Check for updates

Immune reconstitution following stem cell transplantation

Marcel R. M. van den Brink,[1,2,3] Enrico Velardi,[3,4] and Miguel-Angel Perales[1,2]

[1]Department of Medicine, Adult Bone Marrow Transplant Service, Memorial Sloan Kettering Cancer Center, New York, NY; [2]Weill Cornell Medical College, New York, NY; [3]Immunology Program, Memorial Sloan Kettering Cancer Center, New York, NY; and [4]Division of Pharmacology, Department of Medicine, University of Perugia, Perugia, Italy

Learning Objectives

- Understanding immune reconstitution after hematopoietic stem cell transplantation
- Clinical implications of delays in post-transplant immune reconstitution
- Strategies to improve post-transplant immune reconstitution

Delayed immune reconstitution after hematopoietic stem cell transplantation (HSCT) has been associated with significant morbidity and mortality, especially after allogeneic HSCT (allo-HSCT), including infections and relapse.[1-3] In particular T-cell immunity is affected by the combined effects of the conditioning regimen, thymic involution in the host, donor age,[4] type of graft, stem cell dose, ex vivo or in vivo T-cell depletion, donor-host disparity, graft-versus-host disease (GVHD) prophylaxis, and GVHD itself (both acute and chronic).

Innate immunity recovers in the first months after HSCT: first monocytes, followed by granulocytes and natural killer cells.[5] In contrast, adaptive immunity, which consists of cellular (T lymphocytes) and humoral (B lymphocytes) immunity, takes 1-2 years to recover and a significant number of patients will incur even longer-lasting deficits.[6,7] Post-transplant T-cell recovery can occur through 2 mechanisms: (1) survival and peripheral expansion of infused donor (memory) T cells, and (2) de novo generation of donor T cells in the thymus from donor hematopoietic precursors.[6,7] The thymus is the primary site for the development of T cells. Lymphoid precursors traffic from the BM to the thymus and undergo a complex process, including proliferation, differentiation, and positive and negative selection resulting in the export of functional CD4 and CD8 T cells. Thymopoiesis occurs as a crosstalk between developing thymocytes and the stroma, which includes dendritic cells, macrophages, fibroblasts, endothelial cells, B cells, and thymic epithelial cells (TECs). In the first months after a HSCT peripheral expansion of the donor T cells is the dominant mechanism for T-cell recovery (except in the recipients of a T-cell depleted allograft) and results in particular in clonal expansion of CD8+ T cells with a limited repertoire.[8-10]

De novo T cell recovery: (1) gradually increases after a few months, (2) is dependent on a functional thymus, (3) is particularly important for CD4+ T cell recovery, (4) provides a more diverse T cell repertoire, (5) is impaired in older patients due to age-associated thymic involution, and (6) can be measured by T-cell receptor rearrangement excision DNA circles (TRECs; see next section).[11] Similar to HIV patients the risk of post-transplant infections is associated with the CD4 count.[12]

B cell counts recover by 6 months after auto HSCT and by 9 months after allo-HSCT. Recovery of humoral immunity is: (1) initially impaired because of limited antibody repertoire, (2) dependent on T cell help, and (3) decreased due to GVHD prophylaxis and treatment, and GVHD itself.[13]

When comparing immune recovery across graft sources and transplant approaches, the available data suggest that immune recovery occurs most rapidly in recipients of autologous grafts. As noted above several factors influence immune recovery after allogeneic HSCT. In general, immune recovery occurs more rapidly after unmodified graft transplants than in the setting of in vivo or ex vivo T-cell depletion. Recovery after cord blood transplants is also dependent on the use of in vivo T-cell depletion as outlined below and can be on par with unmodified grafts in the absence of anti-thymocyte globulin (ATG). Finally, there are increasing numbers of haploidentical transplants being performed with post-transplant cyclophosphamide being used to abrogate alloreactive T cells. Unpublished data indicate that immune recovery after post-transplant cyclophosphamide is similar in haploidentical or matched unrelated donor transplants, but delayed compared to unmodified HSCT with standard GVHD prophylaxis (McCurdy and Luznik, personal communication, September 21, 2015).

Monitoring of post-transplant immune reconstitution

Several assays are used to assess post-transplant immune recovery, including tests that are performed routinely in clinical laboratories [absolute lymphocyte counts (ALCs), lymphocyte subsets ($CD4^+$ and $CD8^+$ T cells, NK cells, B cells), and antibody titers], as well as assays that are currently performed in the research setting (measures of thymic output and T cell and B cell repertoire). Studies have demonstrated an association between the ALC early after autologous,[14,15] or allogeneic transplant,[16,17] and progression-free survival (PFS) and overall survival (OS). For example, an ALC > 500 cells/mcl at day 15 after autologous HSCT was shown to be an

Conflict-of-interest disclosures: M.R.M.v.d.B. is on the Board of Directors or an advisory committee for Novartis, has consulted for Boehringer Ingelheim and Novartis, has received honoraria from Regeneron and Merck, and is an advisory board attendee for Boehringer Ingelheim and Tobira Therapeutics. E.V. declares no competing financial interests. M.-A.P. is on the Board of Directors or an advisory committee for ImmunId and is an advisory board attendee for Amgen, Seattle Genetics, Merck, and Takeda.

Off-label drug use: None disclosed.

Downloaded from http://ashpublications.org/hematology/article-pdf/2015/1/215/1249620/bep0011500215.pdf by guest on 13 December 2024

independent predictor of improved PFS and OS in patients with multiple myeloma, non-Hodgkin lymphoma, Hodgkin Lymphoma, acute myelogenous leukemia, primary systemic amyloidosis, and metastatic breast cancer.[14] Similarly, in recipients of allografts, a higher ALC at days 21 or 30 was associated with improved OS and disease-free survival (DFS), as well as lower relapse rates.[16-18] More recent studies have shown similar results in recipients of cord blood transplant (UCBT). Lymphocyte populations including T, B, and NK cells are also routinely measured on a clinical basis. Early recovery of $CD4^+$ T cells correlated with OS,[19] non-relapse mortality,[19] as well as the risk of opportunistic infections.[19-21] Higher levels of $CD3^+$ and $CD8^+$ T cells also correlated with improved PFS.[22]

The use of multiparameter flow cytometry enables identification of additional subsets of T, B and NK cells, as well as myeloid subsets such as dendritic cells (DC). T cell subsets include naïve ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), effector memory ($CD45RA^-CCR7^-$) and effector ($CD45RA^+CCR7^-$) T cells, regulatory T cells ($CD4+CD25^{hi}FoxP3^+$), and T helper 17 cells. A recent study showed that effector memory $CD4^+$ and $CD8^+$ T cells were the predominant T-cell subset early after T-cell depleted allogeneic HSCT.[23] Flow cytometry can also asses thymic output by detection of recent thymic emigrants (RTEs) identified by the $CD4^+CD45RA^+CD31^+CD62L^{bright}CD95^{dim}$ and $CD8^+CD103^+CD62L^{bright}CD95^{dim}$ phenotypes. B-cell subsets include $CD27^-IgD^+$ naïve B cells, $CD27^+IgD^+$ "able to class switch" memory cells, and $CD27^+IgD^-$ "isotype switched" memory cells.[24] NK populations include NK and TCR-V-alpha-24^+NKT cells, and dendritic cells include myeloid DCs ($CD123^{low/+}CD11c^+$) and plasmacytoid DCs ($CD123^{bright}CD11c^{neg}$). Flow cytometry can also identify antigen-specific responses using either intracellular cytokine detection or tetramers.

Functional assays can provide important additional information on post-transplant immune recovery. Although T-cell proliferative responses (measured by ^{3}HTdR incorporation) to mitogens (PHA, OKT3), recall (candida, tetanus), viral or allogeneic antigens are still used in clinical laboratories,[25] more quantitative functional assays are now used in the research setting. These tests include the ELISPOT, intracellular cytokine secretion detected by flow cytometry, and tetramers. Cytokine secretion can be elicited by incubation with cells, lysates, proteins, or peptides. The use of protein-spanning pools of overlapping peptides has been used to detect both $CD4^+$ and $CD8^+$ responses without being limited by the patient's HLA.[26] These assays can evaluate viral-specific responses including those to cytomegalovirus (CMV), and Epstein-Barr virus (EBV), as well responses to tumor antigens, such as WT1, and track cells after adoptive transfer. Polyfunctional T cells that secrete multiple cytokines can also be detected by intracellular cytokine secretion. Studies in infectious diseases have shown that the ability to generate polyfunctional T-cell responses correlates with improved control of viral replication. More recently, tumor-specific polyfunctional $CD8^+$ T cells have been demonstrated in patients with advanced melanoma immunized against gp100 and tyrosinase.[27]

Molecular tests can be used to assess thymic output,[22,28,29] as well as newly derived functional bone marrow B cells.[30,31] TRECs are markers of thymopoiesis, and have more rapid recovery in younger patients and in recipients of conventional grafts compared to T-cell depleted grafts.[29] Low TREC values correlate strongly with severe opportunistic infections.[29] Production of B cells is assessed by detection of kappa-deleting recombination excision circles (KRECs).[30,31]

Finally, molecular techniques can also be used to asses T-cell receptor (TCR) repertoire and B-cell receptor (BCR) gene rearrangement diversity.[23,32] With the development of next generation sequencing, an increasingly detailed analysis of T-cell and B-cell diversity is emerging.[22,32-34] We recently reported on the TCR diversity in allogeneic HSCT and found significantly higher diversity in $CD4^+$ T cells than $CD8^+$ T cells, demonstrating the need to study subsets separately.[33] Furthermore, we showed that the most rapid recovery in TCR diversity was seen in cord blood recipients, followed by conventional grafts and T-cell depleted grafts. It should be noted that recipients of cord blood transplant in this study did not receive ATG as part of the conditioning regimen. This likely explains improved immune recovery, but also higher rates of GVHD, than in other series of cord blood transplant where the use of ATG in combination with the graft's naïve immune system has resulted in delayed immune recovery. Next generation sequencing can also be used to identify and monitor individual clonotypes, including known clonotypes specific for viral epitopes.[32,33]

Vaccines

As noted above, HSCT results in T-cell and B-cell deficiencies. In particular GVHD and rituximab use have a profound effect on B-cell recovery, irrespective of the stem cell source (double cord blood, conventional or T-cell depleted peripheral blood or bone marrow).[35] B-cell counts typically recover by 3-12 months post-HSCT, except in patients who received rituximab. CD4+ T cell recovery, which is impacted by factors, such as patient age, GVHD, and the use of T-cell depletion, usually occurs by 6-9 months after HSCT in pediatric patients, and up to twice as long in adult recipients. These delays in immune recovery result in decreased response to vaccines.[36] In the absence of revaccination, antibody titers to vaccine-preventable diseases decline during the first decade after autologous or allogeneic HSCT.[37-39] HSCT recipients are therefore at increased risk for infections, particularly with certain organisms such as pneumococcal infection, Hemophilus influenza type b (Hib) infection, measles, varicella, and influenza. Furthermore, due to recent reductions in vaccination rates, there has been a decrease in heard immunity and resultant outbreaks of measles and mumps. Current guidelines recommend that HSCT recipients undergo revaccination after HSCT.[40]

Although there is limited clinical data on vaccine efficacy in HSCT recipients, it is accepted that there has to be at least partial recovery of T and B cells. Although the timing of recovery differs between autologous and allogeneic HSCT and also based on graft source and manipulation, most guidelines on immunization are based on timing from HSCT. Vaccination with inactivated or toxoid containing vaccines is recommended as early as 3-6 months following HSCT, whereas administration of live-attenuated vaccines is recommended at 24 months post-HSCT.[40] The delayed use of live-attenuated vaccines is based on concerns about transmission of vaccine-mediated disease and the limited data on the safety and immunogenicity of earlier vaccination.[41]

Inactivated vaccines should be preferred over live vaccines for patients receiving immunosuppressants because of their reduced ability of mounting sufficient immune responses and the risks of uncontrolled virus replications. The question remains whether patients with ongoing GVHD should be vaccinated. Guidelines suggest that live vaccines should be avoided in these patients, but

Downloaded from http://ashpublications.org/hematology/article-pdf/2015/1/215/1249620/bep00115000215.pdf by guest on 13 December 2024

there is no conclusive evidence showing that inactivated vaccines exacerbate GVHD.[42]

Recent data from our center has shown the safety and immunogenicity of the live attenuated varicella vaccine when given according to pre-set immune milestones (CD4 cells >200/μL, normal PHA, IgG >500 mg/dL at least 6 weeks post-IVIG).[43] In contrast, we have also shown that despite acquisition of minimal milestones of immune reconstitution, only 15% of patients respond to a single conjugated meningococcal vaccine and 35% of patients did not respond to any of the 4 serotypes. This data suggests that a series of two MCV4 as currently recommended for patients with asplenia, complement deficiency, or HIV should be evaluated in this patient population.[44] Additional research is needed to ascertain the optimal timing of post-HSCT vaccines and immunization based on immune recovery parameters rather than time from HSCT.

Donor vaccination. Pre-HSCT donor vaccination may represent a potentially attractive strategy to boost immunity and prevent infections to pathogens that cause significant morbidity and mortality. However, despite an extensive effort, studies are still inconclusive and have not shown any beneficial effect in preventing infections.[45] In addition, there are ethical issues related to donor immunization. As a result, the 2013 Infectious Diseases Society of America guidelines recommend against immunizing the donor solely for the benefit of the recipient.

Virus-specific T-cell clones. An alternative approach to provide anti-viral immunity for transplanted recipients is through the isolation of donor derived virus-specific T cells or through the ex vivo amplification and expansion of virus-specific T cells stimulated with antigen-presenting cells expressing the viral antigens. Virus-specific T cells for common post-transplant pathogens (including EBV, CMV, and adenovirus) have been successfully generated, showing the safety and efficacy of these strategies in improving immune recovery after HSCT. Furthermore, recent studies have demonstrated that 3rd-party viral-specific T cells against EBV or CMV can be safely administered to allo-HSCT as well as solid organ transplant recipients with encouraging clinical results. Although, several limitations in this approach have to be addressed (such as the costs, the complexity of the manufacturing and the time to produce clinical grade T cells), adoptive transfer of virus-specific T cells still represents an attractive strategy to prevent post-transplant viral infections[46]

Novel strategies

At present there is no "standard-of-care" approach to enhance post-transplant immune reconstitution, however, several strategies are being developed in preclinical models as well as early clinical trials. The following strategies are currently in clinical development.

Interleukin-7 (IL-7). IL-7 has many lymphopoietic effects on both T and B cells through: (1) supporting lymphoid precursors, (2) promotion of T-cell development in the thymus, and (3) anti-apoptotic effects during T-cell development.

Mouse models of allogeneic allo-HSCT have shown that IL-7 enhances thymopoiesis, stimulates T-cell proliferation, increases T-cell numbers, and enhances T-cell diversity.[47,48] Initial clinical trials with recombinant human IL-7 (rhIL-7; CYT99–007, Cytheris) demonstrated a dose-dependent expansion of CD4+ and CD8+ T cells in patients with solid tumors or HIV infection[49,50] We recently completed a phase I trial of rhIL-7 (CYT107, Cytheris) in patients with myeloid hematologic malignancies who underwent a T-cell depleted allogeneic HSCT.[23] Patients were treated with escalating doses of rhIL-7 (3 at 10 mcg/kg, 6 at 20 mcg/kg, 3 at 30 mcg/kg) administered SQ weekly for 3 weeks starting at a median of 103 days post-transplant (range, 60-244 days). IL-7 was well tolerated and no patients have developed GVHD, anti-IL-7 antibodies or neutralizing antibodies. In most patients, we observed an increase in CD4+ and CD8+ T cells with evidence of recent thymic emigrants and TRECs, as well as increased TCR repertoire diversity and functional T-cell responses to viral antigens. A phase I clinical trial with IL-7 in HSCT recipients of a CD34+ selected allograft demonstrated low toxicity and no GVHD at doses, which could increase T-cell recovery and T-cell repertoire diversity.[23]

Keratinocyte growth factor (KGF). KGF has been approved for the prophylaxis of mucositis in patients receiving chemo- and/or radiation therapy, however, preclinical studies have indicated that KGF administration can also enhance thymopoiesis through the induction of proliferation of TECs.[51] A clinical trial to test the effect of KGF in combination with leuprolide (see below) on post-transplant T-cell reconstitution is underway.

Sex steroid ablation (SSA). Both estrogen and testosterone have inhibitory effects on early lymphoid precursors, thymopoiesis and B lymphopoiesis.[52-58] The mechanisms through which this occurs are largely unknown, but studies are underway. For example, sex steroids in the thymus seem to decrease the expression of Notch ligand, which is an important driver of T-cell development.[59] Studies both in man and mouse demonstrated that SSA using castration (in mice) or the luteinizing hormone releasing hormone (LHRH) agonist Leuprolide after auto- and allo-HSCT or cytoablative therapy per se results in: (1) increased numbers of lymphoid precursors and import of thymic precursors into the thymus, (2) improved thymopoiesis, (3) enhanced B lymphopoiesis, and (4) improved recovery of functional immunity.[51] As mentioned above, a clinical trial combining SSA with leuprolide and KGF in HSCT recipients is underway.

Growth hormone (GH). Preclinical studies have shown that GH administration can enhance in thymopoiesis in old animals and improve HSC function. Clinical studies in HIV+ patients demonstrated enhanced thymopoiesis and antiviral immunity.[60,61] In addition, several strategies are being developed in preclinical models, including: (1) Flt3L: administration of Flt3L enhances thymic dependent and independent T-cell recovery and increases Flt3L+ precursors in the BM, but decreases B lymphopoiesis.[62,63] (2) IL-22: upon thymic injury innate lymphoid cells type 3 in the thymus secrete IL-22 to promote endogenous regeneration of TECs[64]; IL-22 administration can promote thymic, as well as intestinal regeneration after injury[65] and a phase I study in patients with GVHD is planned. Other cytokines and growth factors, which can enhance immune reconstitution in animal models include: IGF-1, IL-2, IL-12, IL-15, parathyroid hormone, and retinoic acid.[11]

T-cell precursors. Preclinical studies have shown that T-cell precursors can be generated and expanded from HSCs in an ex vivo culture system using Notch-1 stimulation, as well as IL-7 and Flt3L. Adoptive transfer of these cells with the allograft can be done across MHC barriers and results in enhanced thymopoiesis, chimerism, development of host-tolerant and fully functional T cells, and enhanced NK reconstitution.[66,67]

Thymic tissue transplant has been used for the treatment of children with DiGeorge syndrome (congenital hypoplastic thymus).[68] A

Downloaded from http://ashpublications.org/hematology/article-pdf/2015/1/215/1249620/bep00115000215.pdf by guest on 13 December 2024

number of groups are employing tissue engineering techniques to create an artificial thymus, using various biomaterials, thymic epithelial precursor cells, and/or mesenchymal cells.

Several studies have shown that the regulatory T cell (Treg) content in the allograft is associated with improved immune reconstitution and less GVHD and CMV infection.[69-72] Initial studies regarding adoptive cell therapy with donor-derived regulatory T cells have also shown improved T-cell reconstitution and less GVHD.[73,74]

Correspondence

Marcel van den Brink, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065; Phone: 646-888-2304; Fax: 646-422-0452; e-mail: vandenbm@mskcc.org.

References

1. Pizzo PA, Rubin M, Freifeld A, Walsh TJ. The child with cancer and infection: II. Nonbacterial infections. *J Pediatr*. 1991;119:845-857.
2. Mackall CL, Fleisher TA, Brown MR, et al. Lymphocyte depletion during treatment with intensive chemotherapy for cancer. *Blood*. 1994;84:2221-2228.
3. Mackall CL. T-cell immunodeficiency following cytotoxic antineoplastic therapy: a review. *Stem Cell*. 2000;18:10-18.
4. Kollman C, Howe CW, Anasetti C, et al. Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age. *Blood*. 2001;98:2043-2051.
5. Storek J, Geddes M, Khan F, et al. Reconstitution of the immune system after hematopoietic stem cell transplantation in humans. *Semin Immunopathol*. 2008;30:425-437.
6. Mackall CL, Fleisher TA, Brown MR, et al. Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. *N Engl J Med*. 1995;332:143-149.
7. Hakim FT, Memon SA, Cepeda R, et al. Age-dependent incidence, time course, and consequences of thymic renewal in adults. *J Clin Invest*. 2005;115:930-939.
8. Mackall CL, Fleisher TA, Brown MR, eta al. Distinctions between CD8+ and CD4+ T-cell regenerative pathways result in prolonged T-cell subset imbalance after intensive chemotherapy. *Blood*. 1997;89:3700-3707.
9. Fagnoni FF, Lozza L, Zibera C, et al. T-cell dynamics after high-dose chemotherapy in adults: elucidation of the elusive CD8+ subset reveals multiple homeostatic T-cell compartments with distinct implications for immune competence. *Immunology*. 2002;106:27-37.
10. Heitger A, Neu N, Kern H, et al. Essential role of the thymus to reconstitute naive (CD45RA+) T-helper cells after human allogeneic bone marrow transplantation. *Blood*. 1997;90:850-857.
11. van den Brink MRM, Dudakov JA. Strategies for immune reconstitution following allogeneic hematopoietic cell transplantation. In: DS Basow, Ed. *UpToDate*. Waltham, MA: 2012.
12. Storek J, Gooley T, Witherspoon RP, Sullivan KM, Storb R. Infectious morbidity in long-term survivors of allogeneic marrow transplantation is associated with low CD4 T cell counts. *Am J Hematol*. 1997;54:131-138.
13. Storek J, Wells D, Dawson MA, Storer B, Maloney DG. Factors influencing B lymphopoiesis after allogeneic hematopoietic cell transplantation. *Blood*. 2001;98:489-491.
14. Porrata LF, Markovic SN. Timely reconstitution of immune competence affects clinical outcome following autologous stem cell transplantation. *Clin Exp Med*. 2004;4:78-85.
15. Jimenez-Zepeda VH, Reece DE, Trudel S, et al. Absolute lymphocyte count as predictor of overall survival for patients with multiple myeloma treated with single autologous stem cell transplant. *Leuk Lymphoma*. Prepublished on Feb 24, 2015, as DOI 10.3109/10428194.2014.1003057.
16. Le Blanc K, Barrett AJ, Schaffer M. Lymphocyte recovery is a major determinant of outcome after matched unrelated myeloablative transplantation for myelogenous malignancies. *Biol Blood Marrow Transplant*. 2009;15:1108-1115.
17. Kim HT, Armand P, Frederick D, et al. Absolute lymphocyte count recovery after allogeneic hematopoietic stem cell transplantation predicts clinical outcome. *Biol Blood Marrow Transplant*. 2015;21:873-880.
18. Savani BN, Mielke S, Rezvani K, et al. Absolute lymphocyte count on day 30 is a surrogate for robust hematopoietic recovery and strongly predicts outcome after T cell-depleted allogeneic stem cell transplantation. *Biol Blood Marrow Transplant*. 2007;13:1216-1223.
19. Kim DH, Sohn SK, Won DI, Lee NY, Suh JS, Lee KB. Rapid helper T-cell recovery above 200 x 10 6/l at 3 months correlates to successful transplant outcomes after allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2006;37:1119-1128.
20. Small TN, Avigan D, Dupont B, et al. Immune reconstitution following T-cell depleted bone marrow transplantation: effect of age and posttransplant graft rejection prophylaxis. *Biol Blood Marrow Transplant*. 1997;3:65-75.
21. Servais S, Lengline E, Porcher R, et al. Long-term immune reconstitution and infection burden after mismatched hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2014;20:507-517.
22. Kanda J, Chiou LW, Szabolcs P, et al. Immune recovery in adult patients after myeloablative dual umbilical cord blood, matched sibling, and matched unrelated donor hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2012;18:1664-1676.e1.
23. Perales MA, Goldberg JD, Yuan J, et al. Recombinant human interleukin-7 (CYT107) promotes T-cell recovery after allogeneic stem cell transplantation. *Blood*. 2012;120:4882-4891.
24. Small TN, Robinson WH, Miklos DB. B cells and transplantation: an educational resource. *Biol Blood Marrow Transplant*. 2009;15:104-113.
25. Small TN, Papadopoulos EB, Boulad F, et al. Comparison of immune reconstitution after unrelated and related T-cell-depleted bone marrow transplantation: effect of patient age and donor leukocyte infusions. *Blood*. 1999;93:467-480.
26. Trivedi D, Williams RY, O'Reilly RJ, Koehne G. Generation of CMV-specific T lymphocytes using protein-spanning pools of pp65-derived overlapping pentadecapeptides for adoptive immunotherapy. *Blood*. 2005;105:2793-2801.
27. Perales MA, Yuan J, Powel S, et al. Phase I/II study of GM-CSF DNA as an adjuvant for a multipeptide cancer vaccine in patients with advanced melanoma. *Mol Ther*. 2008;16:2022-2029.
28. Douek DC, Vescio RA, Betts MR, et al. Assessment of thymic output in adults after haematopoietic stem-cell transplantation and prediction of T-cell reconstitution [see comments]. *Lancet*. 2000;355:1875-1881.
29. Lewin SR, Heller G, Zhang L, et al. Direct evidence for new T-cell generation by patients after either T-cell-depleted or unmodified allogeneic hematopoietic stem cell transplantations. *Blood*. 2002;100;2235-2242.
30. van Zelm, M. C., Szczepanski, T., van der Burg, M., and van Dongen, J. J. Replication history of B lymphocytes reveals homeostatic proliferation and extensive antigen-induced B cell expansion. *J Exp Med*. 2007;204:645-655
31. Lev A, Simon AJ, Bareket M, et al. The kinetics of early T and B cell immune recovery after bone marrow transplantation in RAG-2-deficient SCID patients. *PloS One*. 2012;7:e30494.
32. Krell PF, Reuther S, Fischer U, et al. Next-generation-sequencing-spectratyping reveals public T-cell receptor repertoires in pediatric very severe aplastic anemia and identifies a beta chain CDR3 sequence associated with hepatitis-induced pathogenesis. *Haematologica*. 2013;98:1388-1396.
33. van Heijst JW, Ceberio I, Lipuma LB, et al. Quantitative assessment of T cell repertoire recovery after hematopoietic stem cell transplantation. *Nat Med*. 2013;19:372-377.
34. Meyer EH., Hsu AR, Liliental J, et al. A distinct evolution of the T-cell repertoire categorizes treatment refractory gastrointestinal acute graft-versus-host disease. *Blood*. 2013;121:4955-4962.
35. Small TN, Cowan MJ. Immunization of hematopoietic stem cell transplant recipients against vaccine-preventable diseases. *Expert Rev Clin Immunol*. 2011;7:193-203.
36. Pao M, Papadopoulos EB, Chou J, et al. Response to pneumococcal

Downloaded from http://ashpublications.org/hematology/article-pdf/2015/1/215/1249620/bep00115000215.pdf by guest on 13 December 2024

(PNCRM7) and haemophilus influenzae conjugate vaccines (HIB) in pediatric and adult recipients of an allogeneic hematopoietic cell transplantation (alloHCT). *Biol Blood Marrow Transplant*. 2008;14: 1022-1030.
37. Ljungman P, Fridell E, Lönnqvist B, et al. Efficacy and safety of vaccination of marrow transplant recipients with a live attenuated measles, mumps, and rubella vaccine. *J Infect Dis*. 1989;159:610-615.
38. Ljungman P, Wiklund-Hammarsten M, Duraj V, et al. Response to tetanus toxoid immunization after allogeneic bone marrow transplantation. *J Infect Dis*. 1990;162:496-500.
39. Pauksen K, Duraj V, Ljungman P, et al. Immunity to and immunization against measles, rubella and mumps in patients after autologous bone marrow transplantation. *Bone Marrow Transplant*. 1992;9:427-432.
40. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biol Blood Marrow Transplant*. 2009; 15:1143-1238.
41. Forlenza CJ, Small TN. Live (vaccines) from New York. *Bone Marrow Transplant*. 2013;48:749-754.
42. Ljungman P, Engelhard D, de la Cámara R, et al; Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. Vaccination of stem cell transplant recipients: recommendations of the Infectious Diseases Working Party of the EBMT. *Bone Marrow Transplant*. 2005;35:737-746.
43. Chou JF, Kernan NA., Prockop S, et al. Safety and immunogenicity of the live attenuated varicella vaccine following T replete or T cell-depleted related and unrelated allogeneic hematopoietic cell transplantation (alloHCT). *Biol Blood Marrow Transplant*. 2011;17:1708-1713.
44. Mahler MB, Taur Y, Jean R, Kernan NA, Prockop SE, Small TN. Safety and immunogenicity of the tetravalent protein-conjugated meningococcal vaccine (MCV4) in recipients of related and unrelated allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* 2012;18(1):145-149.
45. Harris AE, Styczynski J, Bodge M, et al. Pretransplant vaccinations in allogeneic stem cell transplantation donors and recipients: an often-missed opportunity for immunoprotection? *Bone Marrow Transplant*. 2015;50:899-903.
46. Leen AM, Heslop HE, Brenner MK. Antiviral T-cell therapy. *Immunol Rev*. 2014;258:12-29.
47. Mackall CL, Fry TJ, Gress RE. Harnessing the biology of IL-7 for therapeutic application. *Nat Rev Immunol*. 2011;11:330-342.
48. Alpdogan O, van den Brink MR. IL-7 and IL-15: therapeutic cytokines for immunodeficiency. *Trends Immunol*. 2005;26:56-64.
49. Rosenberg SA, Sportes C, Ahmadzadeh M, et al. E. IL-7 administration to humans leads to expansion of CD8+ and CD4+ cells but a relative decrease of CD4+ T-regulatory cells. *J Immunother*. 2006;29:313-319.
50. Levy Y, Lacabaratz C, Weiss L, et al. Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. *J Clin Invest*. 2009;119: 997-1007.
51. Velardi E, Dudakov JA, van den Brink MR. Clinical strategies to enhance thymic recovery after allogeneic hematopoietic stem cell transplantation. *Immunol Lett*. 2013;155(1-2):31-35.
52. Zoller AL, Kersh GJ. Estrogen induces thymic atrophy by eliminating early thymic progenitors and inhibiting proliferation of beta-selected thymocytes. *J Immunol*. 2006;176:7371-7378.
53. Olsen NJ, Kovacs WJ. (2001) Effects of androgens on T and B lymphocyte development. *Immunol Res*. 2006;23:281-288.
54. Grimaldi CM, Jeganathan V, Diamond B. Hormonal regulation of B cell development: 17 beta-estradiol impairs negative selection of high-affinity DNA-reactive B cells at more than one developmental checkpoint. *J Immunol*. 2006;176:2703-2710.
55. Viselli SM, Reese KR, Fan J, et al. Androgens alter B cell development in normal male mice. *Cell Immunol*. 1997;182:99-104.
56. Igarashi H, Kouro T, Yokota T, Comp PC, Kincade PW. Age and stage dependency of estrogen receptor expression by lymphocyte precursors. *Proc Nat Acad Sci U S A*. 2001;98:15131-15136.
57. Kincade PW, Medina KL, Payne KJ, et al . Early B-lymphocyte precursors and their regulation by sex steroids. *Immunol Rev*. 2000;175: 128-137.
58. Medina KL, Garrett KP, Thompson LF, Rossi MI, Payne KJ, Kincade PW. Identification of very early lymphoid precursors in bone marrow and their regulation by estrogen. *Nat Immunol*. 2001;2:718-724.
59. Velardi E, Tsai JJ, Holland AM, et al. Sex steroid blockade enhances thymopoiesis by modulating Notch signaling. *J Exp Med*. 2014;211(12): 2341-2349.
60. Napolitano LA, Schmidt D, Gotway MB, et al. Growth hormone enhances thymic function in HIV-1-infected adults. *J Clin Invest*. 2008;118:1085-1098.
61. Chen BJ, Cui X, Sempowski GD, Chao NJ. Growth hormone accelerates immune recovery following allogeneic T-cell-depleted bone marrow transplantation in mice. *Exp Hematol.* 2003;31:953-958.
62. Fry TJ, Sinha M, Milliron M, et al. Flt3 ligand enhances thymic-dependent and thymic-independent immune reconstitution. *Blood*. 2004; 104:2794-2800.
63. Wils EJ, Braakman E, Verjans GM, et al. Flt3 ligand expands lymphoid progenitors prior to recovery of thymopoiesis and accelerates T cell reconstitution after bone marrow transplantation. *J Immunol*. 2007;178: 3551-3557.
64. Dudakov JA, Hanash AM, Jenq RR, et al. Interleukin-22 drives endogenous thymic regeneration in mice. *Science*. 2012;336:91-95.
65. Hanash AM, Dudakov JA, Hua G, et al. Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. *Immunity*. 2012;37:339-350.
66. Zakrzewski JL, Kochman AA, Lu SX, et al. Adoptive transfer of T-cell precursors enhances T-cell reconstitution after allogeneic hematopoietic stem cell transplantation. *Nat Med*. 2006;12:1039-1047.
67. Zakrzewski JL, Suh D, Markley JC, et al. Tumor immunotherapy across MHC barriers using allogeneic T-cell precursors. *Nat Biotechnol*. 2008;26:453-461.
68. Markert ML, Marques JG, Neven B, et al. First use of thymus transplantation therapy for FOXN1 deficiency (nude/SCID): a report of 2 cases. *Blood*. 2011;117:688-696.
69. Torelli GF, Lucarelli B, Iori AP, et al. The immune reconstitution after an allogeneic stem cell transplant correlates with the risk of graft-versus-host disease and cytomegalovirus infection. *Leuk Res*. 2011;35:1124-1126.
70. Rezvani K, Mielke S, Ahmadzadeh M, et al. High donor FOXP3-positive regulatory T-cell (Treg) content is associated with a low risk of GVHD following HLA-matched allogeneic SCT. *Blood*. 2006;108:1291-1297.
71. Gaidot A, Landau DA, Martin GH, et al. Immune reconstitution is preserved in hematopoietic stem cell transplantation coadministered with regulatory T cells for GVHD prevention. *Blood*. 2011;117:2975-2983.
72. Winstead CJ, Reilly CS, Moon JJ, et al.CD4+CD25+Foxp3+ regulatory T cells optimize diversity of the conventional T cell repertoire during reconstitution from lymphopenia. *J Immunol*. 2010;184:4749-4760.
73. Brunstein CG, Miller JS, Cao Q, et al. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. *Blood*. 2011;117:1061-1070.
74. Di Ianni M, Falzetti F, Carotti A, et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. *Blood*. 2011;117:3921-3928.

Downloaded from http://ashpublications.org/hematology/article-pdf/2015/1/215/1249620/bep00115000215.pdf by guest on 13 December 2024