona fide*' T-cell reconstitution is dependent on the recapitulation of T-cell ontogeny from progenitors of donor origin in the recipient thymus. This is obviously a non-physiological situation which maybe impacted by GvHD and its immunosuppressive treatments. Recovery through the thymic-dependent pathway accounts for the durable and clonally diverse reconstitution of the T-cell compartment. It is now clear from different studies that this thymic-dependent long-term T-cell reconstitution is a slow process that can be influenced by several factors including:

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age of patients, human leukocyte antigen (HLA) mismatches, source of stem cells or the occurrence of GvHD (1–3). The setting of the graft is critical. Indeed, as TCD and haploidentical HSCT will depend solely on thymopoiesis for IR.

How to assess thymopoiesis in humans: lessons from physiology and aging

The homeostatic maintenance of a diverse repertoire in the peripheral lymphocyte pool is dependent on the generation of functional thymocytes throughout adult life. A direct access to the thymus is obviously impossible for ethical reasons in humans. A direct evaluation of thymic size and cellularity is possible through tomography imaging, showing for instance a rebound in thymic mass in children after chemotherapy and in adults after autologous HSCT (13). However, this technique is inadequate in a routine monitoring of patients and only indirectly tells about functional recovery. Lymphocytes leaving the thymus or 'recent thymic emigrants' (RTE) can now be evaluated directly *ex vivo* by measuring the episomal DNA excision circles of the TCR δ locus deleted during recombination of the α-locus present in the vast majority (>70%) of functional αβ-T-cells (10, 14, 15). This is the TCR rearrangement excision DNA circles or 'sjTREC' assay which is a totally noninvasive technique requiring only a small amount of blood and suitable for cohort follow-up (Figure 2). Excision circles are episomal DNA not replicated during cell division. T-cell receptor excision circles (TREC) values reflect thymic output but may also be influenced by peripheral T-cell proliferation. This should be taken into account especially in the post-transplant period where lymphoid homeostasis is unbalanced. For that reason, sjTREC should be calculated as copy numbers/CD3+ T-cell counts and as absolute numbers of sjTREC/ml of peripheral blood which are directly proportional to the RTE amount. The same principle applies to βTREC produced during the TCR β-chain recombination at an early stage of T-cell differentiation (15, 16). The ratio of sjTREC/βTREC indicates the proliferative ability of thymic progenitors within the thymus (Figure 2). Some phenotypic markers have also been proposed to stain RTE, such as CD31 (PECAM-1), naive CD31+CD4 T-cells having a higher sjTREC content than the CD31-counterpart (17). However, CD31 expression can be maintained during cytokine-driven proliferation of CD4 T-cells, making the expression staining analysis more difficult to interpret in terms of thymopoiesis.

Aging is a major parameter impacting on thymic function. That said, even if thymic involution begins shortly after birth, with a 3%/year decrease through middle age (35–45 years of age), thymopoiesis persists into at least the fifth decade of life, and the involution process can be potentially halted or reversed (18). T-cell maturation requires a special environment structured into an outer cortex and inner medulla. Intrathymic T-cell development requires an intimate contact between various types of stromal cell types interacting with early T-cell progenitors (ETP) at different differentiation steps. ETP commit to the T-cell lineage after engagement of the Notch1 receptor with delta-like 4 (DL4) ligand on cortical thymic epithelial cells (TEC). The loss of a clear morphological demarcation between cortex and medulla is one hallmark of thymus senescence and reflects the disruption of TEC/ETP crosstalk. In addition, crucial cytokines are produced by stromal TEC and their expression level is affected during aging (19): leukemia inhibitory factor (LIF), oncostatin M (OSM), interleukin (IL)-6 and stem cell factor (SCF) are increased with age and their administration to mice causes thymic atrophy. Conversely, growth hormone (GH) and insulin-like growth factor (IGF) decrease with thymic atrophy (20). IL-7 transcripts remain stable with age in unseparated thymi in humans (19) arguing against a major role for this cytokine in thymic involution. However, data in mice showed more precisely that IL-7 expressing TEC are located at the cortico-medullary junction and decrease with age (21). Other factors intricately affect thymic senescence: sex hormones (estrogens and androgens), lutenizing hormone releasing hormone (LHRH) and proinflammatory cytokines (IL-6, IFN-α and TNF-α). Conditions other than aging could therefore affect thymopoiesis such as malnutrition and infection associated with increased levels of corticosteroids and decreased concentrations of leptin (22). Finally, especially relevant in the context of HSCT, γ-irradiation exposure results in damage to thymocytes and TEC through a transforming growth factor (TGF)-β-dependent mechanism (23).

Pre-transplant thymic function is influenced by the primary disease and may be associated with post-transplant clinical outcome

The beneficial impact of young age on HSCT outcomes suggests a strong role of the thymus in post-transplant events. However, as stated before, thymic function decreases with age but also shows a large heterogeneity within a same age range, especially in adults (Figure 3). Thus, it could be questioned whether age or thymic function *per se* is the main parameter associated with HSCT outcome. We measured recipient pre-transplant sjTREC levels in a genoidentical-HSCT setting without T-cell depletion to answer that question, taking into account in multivariate analysis the other known factors of transplant outcome (sex mismatch, disease status, ABO incompatibility, donor/recipient CMV status) (24). Before HSCT, sjTREC levels were significantly lower in patients affected by a hematological malignancy *vs* nonmalignant diseases or healthy controls, as reported in children affected with T-cell malignancies compared with normal age-matched controls (25). However, because sjTREC number was also found to decrease with a higher disease risk, we cannot exclude an influence of pre-graft treatment on sjTREC

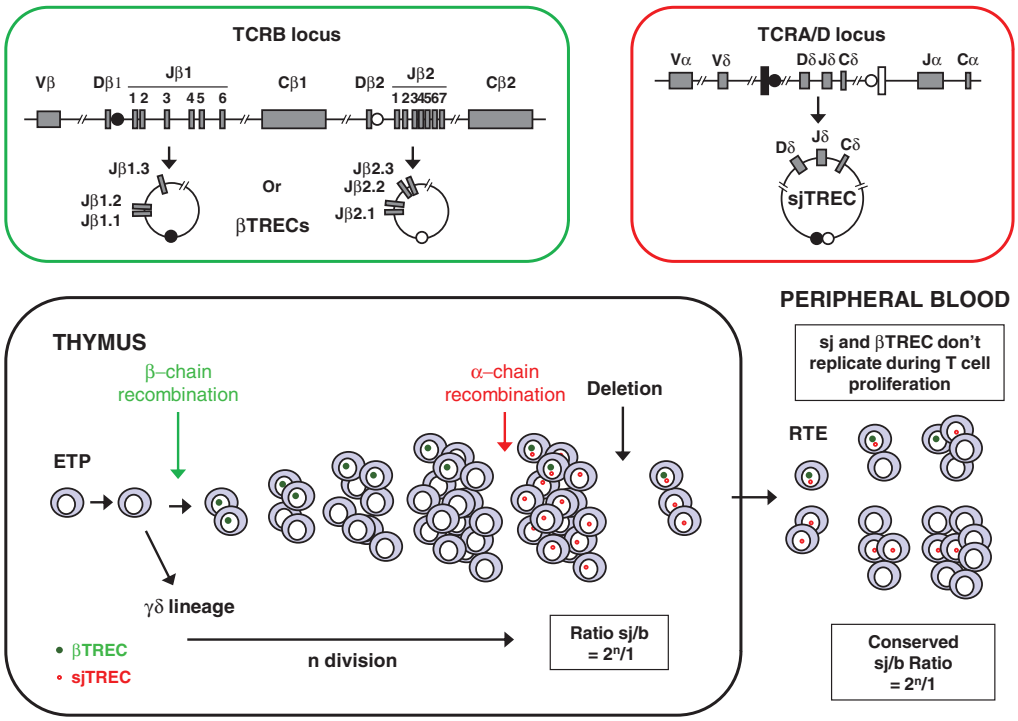

Figure 2 Thymic function can be assessed *ex vivo* by quantification of TREC (T-cell receptor excision circles). sjTREC arising from T-cell receptor (TCR) delta locus excision during alpha chain recombination (TCRA/D locus, upper right) are shown in red and βTREC produced during β-chain recombination (TRCB locus, upper left) are indicated in green. ETP, early thymic progenitors; RTE, recent T-cell emigrants.

levels. Nevertheless, although disease severity was associated with low sjTREC, multivariate analysis showed an independent influence of sjTREC on survival. Patients having low pre-transplant sjTREC had a statistically significant lower survival rate independently of other pre-transplant parameters, especially age. We observed an association between low TREC before graft and acute GvHD (aGvHD), especially grades II–IV, and extensive chronic GvHD (cGvHD). We also found an association between low sjTREC and a higher incidence of bacterial infections and CMV reactivation. A high incidence of infections has also been associated with low sjTREC before autologous HSCT in myeloma patients (26). Considering the strong link between GvHD and the occurrence of opportunistic infections, the impact of host pre-transplant immune function on both is unsurprising.

In all, multivariate analysis highlighted the value of assessing recipient thymic function as a major pre-transplant factor influencing HSCT outcome. Main correlations were found with 3-year survival and the incidence of bacterial and CMV infection after transplant. The simplest explanation is that the residual recipient thymic function after conditioning and transplant is dependent on the pre-transplant thymic activity itself. Residual thymic activity could be a major factor for donor lymphoid progenitors engraftment and differentiation after transplant. This is consistent with the reports in children showing that the rate of thymic-dependent

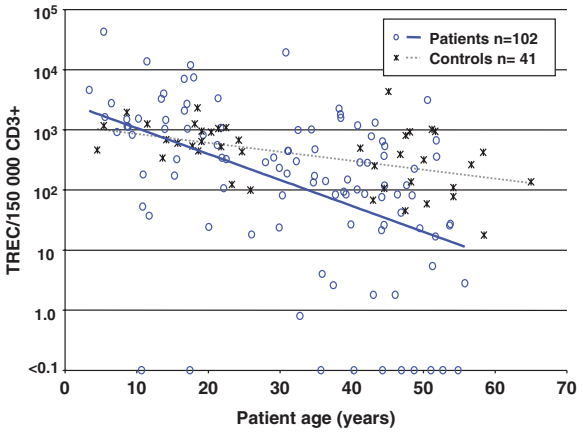

Figure 3 T-cell receptor excision circles (TREC) values/150,000 CD3 positive lymphocytes are expressed in patients given an allo-HSCT (•) or healthy controls (x) in function of age (years).

T-cell reconstitution is dependent of the thymic function before allo-HSCT (27, 28).

The thymus is a sensitive but plastic target in acute GvHD

The sjTREC assay as *ex vivo* markers of thymic function has been used in allo-HSCT monitoring by different groups

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showing that sjTREC levels are low until 3–6 months after allo-HSCT. Low sjTREC values are not only associated with increasing patient age and T-cell depletion but also mainly with GvHD, leukaemia relapse or opportunistic infections (29, 30). As shown by different groups in different myeloablative and non-myeloablative conditioning (31), patients affected with cGvHD have a very low and usually undetectable thymic function. High TREC levels and a broad T-cell repertoire have been associated with an efficient IR after CBT in the long term (10). Interestingly, the level of thymopoiesis recovery has been directly linked to CMV clearance and survival after double CBT (32). Experimental models in mice showed that the thymus is very sensitive to an allogeneic attack, notably through IFN-γ-induced TEC apoptosis (23, 33). We recently precised further some mechanisms of thymic impairement during aGvHD in an intrafamilial HLA-genoidentical HSCT setting in adults (34).

Acute GvHD delays the thymic-dependent T-lymphocyte recovery

Although total T-cell number recovery was not significantly affected by the occurrence of aGvHD, the reconstitution of the CD4+CD45RA+ CD62L+ naive compartment and the level of RTE assessed by sjTREC were significantly lower in patients with aGvHD at 6 and 12 months after graft. T-cell repertoire data confirmed this result showing a deeper oligoclonality in case of aGvHD.

The thymus is very sensitive to acute GvHD

Although grade I aGvHD has limited clinical manifestations and does not require corticosteroids, we observed that thymic output early after graft was significantly affected even in grade I as well as in grades II–IV compared with patients who did not experience aGvHD (Figure 4). This distinguishes the thymic impact of aGvHD from steroids treatment. This also showed that clinical and pathological definition of aGvHD could be in some way different from its actual impact on primary lymphoid organs and supports in humans the concept of a 'thymic GvHD'. This thymic sensitivity to aGvHD may have consequences in terms of HSCT treatment. Decreasing the incidence of aGvHD with aggressive GvHD prophylaxis and treatment, or other approaches such as selective allo T-cell depletion, could temper its effect on thymus.

Acute GvHD effect on thymic function is reversible in younger adult patients

Age-related thymic involution begins with sex steroid increase at puberty (18). Age together with aGvHD could synergize to delay IR after allo-HSCT in adults. Indeed, patients older than 25 years had the lowest sjTREC levels before transplantation and showed also a minimal recovery in the presence or absence of aGvHD up to 1 year after graft. Recovery of

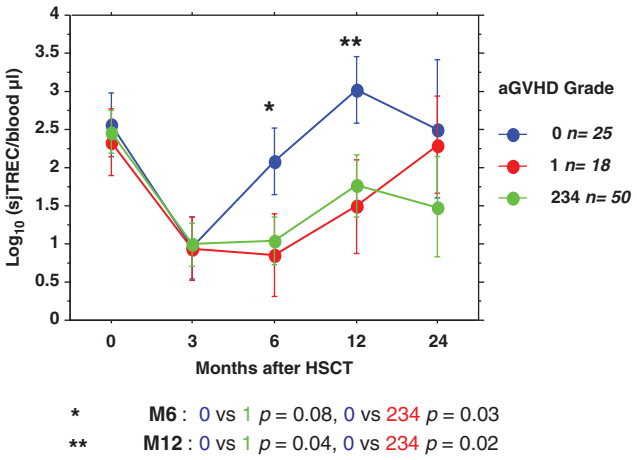

Figure 4 T-cell receptor excision circles (TREC) values were followed before transplant and 3, 6, 12, 24 months after transplant in a series of 93 patients described by Clave et al. (34). Even grade 1 acute graft-versus-host disease (GvHD) statistically impacted thymic function.

sjTREC level was much more efficient in younger patients. aGvHD significantly impacted thymic output at month 6 after HSCT but thereafter thymic function recovered, reaching pre-transplant values at 1 year after graft. Therefore, we concluded that in younger patients (<25 years of age), the thymic impact of an episode of aGvHD could be fully reversible. Conversely, chronic GvHD had a persistent effect on thymic function independent of the recipient's age.

Acute GvHD-induced delay in thymic function recovery is not primarily because of a default in intrathymic proliferation

To study the mechanisms of aGvHD in thymic function recovery after allo-HSCT, we analyzed a series of age-matched patients through the quantification of sj and βTREC, their ratio reflecting the intrathymic αβ-T-cells proliferation rate between β- and α-chain recombination (16). We showed that βTREC levels declined in proportion to sjTREC at month 6 post-transplant, suggesting that the decreased thymic output during aGvHD is not due primarily to a decline in thymocyte proliferation but possibly linked to steps earlier than TCR β-chain recombination. This suggests a different mechanism other than aging (16).

Thymic rejuvenation in allo-HSCT: a matter of niches

The challenge to improve IR through thymopoiesis should take into account the sequential steps from hematopoietic stem cell generation in the BM, ETP homing in the thymus, thymocyte differentiation through the thymic microenvironment, RTE egress and peripheral homeostasis of naive T-cells (Figure 5). Protecting hematopoietic niches from

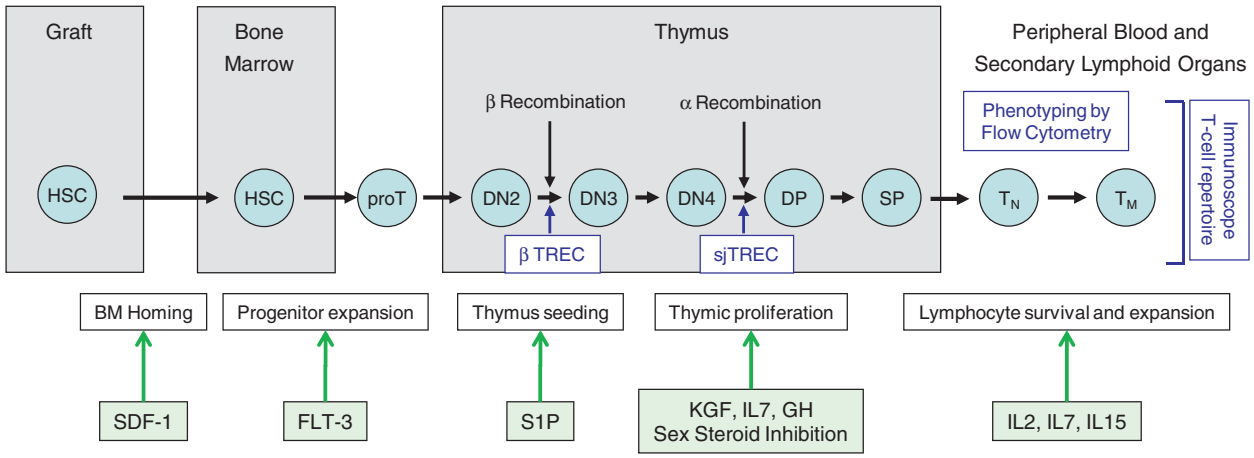

Figure 5 Improving T-cell reconstitution after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Strategies aim to act on hematopoietic stem cells (HSC), lymphoid T-cell progenitors (proT), thymic differentiation and peripheral T-cell homeostasis. The last two aspects can be followed by T-cell excision circles (TREC) quantification (Figure 2), lymphocyte phenotyping by flow cytometry and T-cell diversity by immunoscope (T-cell repertoire) analysis. This could be achieved by cytokines [interleukin (IL)-2, IL-7, IL-15], chemokines (SDF-1, S1P), growth factors (FLT-3) and/or neuroendocrine hormones (GH, growth hormone, sex steroid ablation) acting on thymic epithelium and intratthymic proliferation. Some of these therapies have already been included in human clinical trials (KGF, keratinocyte growth factor, IL-7).

aGvHD (35), *ex vivo* expansion of T-cell progenitors (36) and/or gaining accessibility to thymic niches (37) are straightforward approaches. Experimental models, especially in mice, but also now in human clinical trials, have shown that it is possible to increase thymic function after HSCT through the stimulation of the GH pathway (38) or sex steroid blockade (39), that are supposed to counterbalance the effect of aging on the thymic function. Finally, treatments with exogenous IL7 (40), FLT3L or keratinocyte growth factor (KGF) (41) have been shown to increase thymic output and promote T-cell precursor survival and naive T-cell homeostasis. In allo-HSCT, the use of these therapeutic agents, alone or in combination, could be a way to alleviate the effects of age and GvHD on the restoration of a potent thymic function (42, 43).

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Conflict of interests

The authors have declared no conflicting interests.

References

1. Mackall C, Fry T, Gress R, Peggs K, Storek J, Toubert A. Background to hematopoietic cell transplantation, including post transplant immune recovery. *Bone Marrow Transplant* 2009: **44**: 457–62.
2. Seggewiss R, Einsele H. Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update. *Blood* 2010: **13**: 3861–8.
3. Cavazzana-Calvo M, André-Schmutz I, Dal Cortivo L et al. Immune reconstitution after hematopoietic stem cell transplantation: obstacles and anticipated progress. *Curr Opin Immunol* 2009: **21**: 544–8.
4. Roux E, Dumont-Girard F, Starobinski M et al. Recovery of immune reactivity after T cell-depleted bone marrow transplantation depends on thymic activity. *Blood* 2000: **96**: 2299–303.
5. Storek J, Dawson MA, Storer B et al. Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation. *Blood* 2011: **97**: 3380–9.
6. Bains I, Antia R, Callard R, Yates AJ. Quantifying the development of the peripheral naive CD4+ T-Cell pool in humans. *Blood* 2009: **28**: 5480–7.
7. Bouneaud C, Garcia Z, Kourilsky P, Pannetier C. Lineage relationships, homeostasis, and recall capacities of central-and effector-memory CD8 T cells in vivo. *J Exp Med* 2005: **201**: 579–90.
8. Arstila TP, Casrouge A, Baron V, Even J, Kanellopoulos J, Kourilsky P. A direct estimate of the human alpha beta T cell receptor diversity. *Science* 1999: **286**: 958–61.
9. Gorski J, Yassai M, Zhu X, Kissela B, Keever C, Flomenberg N. Circulating T cell repertoire complexity in normal individuals and bone marrow recipients analyzed by CDR3 size spectratyping. Correlation with immune status. *J Immunol* 1994: **152**: 5109–19.
10. Talvensaari K, Clave E, Douay C et al. Abroad T-cell repertoire diversity and an efficient thymic function indicate a favorable long-term immune reconstitution after cord blood stem cell transplantation. *Blood* 2002: **99**: 1458–64.
11. Wu CJ, Chillemi A, Alyea EP et al. Reconstitution of T-cell receptor repertoire diversity following T-cell depleted allogeneic bone marrow transplantation is related to hematopoietic chimerism. *Blood* 2000: **95**: 352–59.

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12. Michalek J, Collins RH, Hill BJ, Brenchley JM, Douek DC. Identification and monitoring of graft-versus-host specific T-cell clone in stem cell transplantation. *The Lancet* 2003: **361**: 1183–185.
13. Hakim FT, Memon SA, Cepeda R et al. Age-dependent incidence, time course, and consequences of thymic renewal in adults. *J CIin Invest* 2005: **115**: 930–9.
14. Douek DC, Mc Farland RD, Keiser PH et al. Changes in thymic function with age and during the treatment of HIV infection. *Nature* 1998: **396**: 690–5.
15. Dion ML, Sékaly RP, Cheynier R. Estimating thymic function through quantification of T-cell receptor excision circles. *Methods Mol Biol* 2007: **380**: 197–213.
16. Dion ML, Poulin JF, Bordi R et al. HIV infection rapidly induces and maintains a substantial suppression of thymocyte proliferation. *Immunity* 2004: **21**: 757–68.
17. Azevedo RI, Soares MV, Barata JT et al. IL-7 sustains CD31 expression in human naive CD4+ T cells and preferentially expands the CD31+ subset in a PI3K-dependent manner. *Blood* 2009: **113**: 2999–3007.
18. Lynch HE, Goldberg GL, Chidgey A et al. Thymic involution and immune reconstitution. *Trends Immunol* 2009: **30**: 366–73.
19. Sempowski GD, Hale LP, Sundy JS et al. Leukemia inhibitory factor, oncostatin M, IL-6, and stem cell factor mRNA expression in human thymus increases with age and is associated with thymic atrophy. *J Immunol* 2000: **164**: 2180–7.
20. Chen BJ, Deoliveira D, Spasojevic I et al. Growth hormone mitigates against lethal irradiation and enhances hematologic and immune recovery in mice and nonhuman primates. *PLoS One* 2010: **5**: e11056.
21. Aves NL, Richard-Le Goff O, Nicholas D et al. Characterization of the thymic IL-7 niche in vivo. *Proc Natl Acad Sci USA* 2009: **106**: 1512–17.
22. Hick RW, Gruver AL, Ventevogel MS et al. Leptin selectively augments thymopoiesis in leptin deficiency and lipopolysaccharide-induced thymic atrophy. *J Immunol* 2006: **177**: 169–76.
23. Hauri-Hohl MM, Zuklys S, Keller MP et al. TGF-beta signaling in thymic epithelial cells regulates thymic involution and postirradiation reconstitution. *Blood* 2008: **112**: 626–34.
24. Clave E, Rocha V, Talvensaari K et al. Prognostic value of pretransplantation host thymic function in HLA-identical sibling hematopoietic stem cell transplantation. *Blood* 2005: **105**: 2608–13.
25. Petridou E, Klimentopoulou AE, Moustaki M, Kostrikis LG, Hatzakis A, Trichopoulos D. Recent thymic emigrants and prognosis in T- and B-cell Childhood hematopoietic malignancies. *Int J Cancer* 2002: **101**: 74–7.
26. Svaldi M, Lanthaler AJ, Dugas M et al. T-cell receptor excision circles: a novel pronostic parameter for the outcome of transplantation in multiple myeloma patients. *Br J Haematol* 2003: **122**: 795–801.
27. Chen X, Barfield R, Benaim E et al. Prediction of T-cell reconstitution by assessment of T-cell receptor excision circle before allogeneic hematopoietic stem cell transplantation in pediatric patients. *Blood* 2005: **105**: 886–93.
28. Olkinuora H, Talvensaari K, Kaartinen T et al. T cell regeneration in pediatric allogeneic stem cell transplantation. *Bone Marrow Transplant* 2007: **39**: 149–56.
29. Hazenberg MD, Otto SA, de Pauw ES et al. T-cell receptor excision circle and T-cell dynamics after allogeneic stem cell transplantation are related to clinical events. *Blood* 2002: **99**: 3449–53.
30. Poulin JF, Sylvestre M, Champagne P et al. Evidence for adequate thymic function but impaired naive T-cell survival following allogeneic hematopoietic stem cell transplantation in the absence of chronic graft-versus-host disease. *Blood* 2003: **102**: 4600–7.
31. Castermans E, Hannon M, Dutrieux J et al. Thymic recovery after allogeneic hematopoietic cell transplantation with non-myeloablative conditioning is limited to patients younger than 60 years of age. *Haemetologica* 2011: **96**: 298–306.
32. Brown JA, Stevenson K, Kim HT et al. Clearance of CMV viremia and survival after double umbilical cord blood transplantation in adults depends on reconstitution of Thymopoiesis. *Blood* 2010: **115**: 4111–9.
33. Na IK, Lu SX, Yim NL, Golberg GL et al. The cytolytic molecules Fas ligand and TRAIL are required for murine thymic graft-versus-host disease. *J Clin Invest* 2010: **12**: 343–56.
34. Clave E, Busson M, Douay C et al. Acute graft-versus-host disease transiently impairs thymic output in young patients after allogeneic hematopoietic stem cell transplantation. *Blood* 2009: **113**: 6477–84.
35. Shono Y, Ueha S, Wang Y et al. Bone marrow graft-versus-host disease: early destruction of hematopoietic niche after MHC-mismatched hematopoietic stem cell transplantation. *Blood* 2010: **115**: 5401–11.
36. Zakrzewski JL, Kochman AA, X Lu S et al. Adoptive transfer of T-cell precursors enhances T-cell reconstitution after allogeneic hematopoietic stem cell transplantation. *Nature Med* 2006: **12**: 1039–47.
37. Fewkes NM, Krauss AC, Guimond M, Meadors JL, Dobre S, Mackall CL. Pharmacologic modulation of niche accessibility via tyrosine kinase inhibition enhances marrow and thymic engraftment after hematopoietic stem cell transplantation. *Blood* 2010: **115**: 4120–9.
38. Napolitano LA, Schmidt D, Gotway MB et al . Growth hormone enhances thymic function in HIV-1-infected adults. *J Clin Invest* 2008: **118**: 1085–98.
39. Sutherland JS, Spyroglou L, Muirhead JL et al. Enhanced immune system regeneration in humans following allogeneic or autologous hemopoietic stem cell transplantation by temporary sex steroid blockade. *Clin Cancer Res* 2008: **14**: 1138–49.
40. Sportès C, Hakim FT, Memon SA et al. Administration of rhIL-7 in humans increases in vivo TCR repertoire diversity by preferential expansion of naive T cell subsets. *J Exp Med* 2008: **205**: 1701–14.
41. Kelly RM, Goren EM, Taylor PA et al. Short-term inhibition of p53 combined with keratinocyte growth factor improves thymic epithelial cell recovery and enhances T-cell reconstitution after murine bone marrow transplantation. *Blood* 2010: **115**: 1088–97.
42. Holland AM, Van Den Brink MRM. Rejuvenation of the aging T cell compartment. *Curr Opin Immunol* 2009: **21**: 454–59.
43. Holländer GA, Krenger W, Blazar BR. Emerging strategies to boost thymic function. *Curr Opin Pharmacol* 2010: **10**: 443–53.