T cell regeneration after immunological injury

Enrico Velardi[1]✉, *Jennifer J. Tsai*[2,3] *and Marcel R. M. van den Brink*[2,4,5]✉

Abstract | Following periods of haematopoietic cell stress, such as after chemotherapy, radiotherapy, infection and transplantation, patient outcomes are linked to the degree of immune reconstitution, specifically of T cells. Delayed or defective recovery of the T cell pool has significant clinical consequences, including prolonged immunosuppression, poor vaccine responses and increased risks of infections and malignancies. Thus, strategies that restore thymic function and enhance T cell reconstitution can provide considerable benefit to individuals whose immune system has been decimated in various settings. In this Review, we focus on the causes and consequences of impaired adaptive immunity and discuss therapeutic strategies that can recover immune function, with a particular emphasis on approaches that can promote a diverse repertoire of T cells through de novo T cell formation.

Thymic epithelial cells (TECs). The major component of thymic stroma that supports all stages of thymocyte development. They are further divided into cortical and medullary TECs on the basis of their localization within the thymus and are crucial for the positive and negative selection of thymocytes, respectively.

[1]*Department of Pediatric Hematology and Oncology, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy.*

[2]*Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA.*

[3]*Department of Medicine, State University of New York, Downstate Medical Center, Brooklyn, NY, USA.*

[4]*Department of Immunology, Memorial Sloan Kettering Cancer Center, New York, NY, USA.*

[5]*Department of Microbiology and Immunology, Weill Cornell Medical College, New York, NY, USA.*

✉*e-mail: enrico.velardi@opbg.net; vandenbm@mskcc.org*

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Recovery of immunocompetence after periods of haematopoietic stress or injury is crucial not only for efficient responses against pathogens and tumour antigens but also for optimal responses to immunotherapy for cancer. In contrast to the early recovery of innate cells, including neutrophils, natural killer (NK) cells and monocytes, adaptive immune cells, in particular T cells, recover at a much slower pace and are particularly sensitive to negative insults caused by infections or cytoreductive chemotherapy and radiotherapy. Constrictions in the diversity of the T cell pool have been associated with impaired immune responses to several antigens[1–3] and adverse clinical outcomes in patients receiving haematopoietic cell transplantation (HCT)[4,5].

The capacity of T cells to mount and maintain effective responses to a wide variety of antigens depends on a large repertoire of unique T cell receptors (TCRs) generated in the thymus during the process of T cell development. This process is dependent on crosstalk between bone marrow (BM)-derived T cell progenitors and the supportive thymic stromal microenvironment, which primarily consists of thymic epithelial cells (TECs), endothelial cells, mesenchymal stromal cells, dendritic cells and macrophages[6]. Although, for example, T cell proliferation, driven by interleukin-7 (IL-7) and IL-15, in response to lymphopenic conditions can contribute to numerical reconstitution of T cells, complete long-term recovery of a diverse and functional T cell pool requires reactivation of thymic function and de novo T cell generation (FIG. 1). However, the thymus is sensitive to various injuries, such as those caused by cytoreductive treatments, infection, septic shock and graft-versus-host disease (GVHD). Furthermore, progressive involution of thymic tissue during ageing leads to a decline in T cell output and T cell senescence with restricted TCR repertoire diversity and impaired immune responses.

Thymic damage and impaired T cell reconstitution are particularly detrimental in HCT recipients[7]. Defective quantitative and functional recovery of T cells, in particular of $CD4^+$ T cells[8–11], has been directly linked to increased risks of opportunistic infections[9,12], malignant relapse[13] and overall adverse clinical outcomes[14,15]. Defective T cell responses are a clinical hurdle not only for patients receiving HCT but also for patients receiving other modalities of cancer immunotherapy, including immune checkpoint inhibitors, that exert their antitumour effects primarily through the activation of T cell effector function. Although the prognostic significance of this association has still to be further characterized in larger studies, a highly diverse pool of T cells before therapy correlates with improved outcome after immune checkpoint blockade therapy[16–19]. Thus, there is considerable interest in developing approaches to evaluate the quantity and quality of T cells before and during different forms of immunotherapy to guide treatment directions, monitor immune responses and ultimately identify functional biomarkers to predict clinical outcomes[20].

In this Review, we highlight the primary causes of impaired immune function, with special emphasis on HCT recipients, and discuss regenerative approaches that have been clinically translated to facilitate the recovery of adaptive immune function. We also provide an update on emerging new immune-boosting approaches that have demonstrated promising regenerative properties in preclinical models. We focus on approaches that can broaden

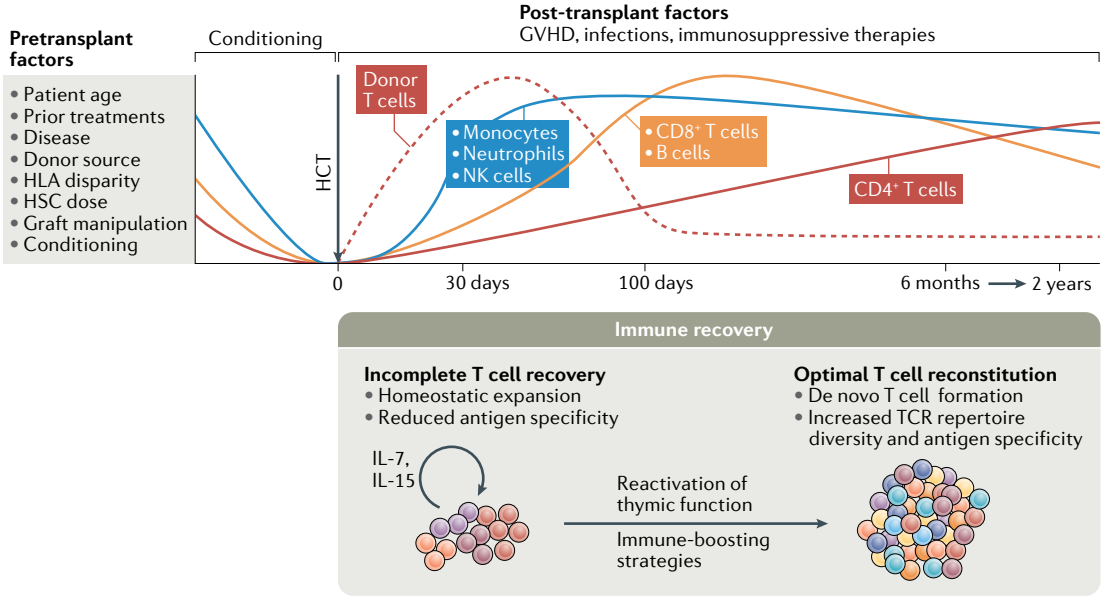

Fig. 1 | **Overview of the dynamics and determinants of T cell reconstitution after haematopoietic cell transplantation.** In the first period following haematopoietic cell transplantation (HCT), immune cells follow a predictable course of reconstitution. In contrast to the relatively early recovery of innate immune cells, recipients of HCT experience prolonged deficiencies in T cells and B cells, which can take more than 2 years to fully recover. This is particularly evident in adult patients, whose thymic function is lessened owing to age-related thymic involution. The 'first wave' of T cells after HCT comprises donor T cells that undergo lymphopenia-induced homeostatic proliferation and alloactivation. This results in polyclonal T cells with a restricted T cell receptor (TCR) repertoire and limited antigen specificity, or with alloreactivity causing graft-versus-host disease (GVHD). Overall, the incomplete recovery of the T cell pool has been directly linked to increased risks of infection, malignancy relapse and adverse clinical outcomes. Optimal and complete T cell reconstitution requires the regeneration of thymic function and the reactivation of endogenous T cell development. This allows the generation of a new T cell pool with broad TCR diversity. Multiple pretransplant and post-transplant factors influence the overall process of T cell reconstitution. HSC, haematopoietic stem cell; IL, interleukin; NK, natural killer.

Graft-versus-host disease (GVHD). Following allogeneic bone marrow transplantation, donor-derived T cells can be activated by residual host-derived antigen-presenting cells. The resulting T cell reactivity can escalate into the life-threatening condition known as GVHD, which targets mainly the skin, liver and intestines. Acute GVHD is a rapid response against recipient tissues that usually manifests itself within 100 days following haematopoietic cell transplantation, whereas chronic GVHD is reactions that occur after 100 days.

Sepsis
A severe, life-threatening form of infection characterized by systemic inflammatory response with resultant multi-organ failure followed by immunosuppression.

Glucocorticoids
A group of compounds that belong to the corticosteroid family. These compounds can be either naturally produced (hormones) or synthetic. They affect metabolism and have anti-inflammatory and immunosuppressive effects. Many synthetic glucocorticoids (for example, dexamethasone) are used in clinical medicine as anti-inflammatory drugs.

the diversity of the T cell pool through the restoration of de novo T cell formation in the thymus and discuss the implications for other cancer immunotherapies. While this Review primarily concentrates on T cell immunity, a brief summary of the B cell defects associated with immunological insults is provided in BOX 1.

Conditions leading to immune dysfunction

Infection. In the healthy state, homeostasis of the immune system relies on a fine balance between cell production and cell death. During an infection, this dynamic equilibrium is altered to ensure pathogen clearance without unrestrained immune responses. Haematopoietic stem and progenitor cells (HSPCs) replenish immune cells by responding to infections either indirectly through sensing a depletion of downstream cells (a process termed 'emergency haematopoiesis') or directly through sensing pathogen-specific systemic inflammatory signals (such as cytokines or Toll-like receptor ligands)[21]. During acute inflammation, lineage commitment of HSPCs favours granulopoiesis over lymphopoiesis[22]. During sepsis, migration of thymic precursors from the BM to the thymus is decreased, leading to a depletion of early thymic progenitors and contributing to lymphopenia[23].

In addition, the thymus itself is a target organ of various pathogens, leading to thymic atrophy and lymphocyte depletion, which are both common features of infectious diseases[24]. Thymic haematopoietic and stromal compartments can both be directly targeted in viral and parasitic infections. $CD4^+CD8^+$ double-positive (DP) thymocytes and their immediate precursors $CD24^{hi}CD3^{low}CD8^+$ single-positive thymocytes are particularly vulnerable, whereas mature $CD24^{mid/low}CD8^+$ SP cells are the most resistant thymic subsets during infection[25,26]. Although the precise mechanisms for infection-induced acute thymic involution remain to be further elucidated, stress-responsive hormones (such as glucocorticoids), pro-inflammatory mediators (such as interferon-γ (IFNγ) and tumour necrosis factor (TNF)) and apoptosis pathways (such as those mediated by BAX, BCL-2, JUN amino-terminal kinase, p53, caspase 8 and caspase 9) have all been implicated[27]. DP thymocytes are particularly sensitive to the increased level of glucocorticoids occurring in infection, providing a potential mechanism by which infections induce depletion of this particular cell subset[28].

Functional changes in the thymic microenvironment are also observed following infection[24]. The thymic epithelium undergoes substantial phenotypic and functional changes after infection with the parasite *Trypanosoma cruzi*[29] and viruses such as HIV[30], measles virus[31] and Zika virus[32]. For instance, localization of the TR5 and CK18 antigens restricted to medullary TECs (mTECs) and cortical TECs (cTECs), respectively, was altered in *T. cruzi*-infected mice along with increased extracellular matrix production by in vitro cultured

Conditioning regimens
Also known as preparative regimens. A combination of chemotherapy, radiotherapy and/or immunosuppressive medications that is designed not only to destroy residual malignant cells but also to provide space for donor stem cell engraftment and to provide immunosuppression to prevent host rejection of the donor stem cells.

TCR excision circles
(TRECs). Non-replicative DNA episomes that are normally produced as by-products during T cell receptor (TCR) rearrangement in thymocytes. They are therefore expressed only in T cells of thymic origin and provide a useful tool to assess thymic function and recovery of the T cell pool.

TECs[33,34]. Other functional changes in the thymic epithelium, including cell cycle arrest, terminal cell differentiation and abnormal expression of cell adhesion and migration genes, were observed in experimental models of measles virus[31] and Zika virus[32].

The increases in the levels of pro-inflammatory cytokines, including IL-6, TNF and IFNγ[35] (which was previously shown to cause BM and thymic aplasia[36–38]), together with other putative mechanisms, including direct damage of lymphoid tissues and apoptosis of lymphocytes, are presumably the primary causes of the profound lymphopenia observed in patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[39,40].

Thymic atrophy during acute infection is usually a rapid yet transient response, with thymic rebound occurring within 2 weeks after infection[41,42]. Nonetheless, delayed or incomplete thymic recovery has been reported and is particularly associated with pathogens that cause chronic infections such as HIV[43,44]. Sustained immune activation and alteration in T cell homeostasis during chronic infections have been associated with progressive and possibly irreversible loss of thymic function as seen in advanced age. Thymopoiesis, as reflected by the levels of TCR excision circles (TRECs) and naive $CD4^+$ T cells in the periphery (BOX 2), is significantly impaired in HIV-infected patients[43,44]. In addition, the reductions in the ratios of $CD4^+$ T cells to $CD8^+$ T cells and naive T cells to memory T cells, the expansion in $CD57^+CD8^+$ and $CD28^-CD8^+$ senescent T cell populations, and the overall reduction in vaccine responsiveness have all been correlated with HIV-related disease progression and ageing[45]. This suggests that HIV-associated immunological abnormalities can induce early onset of immunosenescence and correlate with the higher risk of age-associated diseases typically observed in these patients[46].

Cytoreductive therapy. Cytoablative treatments, such as radiotherapy, cell-depleting antibodies and chemotherapy, are designed to target malignant cells as well as the host haematopoietic system to allow successful HCT and markedly deplete all haematopoietic lineages, especially T and B cells[47]. Whereas many cytoablative therapies target highly proliferative cells while sparing the quiescent haematopoietic stem cells (HSCs) numerically, the resultant immunodepletion impairs haematopoietic cell function by selectively inducing premature senescence of HSCs through upregulation of the cyclin-dependent kinase inhibitors $p19^{ARF}$ and $p16^{INK4a}$ (REFS[48,49]). Recent studies demonstrate that chemotherapy induces fundamental changes in the expression of prohaematopoietic factors by the BM stromal niche, including downregulation of the Notch ligands Delta-like 4 (DLL4) and DLL1 by the vascular endothelium[50]. Consistent with the key role of DLL4 in sustaining common lymphoid progenitors and providing T-lineage specification before thymic entry[51], reduced Notch signalling induces a premature myeloid skewing of HSPCs and can contribute to delayed recovery of lymphoid cells following chemotherapy.

In addition to depleting haematopoietic and lymphoid precursors, cytoablative therapies induce prolonged deficiency in adaptive immunity by damaging the normal process of T cell development in the thymus and impairing B cell lymphopoiesis in the BM (BOX 1). The reduction in BM progenitors and T cell development can lead to long-term suppression of thymic function even after a single sublethal irradiation dose and can accelerate age-associated thymic involution[52,53]. In one study, chemotherapy induced transient thymic atrophy in as many as 90% of patients[54]. Both alkylating agents, such as cyclophosphamide, and irradiation reduce the numbers of all thymocyte subsets in mice, with DP thymocytes and their immediate progenitors $CD4^-CD8^-$ double-negative thymocytes being particularly sensitive to such insults[55,56].

Further contributing to impaired thymopoiesis, cytoablative agents damage TECs[57]. Irradiation and cyclophosphamide both deplete cTECs and mTECs which are important for, respectively, positive and negative selection of developing T cells, and this interferes with the generation of a broadly reactive TCR repertoire[58,59]. Activation of signal transducer and activator of transcription 3 (STAT3) signalling has been linked to the induction of apoptosis in TECs after irradiation[60]. However, more recent studies show that STAT3-mediated signalling is essential for growth and architectural organization of

Box 1 | Mechanisms regulating B cell recovery after immunological injury

Compared with our understanding of T cell reconstitution, less information is available regarding the kinetics of B cell recovery after immunological insults and the factors regulating this process[258]. Optimal B cell function is important not only for the generation of protective antibodies and efficient antigen presentation but also for proper immune tolerance. This is particularly crucial for recipients of haematopoietic cell transplantation (HCT) as delays in B cell reconstitution are associated with increased risk of infection[259] and development of chronic graft-versus-host disease (GVHD)[260]. B cell numbers generally return to normal levels within 12 months after HCT, but it can take up to 2 years for complete recovery of the B cell compartment[259,261]. Several factors such as conditioning regimens, total body irradiation, corticosteroid treatment and GVHD negatively impact B cell reconstitution[77,258,262]. B cell-targeting therapies such as the monoclonal anti-CD20 agent rituximab also significantly delay B cell recovery[263].

In allogeneic HCT recipients, immunoglobulins can be derived from recipient plasma cells that survive conditioning regimens and donor-derived mature B cells[261]. However, restoration of the B cell compartment is primarily mediated by de novo regeneration from bone marrow progenitors. B cell recovery following HCT is reminiscent of B cell ontogeny[264,265]. Transitional $CD19^+CD21^{low}CD38^{hi}$ B cells are the first B cells to emerge following HCT. Their percentage decreases in the first 12 months as the proportion of mature $CD19^+CD21^{hi}CD27^-$ naive B cells increases[262]. However, even when the total number of B cells recovers, their functionality can remain compromised for 1–2 years. Indeed, in addition to environmental or vaccine-based antigen exposure, insufficient recovery and signalling from donor $CD4^+$ T cells can result in B cell maturation arrest and decreased responses to vaccines[259,266]. Thus, the degree of $CD4^+$ T cell recovery impairs B cell differentiation and functional reconstitution. In the first period following HCT, due to lack of T cell help, memory B cells display a limited diversity of the IgH complementarity-determining region 3 repertoire compared with naive B cells[267]. However, B cell autonomous defects are also evident in HCT recipients as the capacity to accumulate somatic mutations is decreased in mature B cells even in the presence of an adequate pool of $CD4^+$ T cells[268]. Limited information is available regarding the dynamics of B cell receptor repertoire evolution following HCT. Similarly to the T cell receptor repertoire, the sequencing of the VH1 repertoire of class-switched B cells revealed lower repertoire diversity after HCT than the pretransplantation status[269]. However, a more comprehensive study of B cell receptor reconstitution and repertoires after allogeneic HCT is needed.

Analogously to the T cell compartment, the naive B cell pool also declines with ageing. In old individuals, B cells are replaced by antigen-experienced memory cells carrying substantial functional changes, including impaired affinity maturation and isotope switching[88].

Box 2 | **Methods to estimate the dynamics of T cell reconstitution**

The close association between T cell function and clinical response to cancer immunotherapy highlights the need for comprehensive methods to monitor a patient's immune function and T cell receptor (TCR) repertoire changes and develop potential biomarkers predictive of clinical responses.

Characterization of the main lymphocyte populations in peripheral blood is performed classically by flow cytometry and, more recently, by mass cytometry[270] using a combination of cell surface markers such as CD4, CD8, CD45RA, CD45RO, CCR7, CD62L and CD27 (REF.[271]). Nonetheless, there are significant disparities among centres regarding the combination of markers to use and the tests to characterize reactivation of thymic function, TCR diversity and T cell function.

Analysis of thymic output

Analysis of recent thymic emigrants (RTEs) is performed in most immune monitoring studies. Several markers can be used to phenotypically characterize RTEs, when combined with CD45RA and CD45RO, including CD31, CCR7, PTK7 for $CD4^+$ RTEs[272] and CD103 for $CD8^+$ RTEs[273], which can be used to enrich for RTEs carrying TCR excision circles (TRECs). However, not all $CD31^+$ naive $CD4^+$ T cells are RTEs, as T cells can undergo interleukin-7-driven homeostatic proliferation without downregulating their CD31 expression[146]. In addition, CD31 can be re-expressed by activated $CD4^+$ T cells[274]. As an alternative, recent studies suggest that complement receptor 1 (CR1) and CR2 are novel markers enriched in RTEs and allow identification of this population with high purity[275].

Other approaches to monitor T cell output include molecular quantification of TRECs. Detectable by standard or real-time PCR, these non-replicating circles of DNA are enriched in RTEs[276]. However, division of T cells in response to cytokine stimulation in the periphery can complicate the assessment of RTEs and thymic output[141,217].

Imaging thymic mass by computed tomography scanning or magnetic resonance imaging has also been used to evaluate thymic regeneration in patients following antineoplastic therapy[66,70] and after haematopoietic cell transplantation[69]. A major limitation of this approach is its semiquantitative estimation of thymic output based on the size of the thymus, as these two parameters often do not follow a linear correlation.

Analysis of TCR diversity

A diverse TCR repertoire is generally associated with a favourable clinical outcome of immunotherapy for cancer. TCR diversity can be assessed by flow cytometry, which allows measurement of use of different TCR variable (V) genes at the protein level[277], and spectratyping, which reveals clonal length polymorphism in the complementarity-determining region 3 of each TCR V gene family at a molecular level[278]. However, these methods estimate total TCR diversity of the whole αβ T cell population and do not measure the frequency of individual TCRs. The rapid advances in next-generation sequencing-based high-throughput TCR analysis have offered the opportunity to measure TCR diversity with increased resolution. As TCR sequencing is an indirect measurement of thymic function, other approaches should be used to gain more direct insights into the endogenous process of T cell development. In addition, measurement of total TCR diversity also comes with significant challenges due to the high heterogeneity and dynamism of naive T cells.

mTECs but is dispensable for the biology of cTECs[61,62]. Coinciding with their higher rate of proliferation[57], the greatest numerical reduction occurs in the subset of TECs expressing the highest levels of MHC class II, particularly the tolerance-inducing $AIRE^+MHC\text{-}II^{hi}$ mTECs[63]. This imbalance in TEC subsets persists even after thymic cellularity is restored and may promote the development of autoimmunity[58]. Importantly, certain stromal subsets, such as innate lymphoid cells (ILCs) and endothelial cells, are relatively resistant to cytoreductive therapies and play an important role in thymic regeneration by secreting factors, such as IL-22 and bone morphogenetic protein 4 (BMP4), which promote the survival, proliferation and maintenance of TECs[64,65]. Full, albeit delayed, recovery of thymic function is possible up until middle age, but the peripheral naive repertoire is never fully restored in older patients[66].

Common lymphoid progenitors
Progenitors of lymphoid cell lineages, which include B cells, T cells, natural killer cells and innate lymphoid cells. Bone marrow common lymphoid progenitors are defined by their expression of the interleukin-7 receptor, FMS-related tyrosine kinase 3 (FLT3) and KIT, and the absence of all conventional lineage markers.

Peripheral T cell depletion, especially of $CD4^+$ T cells, is the main cause of clinical immunodeficiency observed in patients with cancer[67]. T cell recovery following cytoablative therapy is predominantly achieved through two mechanisms: de novo production in the thymus, particularly for $CD4^+$ T cells, or homeostatic expansion of peripheral T cells, preferentially for $CD8^+$ T cells[68,69] (FIG. 1). The relatively rapid $CD8^+$ T cell recovery through extrathymic clonal expansion[70,71] is typically associated with limited breadth of the TCR repertoire and diminished immune responses[72].

Graft-versus-host disease. Although allogeneic HCT offers a potential cure for various malignant and non-malignant disorders, its wider application is limited by the significant morbidity and mortality associated with GVHD and prolonged post-transplantation immunodeficiency[73]. In GVHD, donor alloreactive T cells in addition to targeting the host gastrointestinal tract, liver and skin also directly destroy primary lymphoid organs (BM and thymus) and delay immune reconstitution after allogeneic HCT. Both mouse models and clinical studies have demonstrated that GVHD targets the BM[74], and the resultant medullary aplasia delays donor-derived haematopoiesis, especially of the lymphoid lineage[75]. IFNγ and TNF disrupt donor haematopoiesis both directly and indirectly through their effects on host BM stromal cells, including osteoblasts and mesenchymal stem cells. As a result, acute GVHD is associated with a significant reduction in the numbers of residual and de novo B cell precursors, in the numbers of mature B cells and in antibody production[76,77] (BOX 1).

During acute GVHD, the thymus is exquisitely sensitive to damage by alloreactive T cells, and its acute atrophy reflects the precipitous contractions of the lymphocytic and stromal compartments[78,79]. Mouse models of acute GVHD have demonstrated that, in addition to the reduced number of early thymic progenitors migrating from the BM to the thymus, reduction in thymic cellularity is primarily due to loss of the large DP thymocyte population via glucocorticoid-independent apoptotic cell death[78].

Within the stromal cell subset, TECs are targets of GVHD-mediated apoptosis. TECs also act as antigen-presenting cells and prime alloreactive T cells directly through their intrinsic expression of MHC class I and class II molecules, such that depletion of host-derived professional antigen-presenting cells does not prevent activation of alloreactive donor T cells and thymic injury[79]. This raises the possibility that GVHD is restricted to the thymus and remains subclinical despite detrimental consequences for T cell reconstitution. In addition, alloreactive T cells eliminate IL-22-producing thymic group 3 ILCs (ILC3s), resulting in impaired thymic recovery[80]. Moreover, donor alloreactive $CD8^+$ T cells preferentially target tolerance-inducing mature mTECs, thereby impairing negative selection and allowing de novo generation of autoreactive $CD4^+$ T cells, linking alloimmunity during acute GVHD to the development of autoimmunity during chronic GVHD[81,82]. These studies underline the importance of functional preservation of TECs as well as numerical restoration

Innate lymphoid cells
(ILCs). A group of innate immune cells that are lymphoid in morphology and developmental origin but lack properties of adaptive B cells and T cells such as recombined antigen-specific receptors. They function in the regulation of immunity, tissue homeostasis and inflammation in response to cytokine stimulation.

Allogeneic HCT
Transplantation approach involving transfer of haematopoietic cells from a healthy donor to a patient after conditioning with high-intensity chemotherapy or irradiation. This approach can be used to treat either malignant or non-malignant disorders. Mismatches between the histocompatibility antigens of the donor and the patient can lead to adverse events, such as rejection of the transplanted graft or pathological immune responses to normal tissues in the patient.

of lymphocytes as the goals for immune-regenerative strategies following allogeneic HCT.

Clinically, GVHD-induced immunosuppression has been linked to decreased thymic output of naive T cells, as measured by TRECs, and a distorted TCR repertoire[83–86]. Paradoxically, pharmacological immunosuppressants, including corticosteroids and cyclosporine A, used in allogeneic HCT to prevent allograft rejection, as well as GVHD prophylaxis and/or treatment, can induce thymic involution, deplete thymocytes and ablate AIRE^{+}MHC-II^{hi} mTEC subsets, further contributing to thymic damage[58,87]. These findings highlight the multifactorial nature of post-transplantation immune disorders and represent the clinical challenges in preventing and treating GVHD after allogeneic HCT.

Immunosenescence. The functionality of the immune system progressively declines with age[88], contributing to increased incidence of infections, inadequate vaccine responses and decreased immunosurveillance of malignant cells observed in the elderly population[89,90]. It is estimated that only ~30–40% of elderly people are capable of mounting sufficient immune responses to the influenza vaccine[91,92].

Ageing impairs the normal process of thymic and BM lymphopoiesis at multiple levels. With age, there is an accumulation of HSCs with reduced homing and engraftment capacity[93–95]. In patients receiving allogeneic HCT, younger donor age has been associated with faster immune reconstitution[96]. Although several intrinsic changes in HSCs and lymphoid progenitors have been identified (including myeloid skewing[97,98], defects in DNA repair[99,100], epigenetic alterations[101] and loss of cell polarity[102]), substantial changes in the BM stromal microenvironment also contribute to defective lymphopoiesis[103]. Transplantation of old HSCs into a young microenvironment is sufficient to partially reverse myeloid skewing[104,105].

As T cell development depends on the constant output of T cell progenitors from the BM, defective lymphopoiesis, along with impaired developmental potential of intrathymic progenitors[106], results in a significant reduction in the number of intrathymic lymphoid progenitors contributing to the reduced T cell output observed in older individuals[97,106].

The progressive decline of thymus function during ageing represents one of the most important causes of immune degeneration in the elderly population[107]. However, the precise kinetics of this chronic physiological process is still under debate. Compared with the mouse thymus, which undergoes a progressive reduction in its volume with age, the human thymus remains almost unchanged in size under normal circumstances and, instead, it is characterized by profound perturbation of the thymic stromal cell microenvironment, including loss of epithelial cells, increased volume of the perivascular space and progressive replacement of healthy tissue with adipose tissue[108].

The thymic stroma is one of the main targets of the effects of ageing, as demonstrated by HCT and parabiosis experiments in mice with different age-mismatched donors[109,110]. For example, intrathymic injection of early thymic progenitors derived from young mice into old mice is not sufficient to restore normal thymic lymphopoiesis[111]. At a molecular level, several studies have revealed extensive transcriptional changes in mouse thymic stromal cells as early as 3 months of age, particularly in genes associated with cell cycling and inflammatory responses[112–114]. Although ageing significantly erodes its functionality, the thymus maintains a proportion of functional cortical and medullary regions and active thymopoiesis. Indeed, T cell output, as measured by TREC level in the peripheral blood, is maintained in older individuals, albeit substantially reduced[115,116]. In addition, recent studies demonstrate that the involution of thymic tissue is not as dramatic as previously reported and that residual thymic tissue can be detected by computed tomography scanning in individuals up to 70 years of age[117].

As the thymus involutes, the production of T cells is progressively impaired, and with age the naive T cell pool is increasingly maintained by homeostatic expansion[3,118,119]. Mathematical modelling suggested that thymic T cell export declines exponentially over time with a half-life of 15.7 years; therefore, by the age of 55 years only 5% of naive T cell production is derived from thymic export[120]. More recently, the average TREC content of the naive T cell population was used to estimate thymic T cell output. This study demonstrated that up to 90% of naive T cells in healthy human adults are generated through proliferation[118]. By measuring the average TREC content in naive T cells as well as the turnover of T cells, this work also showed that, unlike in humans, the naive T cell pool in mice is almost exclusively sustained throughout life by thymus output[118].

The decline in naive T cell production results in a shift towards an oligoclonal pool of memory T cells and an almost linear decrease of T cell diversity with age[119,121]. These effects are particularly pronounced after the age of 60 years[3,122]. Some long-lived individuals (average age of 82 years) display a significantly higher percentage of naive CD4^{+} T cells, decreased abundance of expanded clones and increased TCR diversity compared with a younger cohort, suggesting that these key immune parameters may represent hallmarks of longevity[122]. Mathematical modelling studies demonstrate a significant inverse correlation between thymic export and the incidence of both infectious diseases and cancer[123]. During the coronavirus disease 2019 (COVID-19) pandemic, being older than 65 years was identified as a risk factor for morbidity and death from COVID-19 (REF.[124]).

The age-associated decline of thymic function significantly impairs and delays the endogenous process of thymic repair after immunological insults, resulting in a prolonged recovery time following common cancer cytoreductive therapies[12,15]. This is particularly problematic in the HCT setting, which is increasingly used in older patients. An inverse relationship exists between the transplant recipient's age and T cell recovery after transplantation[9,125]. Insufficient recovery of thymopoiesis has been directly correlated with an increased risk of opportunistic infections, leukaemia relapse and adverse clinical outcome[126,127].

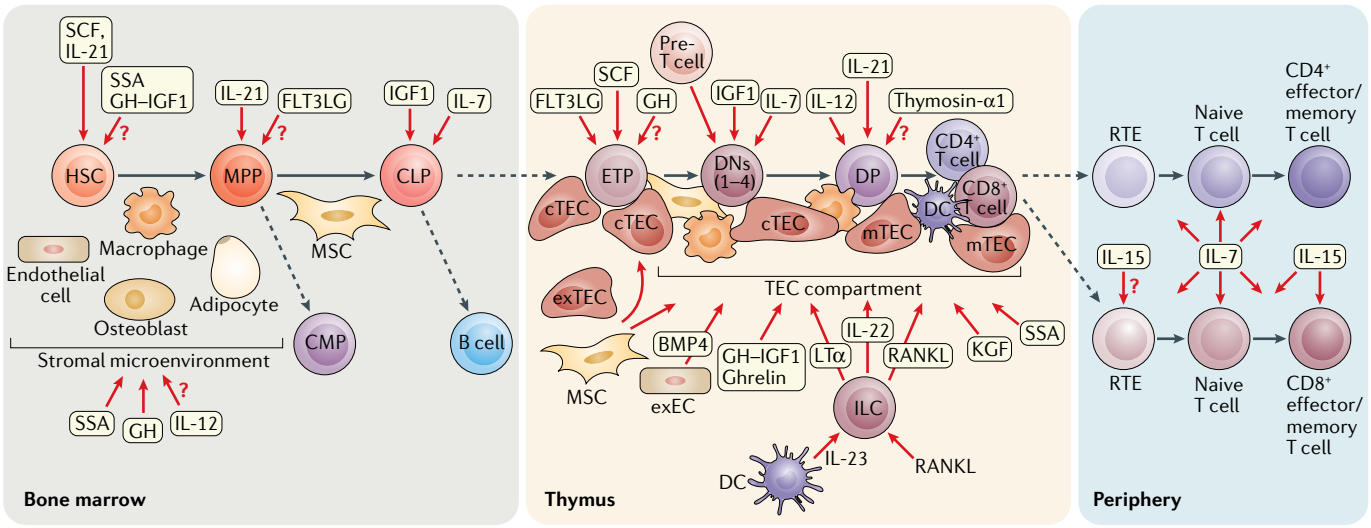

Fig. 2 | **Simplified overview of T cell generation with regenerative strategies after immune injury.** T cell development begins when T cell progenitors, originating from common lymphoid progenitors (CLPs) in the bone marrow, migrate into the thymus and progress through a series of well-characterized developmental steps. Thymocytes go through the double-negative (DN; $CD4^-CD8^-$) and double-positive (DP; $CD4^+CD8^+$) stages to form single-positive ($CD4^+CD8^-$ or $CD4^-CD8^+$) T cells. During this process, approximately 95% of developing thymocytes produced daily are deleted through β-selection, positive selection and negative selection, resulting in the formation of self-restricted and self-tolerant naive $CD4^+$ and $CD8^+$ single-positive cells that can exit the thymus and migrate to peripheral lymphoid organs. Approaches to enhance T cell recovery act at multiple levels. Factors and approaches such as interleukin-7 (IL-7), IL-12, IL-15, IL-21, FMS-like tyrosine kinase 3 ligand (FLT3LG), growth hormone (GH), insulin-like growth factor 1 (IGF1), sex steroid ablation (SSA), thymosin-α1, stem cell factor (SCF), administration of precursor T cells (pre-T cells) and delivery of ex vivo-generated thymic epithelial cells (exTECs) primarily promote recovery of the haematopoietic compartment. By contrast, keratinocyte growth factor (KGF), IL-22, receptor activator of nuclear factor-κB ligand (RANKL), IGF1, lymphotoxin-α (LTα) and bone morphogenetic protein 4 (BMP4) produced by ex vivo-generated endothelial cells (exECs) enhance reconstitution of the thymic stromal compartment. Question marks denote approaches where the effects on specific targets are not fully understood. ETP, early thymic progenitor; CMP, common myeloid progenitor; cTEC, cortical thymic epithelial cell; DC, dendritic cell: ILC, innate lymphoid cell; HSC, haematopoietic stem cell; MPP, multipotent progenitor; MSC, mesenchymal stromal cell; mTEC, medullary thymic epithelial cell; RTE, recent thymic emigrant.

Strategies to enhance immune recovery

Over the past few years, several approaches have been proposed to enhance immune function through recovery of the T cell pool (FIG. 2; TABLE 1). These strategies include the stimulation of T cell development and expansion using cytokines, such as IL-7, IL-12 and IL-21; the administration of cytokines and growth factors, such as stem cell factor (SCF; also known as KITLG), keratinocyte growth factor (KGF; also known as FGF7), IL-22 and FMS-like tyrosine kinase 3 ligand (FLT3LG); the modulation of hormone levels by suppression of sex steroids or by administration of thymosin-α1, growth hormone (GH; also known as somatotropin), insulin-like growth factor 1 (IGF1) and ghrelin; the adoptive transfer of lymphoid progenitors such as precursor T cells and ex vivo expanded thymus-derived endothelial cells; and the use of artificial BM or thymus-like grafts.

Here, we focus on immune-boosting strategies that have been translated into clinical studies and provide a brief update on novel approaches that have shown regenerative potential in preclinical models. We put particular emphasis on approaches that can promote de novo T cell formation through the regeneration of thymic function, which is the primary mechanism to generate a pool of naive T cells with a diverse TCR repertoire. We also provide a brief summary of the different methods used to estimate thymic function and the dynamics of T cell reconstitution in BOX 2.

Interleukin-7. IL-7 is classified as a type 1 short-chain cytokine crucial for the development of innate and adaptive immune cells[128]. It is secreted mainly by non-haematopoietic cells, including epithelial cells and fibroblasts in the thymus, BM stromal cells, lymphatic endothelial cells, fibroblastic reticular cells and enterocytes[129–131]. IL-7 is particularly important for the differentiation of T and B cells from common lymphoid progenitors and for the maintenance and survival of mature T cells. IL-7 receptor (IL-7R) is a heterodimer of two chains: IL-7Rα (also known as CD127) and cytokine receptor common subunit-γ (also known as CD132 or IL-2RG). The γ-chain is expressed by all haematopoietic cell types, whereas IL-7Rα is expressed mainly by developing B and T cells, naive and memory T cells, NKT cells, ILC2s and ILC3s.

The crucial role of IL-7 in lymphopoiesis is demonstrated by the development of severe combined immunodeficiency disease in patients carrying mutations affecting the α-chain[132,133] or the γ-chain of IL-7R[134]. While studies in *Il7*$^{-/-}$ mice showed that IL-7 is a non-redundant cytokine for both T and B cell lymphopoiesis, B cell development in humans does not appear to require IL-7, as B cells are maintained in patients with severe combined immunodeficiency disease with mutations in the gene encoding IL-7Rα[132].

The important role of IL-7 in T cell biology is also supported by the inverse correlation between circulating IL-7 levels and peripheral T cells observed in

Cytokine receptor common subunit-γ
A chain common to type I cytokine receptors. It was first discovered as the γ-chain of the interleukin-2 (IL-2) receptor and was subsequently shown also to be present in the receptors for IL-4, IL-7, IL-9, IL-15 and IL-21. Gene mutations affecting this γ-chain in humans result in absence of T cells and natural killer cells, a condition termed X-linked severe combined immunodeficiency.

Recent thymic emigrants (RTEs). Semimature T cells that have left the thymus but have yet to undergo the final stages of maturation. Typically a window of around 2 weeks after thymic maturation is used to differentiate between RTEs and fully mature T cells.

patients with lymphopenia[135]. The degree of available IL-7 controls the size of the peripheral T cell pool and plays an important role in regulating overall T cell homeostasis.

The effects of exogenous administration of IL-7 on immune reconstitution have been widely investigated[128]. Several preclinical mouse models have demonstrated the beneficial effects of exogenous IL-7 in promoting immune reconstitution through thymus-dependent and thymus-independent mechanisms. In the setting of HCT, exogenous IL-7 accelerates the reconstitution of donor-derived thymocytes and the peripheral T cell pool, leading to enhanced T cell recovery after both syngeneic and allogeneic HCT[135–139]. A phase I/IIa dose-escalation study (NCT00477321) of repeated administration of a glycosylated recombinant human IL-7 (rhIL-7; CYT 107) in HIV-1-infected patients demonstrated that rhIL-7 treatment was safe, well tolerated and transiently promoted the expansion of naive and memory $CD4^+$ and $CD8^+$ T cells, and decreased the proportion of exhausted $PD1^+$ T cells[140]. Importantly, rhIL-7 therapy also increased the numbers of $CD4^+$ recent thymic emigrants (RTEs), the signal joint to β TREC ratio and TCR repertoire diversity in some participants, effects that imply enhanced thymic activity (BOX 2). Subsequently, two other clinical trials (NCT01190111 and NCT01241643) demonstrated that repeated doses of rhIL-7 were well tolerated and resulted in sustained $CD4^+$ T cell numbers in the majority of HIV-infected participants[140–143]. Similarly, in a phase I/IIa dose-escalation trial (NCT00839436) in patients with idiopathic $CD4^+$ lymphopenia at risk of disease progression, rhIL-7 led to an increase in the number of circulating $CD4^+$ and $CD8^+$ T cells and tissue-resident $CD3^+$ T cells in the gut mucosa and BM. Importantly, enhanced thymopoiesis, measured by TRECs, was observed only in the youngest patients of the cohort (aged 23 and 34 years)[144].

In a phase I clinical trial (NCT00684008) that evaluated the immune-regenerative properties of rhIL-7 in patients receiving T cell-depleted allogeneic HCT[138],

Table 1 | **Approaches to enhance T cell recovery, their targets and progress towards the clinic**

Approach	Target cells	Clinical trials	Refs
Cytokines			
IL-7	HSPCs, thymocytes, peripheral T cells	NCT00477321, NCT01190111, NCT01241643, NCT00839436, NCT00684008	136–144,216,217
IL-12	Thymocytes, HSPCs?	Not currently in clinical trials	185,186
IL-15	NK cells, NKT cells, $CD8^+$ T cells	Not currently in clinical trials	187–189
IL-21	Thymocytes, HSPCs	Not currently in clinical trials	190–192
IL-22	TECs	Not currently in clinical trials	64,80,193
RANKL	TECs	Not currently in clinical trials	197,218,219
Growth factors			
KGF	TECs	NCT01233921, NCT03042585, NCT02356159, NCT00593554, NCT01712945	150–155,157, 159,220–223
FLT3LG	BM HSPCs, thymocytes	Not currently in clinical trials	224–226
IGF1	TECs	Not currently in clinical trials	227,228
SCF	Thymocytes	Not currently in clinical trials	229–231
Hormones and hormone-like mediators			
Thymosin-α1	Thymocytes	NCT00580450	164,232
GH and ghrelin	TECs, thymocytes	NCT00071240, NCT00287677, NCT00119769, NCT00050921	180–182, 184,233–235
Sex steroid ablation	TECs, BM HSPCs, thymocytes	NCT01746849, NCT01338987	55,58,167,169–173, 175,176,236–240
Cell-based approaches			
Precursor T cells	TECs, thymocytes	Not currently in clinical trials	241–246
Ex vivo expanded endothelial cells	TECs	Not currently in clinical trials	65
Ex vivo expanded MSCs	HSPCs, TECs, T cells	Not currently in clinical trials	247,248
Ex vivo generated TEC graft	TECs, thymocytes	Not currently in clinical trials	249–254
Injectable thymus-like scaffolds	CLPs, peripheral T cells	Not currently in clinical trials	253,255–257

BM, bone marrow; CLP, common lymphoid progenitor; FLT3LG, FMS-like tyrosine kinase 3 ligand; GH, growth hormone; HSPCs, haematopoietic stem and progenitor cells; IGF1, insulin-like growth factor 1; KGF, keratinocyte growth factor; IL, interleukin; MSC, mesenchymal stromal cell; NK, natural killer; NKT cells, natural killer T cells; RANKL, receptor activator of nuclear factor-κB ligand; SCF, stem cell factor; TEC, thymic epithelial cell.

rhIL-7 induced a rapid increase in peripheral CD4$^+$ and CD8$^+$ T cell numbers. While the estimated half-life of rhIL-7 in this study was 9–35 hours, the biological effects on T cell numbers persisted for several weeks after the circulating levels of IL-7 returned to the baseline. In addition, although rhIL-7 administration resulted in increased numbers of RTEs only in some young patients, most participants had enhanced TCR repertoire diversity that persisted several weeks after the end of rhIL-7 therapy[138]. The limited effects on thymic output in this study, as represented by minimal changes not only in the numbers of RTEs but also in the levels of TRECs, could be explained by the age and lymphopenic condition of the patients at the time points analysed. It is also possible that extended duration of rhIL-7 administration is necessary to have a greater effect on thymic function. However, compared with mice, in which IL-7 has both thymic-dependent and thymic-independent regenerative effects[135], it is still unclear what the direct impact of exogenously administered IL-7 is on the thymus in humans and non-human primates. On the basis of clinical and preclinical observations, it appears that most of the effects on TCR diversity following rhIL-7 treatment are primarily driven by extrathymic sources, including the expansion of less frequent but highly diverse RTEs and naive T cells, which preferentially respond to IL-7 stimulation[145,146], as well as their recirculation from lymphoid organs[141,147,148].

Lymphopenia, particularly with regard to CD4$^+$ and CD8$^+$ T cell subsets, in patients with COVID-19 on admission to hospital is emerging as one of the key clinical signs of COVID-19 and is closely associated with disease progression[39]. Enhancing T cell immunity could be a worthwhile strategy for treating these patients. Thus, a clinical trial has recently begun to investigate the possibility that IL-7 can restore T cell immunity in patients with COVID-19.

Keratinocyte growth factor. KGF is a potent growth factor for TECs and is expressed under physiological conditions in the thymus primarily by mesenchymal cells[149]. KGF binds to its receptor, fibroblast growth factor 2 variant IIIb (FGFR2IIIb), on TECs and induces TEC proliferation through activation of the PI3K–AKT–nuclear factor-κB and p53 pathways[150–152].

Studies using knockout animals found that although KGF is redundant for thymopoiesis in steady-state conditions, it is crucial for thymic regeneration and peripheral T cell reconstitution after injury such as that caused by total body irradiation and syngeneic or allogeneic HCT[153]. The impact of exogenous administration of KGF on TEC function and thymic regrowth has been extensively evaluated in several mouse studies. Administration of recombinant KGF transiently accelerated thymic recovery after immune insults such as irradiation, cyclophosphamide therapy and dexamethasone therapy, and enhanced recovery of thymic and peripheral T cell numbers after HCT[150,152,154,155]. KGF reversed thymic involution and restored thymopoiesis in aged mice for up to 2 months after treatment[156]. A study performed in rhesus macaques showed that KGF-treated animals displayed accelerated haematological recovery, improved thymopoiesis and enhanced naive T cell recovery following HCT[157]. Although the effects on thymic function were modest, as measured by minimal changes in thymus mass, KGF-treated animals showed increased numbers of TREC-positive T cells up to 3 months following KGF treatment.

Human recombinant KGF (palifermin; trade name Kepivance, marketed by Biovitrum) is approved by the US Food and Drug Administration for the prevention of mucositis in patients receiving high-dose chemotherapy. Several trials (NCT01233921, NCT03042585, NCT02356159 and NCT00593554) are exploring its effects on T cell reconstitution, but no results have been reported yet. As demonstrated in previous preclinical work[158], the benefits of palifermin on immune reconstitution in transplant recipients may derive from its synergistic effects with other immune-boosting therapies rather than as a sole therapeutic agent. However, a recent study of the use of KGF to promote immune reconstitution in patients with relapsing–remitting multiple sclerosis treated with the anti-CD52 lymphocyte-depleting agent alemtuzumab showed reduced thymic output in KGF-treated patients as measured by evaluation of naive CD4$^+$ T cells, RTEs and TRECs (NCT01712945)[159]. Given that human cTECs express CD52, one possible explanation for these clinical data is that palifermin exacerbates the negative effects of alemtuzumab on thymic function, perhaps through upregulation of CD52 expression on cTECs, rendering these cells more susceptible to antibody-mediated elimination. Thus, the combination of KGF with other drugs should be assessed cautiously as synergistic or deleterious effects on immune regeneration may occur depending on dosing, timing and mechanisms.

Thymic hormones. Thymosins are a group of low molecular weight peptides originally isolated from bovine thymus[160]. Thymosin-α1, derived from prothymosin-α, is produced by TECs[161] and can increase lymphocyte maturation, boost T cell function and promote recovery following immune insults, although its mechanism of action is not completely understood. The receptor for thymosin-α1 is expressed by developing thymocytes, in which it regulates their survival and proliferation. Thymosin-α1 can antagonize dexamethasone-induced apoptosis of DP thymocytes in vitro, as well as the hydrocortisone-induced decrease in thymus and spleen mass[162]. Thymosin-α1 can also enhance the production of IL-7 by TECs[163]. Given promising preclinical results, multiple clinical trials have been initiated to evaluate the immunomodulatory effects of thymosin-α1 in the treatment of patients experiencing viral infections, immunodeficiency or haematological malignancies. Safety and efficacy of thymosin-α1 administration were evaluated in recipients of allogeneic HCT in a phase I/II clinical study (NCT00580450). Treatment with thymosin-α1 increased T cell numbers and resulted in earlier appearance of pathogen-specific T cell responses against pathogens such as cytomegalovirus and *Aspergillus* species. Importantly, thymosin-α1 did not exacerbate acute or chronic GVHD and was associated with significant improvement in phagocytosis and dendritic cell function[164].

Recently, thymosin-α1 was given to patients with COVID-19 showing severe lymphopenia to enhance immunity. Thymosin-α1 treatment increased T cell numbers and recovery of thymic function, measured by TREC analysis[165]. Importantly, thymosin-α1 administration was also associated with increased survival of patients with severe COVID-19 (REF.[165]).

Sex steroid ablation. In addition to their fundamental role in regulating sex dimorphism, sex hormones can impact haematopoiesis at multiple levels. One of the first observations regarding a relationship between T cell development and sex hormones dates back to 1898, when it was reported that the thymus enlarged after castration of male rabbits[166]. Several studies confirmed the enlargement of thymic tissue after gonadectomy in both sexes in different experimental animal models. Conversely, androgens and oestrogens induce atrophy of the thymus[167,168].

The increase in the levels of sex steroids, and in particular of androgens, during puberty has been directly linked to the age-associated deterioration of immune function and to the process of thymic involution. Although the connection between the increase in the levels of sex steroids after puberty and the initiation of thymic involution is still debated, the regenerative impact of the removal of sex steroids on both thymic and BM lymphopoiesis has been extensively characterized. Indeed, through the use of clinically relevant mouse models of immune reconstitution after haematopoietic injuries, such as chemotherapy and radiotherapy, it has been demonstrated that sex steroid ablation enhances HSC self-renewal and lymphoid differentiation capacity and increases the number of common lymphoid progenitors in the BM[169–171]. Sex steroid ablation also has a direct effect on the BM microenvironment, restoring expression of key haematopoietic factors that are downregulated with age, such as FOXO1 (REF.[169]). Considerable rejuvenation effects in the thymus have been extensively characterized, demonstrating that sex steroid ablation reverses thymic atrophy, accelerates the recovery of all thymocyte subsets and elicits potent regenerative signals to the thymic stromal microenvironment[55,172–174]. At a molecular level, sex steroid ablation promotes the upregulation of the key thymopoietic factors CC-chemokine ligand 25 (CCL25)[175] and DLL4 (REF.[167]) in mTECs and cTECs, respectively.

Several drugs have been developed to transiently and reversibly block sex steroids for the treatment of precocious puberty, endometriosis, hormone-sensitive prostate cancer and breast cancer. Some of these sex steroid blockers have been tested clinically to boost immune reconstitution after HCT. A non-randomized pilot study demonstrated that administration of the luteinizing hormone-releasing hormone (LHRH) agonist goserelin (Zoladex) before HCT significantly increased neutrophil engraftment, as well as total lymphocyte numbers, particularly those of naive $CD4^+$ T cells, and levels of TRECs and improved recovery of TCR repertoire diversity[176]. Importantly, an increase in disease-free survival was observed in autologous HCT recipients treated with goserelin. Two trials (NCT01746849 and NCT01338987) are ongoing to evaluate the effects of the LHRH agonist leuprolide (Leuprorelin) and the LHRH antagonist degarelix (Firmagon) to promote immune reconstitution following allogeneic HCT. Notably, the latest androgen receptor inhibitors and LHRH antagonists have the advantage of immediately blocking sex steroids without an initial surge of sex steroids as seen with LHRH agonists[167]. These novel approaches may provide better therapeutic tools to suppress sex steroids and mediate immune reconstitution.

The regenerative effects of sex steroid ablation on T cell development might continue only as long as the levels of sex steroids are suppressed. However, the duration of such effect, particularly in the setting of surgical castration, remains a subject of debate in the field. After the initial regrowth following castration, the thymus of aged animals has been reported to decline and return approximately to its pretherapy condition 1 month after sex steroid ablation therapy[177]. While these results support a model in which the regenerative effects induced after surgical sex steroid ablation are transitory and dynamic, additional studies should be done to better characterize the nature of these 'transient' effects and the precise kinetics of thymic regeneration, in particular, at later time points. For example, it would be interesting to evaluate whether removal of the gonads, in the long term, can induce additional hormonal changes that negatively impact the process of lymphopoiesis.

Growth hormone. GH is a small peptide hormone secreted primarily in the bloodstream by somatotropic cells in the anterior pituitary gland[178]. Apart from its anabolic effects and impact on height, GH is also implicated in the regulation of haematopoietic function. Expression of GH receptor (GHR), by which GH mediates most of its effects, has been found on T cells, B cells, NK cells, monocytes, thymocytes and HSCs in humans and in other species[179]. While the impact of its signalling seems to be dispensable for HSC function, as suggested by the lack of phenotypic defects in HSCs of GHR-deficient mice, GH has important effects on immune function in mice and humans, either directly or through its principal mediator, IGF1. In vivo administration of GH can reverse thymic involution[180–182]. Transgenic mice that overexpress GH have an enlarged thymus. Similarly, the administration of a recombinant form of GH or IGF1 promotes thymic regeneration, increases TCR diversity and enhances recovery of haematopoietic compartments in immunocompromised and aged animals[183].

GH administration to immunocompromised patients has been studied in several clinical trials. Daily administration of human recombinant GH (somatropin) enhances thymic function and peripheral immune function in HIV-infected patients (NCT00071240)[180,181]. The effects on thymic output appear to be transient, as discontinuation of GH treatment is associated with the recurrence of thymic atrophy[181]. Results from the recently completed studies (NCT00287677, NCT00119769 and NCT00050921) are still pending. The use of GH to reverse chronological ageing of the immune system was assessed in a recent pre-phase-I study performed in volunteers aged between 51 and 65 years. Magnetic

Lymphoid tissue inducer (LTi) cells
Cells that are present in developing lymph nodes, Peyer's patches and nasopharynx-associated lymphoid tissue. They are required for the development of these lymphoid organs and are characterized by expression of the transcription factor RORγt, interleukin-7 receptor-α and lymphotoxin-$\alpha_1\beta_2$.

resonance imaging of thymic density showed that the accumulated fat tissue in the thymus was replaced with regenerated tissue in seven of the nine participants receiving the treatment[184]. In the periphery, GH treatment was associated with a significant increase in both naive CD4$^+$ T cells and naive CD8$^+$ T cells, with a concomitant decrease in PD1$^+$CD8$^+$ T cells[184]. However, there are several concerns about the side effects associated with GH treatment, such as increased risk of heart disease, diabetes, elevated cholesterol levels and, more importantly, tumour progression. Thus, additional studies are needed to evaluate its potential use in humans.

Emerging approaches for T cell regeneration

Over the past decade, intensive work has been done to further optimize the efficacy of already identified approaches and to identify alternative regenerative mechanisms (FIG. 2). In addition to IL-7, other cytokines have demonstrated efficacy in preclinical mouse models to restore thymic function and/or expand immune cells in the periphery following immune insults. Administration of IL-12 not only induces thymocyte proliferation through increased IL-7 and IL-2 signalling but also enhances engraftment and haematopoietic reconstitution after transplantation[185,186]. IL-15 can also boost immunity primarily by promoting NK cell, NKT cell and CD8$^+$ T cell proliferation and function. Overall, these effects enhance reconstitution of these cell subsets and graft-versus-tumour responses in mouse models of allogeneic HCT[187–189].

IL-21 can enhance thymic function in young and aged mice. These effects are primarily mediated by the impact of IL-21 on DP thymocytes, which express high levels of IL-21 receptor after glucocorticoid-induced thymic atrophy, and by activation of the IL-21 downstream target BCL-6 (REF.[190]). Administration of recombinant IL-21 improved thymic regeneration and reconstitution of the peripheral naive T cell compartment in different models of immune damage, including glucocorticoid-induced thymic atrophy, ageing and allogeneic HCT[190–192].

IL-22 can also mediate thymic regeneration[64,193]. Following thymic damage, the loss of DP thymocytes can trigger the production of IL-22 by thymic ILCs in an IL-23-dependent manner (FIG. 2). IL-22 then acts on TECs to promote their survival and proliferation through activation of STAT3 and STAT5 and expression of the downstream antiapoptotic molecule MCL1 (REFS[64,193]). Administration of recombinant IL-22 to sublethally irradiated mice or allogeneic HCT recipients can promote the recovery of thymic function and the development of new thymic-derived peripheral T cells[64,80,193].

ILCs play a fundamental role in thymic reconstitution not only via IL-22 production but also through production of receptor activator of nuclear factor-κB ligand (RANKL), a potent factor known for its fundamental role in TEC maintenance and maturation[194–196]. Recent studies have characterized the role of RANKL in the regeneration of the thymic microenvironment and T cell recovery in mouse models of allogeneic HCT. RANKL is expressed early after thymic damage by CD4$^+$ thymocytes and, to a great extent, by lymphoid tissue inducer (LTi) cells[64,197]. RANKL acts on its cognate receptor, RANK, expressed on LTi cells, and induces upregulation of lymphotoxin-α (LTα). Following thymic damage, LTα can bind to LTβ receptor on thymic epithelial progenitor cells and TECs and promote their regeneration. Exogenous administration of recombinant RANKL boosts regeneration of thymic epithelial progenitor cells and TECs and improves T cell progenitor homing and de novo thymopoiesis. Overall, these effects lead to enhanced peripheral T cell reconstitution[197]. LTα can activate LTβ receptor on intestinal dendritic cells to induce IL-23 production, which in turn acts on intestinal ILCs to promote IL-22 production[198].

As cells producing RANKL, IL-22, LTα or LTβ are responsive to IL-7R signalling, which can promote their expansion and function[199,200], it is possible that these molecules contribute to the regenerative effects mediated by IL-7 in the thymus. Although IL-7 has not been shown to directly regulate IL-22, it can regulate $LT\alpha_1\beta_2$ expression by LTi cells[201], thus providing a mechanism by which IL-7R signalling integrates regenerative pathways. In addition, IL-7 can directly induce RANKL expression by T cells and aid in thymic regeneration[202].

In addition to its well-described role during thymus organogenesis[203,204], BMP4 can promote thymic regeneration after thymic damage. Indeed, BMP4 produced by thymic endothelial cells drives thymic regeneration by binding to its receptor expressed on TECs and stimulating the upregulation of FOXN1 and its target genes[65,205]. Importantly, adoptive transfer of ex vivo expanded thymic endothelial cells improves thymic reconstitution after a sublethal dose of total body irradiation through the delivery of BMP4.

Concluding remarks

At present, there are no approved therapies to enhance T cell function in patients with lymphopenia. The development of such approaches would not only benefit patients whose immune system has been decimated by multiple cycles of chemotherapy and radiotherapy or by viral infections but could also improve T cell responses in other clinical settings.

Cancer immunotherapy with immune checkpoint inhibitors is emerging as one of the most promising new treatments for a variety of solid and liquid malignancies. To be effective, these treatments rely on the presence of an adequate pool of T cells capable of recognizing specific tumour antigens[206]. Previous studies demonstrated that the limited response rate to checkpoint inhibitor therapy may be linked to a restricted TCR repertoire observed in the periphery of patients with cancer before therapy. Non-synonymous mutations and neoantigens are associated with clinical efficacy of immune checkpoint blockade[207–209]. Thus, treatments capable of improving immune functions and enhancing TCR repertoire diversity may have the potential to significantly extend the clinical benefit of immune checkpoint blockade.

Therapeutic approaches that can rejuvenate the peripheral T cell pool will also be relevant for the treatment of elderly patients not only to enhance responses to pathogens but also to increase the efficacy of vaccines. The progressive expansion of peripheral TCR clonality

and loss of specific T cell clones observed during ageing contribute to the moderate success rate of vaccination in elderly patients.

However, important aspects should be taken in consideration when one is designing approaches to restore immunocompetence in aged individuals. Recent work performed in old mice and non-human primates demonstrated that additional barriers limit the impact of immune regeneration in the periphery even when thymic regeneration is achieved[210]. Although the thymuses of old mice treated with sex steroid ablation or KGF can be rejuvenated, this did not translate into increased frequencies of naive $CD8^+$ T cells and naive $CD4^+$ T cells in peripheral blood. Age-related intrinsic defects of RTEs[210,211] and a defective thymic stromal microenvironment[113,114,177], together with reduced responses to homeostatic cytokines[212], could explain the defective maintenance and function of naive T cells in old recipients.

One promising, recently identified strategy to expedite immune reconstitution following HCT is the use of non-genotoxic conditioning approaches. The use of depleting antibodies, such as anti-CD117 and anti-CD45, which can recognize and eliminate HSCs and other haematopoietic cells in a targeted manner, allows remarkably efficient HSC engraftment while sparing non-haematopoietic cells, with minimal off-target toxicity[213–215]. This novel approach has the potential to reduce HCT-related toxicity, promote faster immune recovery and significantly improve patient clinical outcome.

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The authors contributed equally to all aspects of the article.

Competing interests
M.R.M.v.d.B. has received research support and stock options from Seres Therapeutics, has received royalties from Wolters Kluwer, has consulted for, received honoraria from or participated in advisory boards for Seres Therapeutics, Jazz Pharmaceuticals, Rheos, Therakos, WindMIL Therapeutics, Amgen, Merck & Co. Inc., Magenta Therapeutics, DKMS Medical Council (board), Forty Seven Inc. (spouse), Pharmacyclics (spouse) and Kite Pharmaceuticals (spouse) and has intellectual property licensing agreements with Seres Therapeutics and Juno Therapeutics. E.V. has acted as a consultant for and received honoraria from Ferring Pharmaceuticals. M.R.M.v.d.B. is an inventor on a patent application (US2015/058095) submitted by Memorial Sloan Kettering Cancer Center. Two provisional patent applications have been filed (US 15/033,178 and US 62/566,897) with E.V. and M.R.M.v.d.B. listed as inventors. J.J.T. declares no competing interests.

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