Bone Marrow Transplantation
https://doi.org/10.1038/s41409-019-0445-0

REVIEW ARTICLE

Targeting the niche: depleting haemopoietic stem cells with targeted therapy

Edward Abadir[1,2] · Christian Bryant[1,3] · Stephen Larsen[2,3] · Georgina J. Clark[1,2]

Received: 14 September 2018 / Revised: 4 December 2018 / Accepted: 30 December 2018

Abstract
Haemopoietic stem cell transplantation is an expanding procedure worldwide but is associated with significant morbidity and mortality. Depletion of resident haemopoietic stem and progenitor cells (HSPC) is required for both autologous and allogeneic haemopoietic stem cell transplantation. Current conditioning protocols utilise chemotherapy or radiation to effectively reduce HSPC but are toxic in both the short and long term. The initial trials to use monoclonal antibodies to target HSPC were limited with marginal efficacy but platforms including antibody drug conjugates and chimeric antigen receptor T cells have made targeted conditioning strategies achievable. In this review we summarise the work developing targeted conditioning that may replace or reduce alkylating agents and total body irradiation. The prospect of conditioning with significantly reduced toxicity will improve outcomes and open transplantation to patients unable to tolerate current conditioning protocols.

Targeting the niche: depleting haemopoietic stem cells with targeted therapy

Haemopoietic stem cell transplantation (HSCT) is a rapidly expanding procedure with over 20,000 and 40,000 transplants performed annually in the United States and Europe, respectively [1, 2]. The majority of HSCT are performed for haematological malignancies but there is increasing use in non-malignant conditions. Substantial donor engraftment is only possible when the haemopoietic stem cell (HSC) bone marrow niche has been cleared of recipient cells to allow space for donor cells to reside [3, 4]. While allogeneic grafts can use graft-versus-host effects to generate their own bone marrow niches without prior depletion of haemopoietic stem and progenitor cells (HSPC), this is characterised by incomplete engraftment and very slow kinetics [5]. To achieve complete engraftment and rapid kinetics, conditioning regimens traditionally incorporate radiation and/or alkylating agents [6–8]. Conditioning regimens have three functions: depletion of HSPC from the niche, suppression of the anti-graft immune response in allogeneic transplantation and, if performed to treat malignancy, to reduce the burden of residual disease. Replacing radiation/alkylating agents with targeted therapy to clear the HSC niche may reduce both short and long-term toxicity, especially in high-risk patients which include those at the extremes of age or with primary immunodeficiencies. The rapidly expanding procedure of genetically modified autologous HSCT would become available to more patients with the development of less toxic conditioning regimens.

Chemotherapy and radiotherapy meet all three functions of conditioning but, as nonspecific agents, are associated with significant toxicity. The initial regimens for allogeneic (allo)-HSCT used myeloablative conditioning (MAC) but the toxicity limited use in the older patients and those with significant comorbidities. Reduced intensity conditioning (RIC) and non-MAC regimens have been developed opening allo-HSCT to those patients for whom MAC is too toxic [9]. RIC leads to both partial and full donor chimerism and is associated with reduced non-relapse mortality (NRM) compared to MAC [10]. Targeted conditioning has the potential to be incorporated into RIC protocols, reducing or replacing alkylating agents to further diminish their toxicity.

✉ Georgina J. Clark
georgina.clark@sydney.edu.au

[1] Dendritic Cell Research, ANZAC Research Institute, Sydney, NSW, Australia

[2] Sydney Medical School, The University of Sydney, Sydney, NSW, Australia

[3] Institute of Haematology, Royal Prince Alfred Hospital, Sydney, NSW, Australia

Published online: 21 January 2019

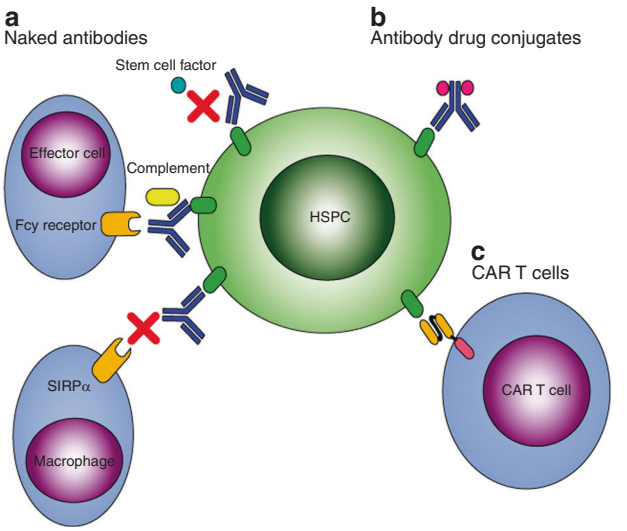

Fig. 1 Methods studied for targeted conditioning modalities. **a** Naked mAbs to CD45 and CD117 deplete HSPC through complement and cellular mediated mechanisms of cytotoxicity. Antibodies to CD117 block the binding of SCF which inhibits proliferation and differentiation of HSPC. Blockade of CD47 prevents the binding of the inhibitory molecule SIRPα increasing the ADCC potential of other co-administered mAb. **b** ADC against CD45 and CD117 are internalised upon binding to their target and administer their cytotoxic payload. **c** CAR T cells bind to CD117 or CD123 where they are activated leading to HSPC depletion

Despite improvements in HSCT and the adoption of RIC, the 100 day mortality remains high in allo-HSCT [11]. Short term toxicity associated with conditioning regimens include myelosuppression, mucositis, organ toxicity and sinusoidal obstruction syndrome [12]. Many patients are ineligible to undergo HSCT due to the toxicity, overcoming the short term toxicity would increase access of allo-HSCT for patients who otherwise have poor long term outcomes. Long-term toxicities resulting from HSCT conditioning include secondary malignancies, cardio-pulmonary toxicity, endocrinopathies and musculoskeletal disorders [13]. Long term survivors have high rates of infertility and increased rates of spontaneous abortion during pregnancy [14, 15]. Conditioning agents are associated with therapy related leukaemia, myelodysplasia and solid organ malignancies [16, 17]. The mortality in allo-HSCT patients who have survived for more than 5 years post-transplant remains 5–9 times higher than the general population, with a 30% reduction in life expectancy in the absence of relapse [18].

The advent of gene therapy for inherited disorders, including haemoglobinopathies and primary immunodeficiencies, has resulted in a new indication for autologous stem cell transplantation. Prior to the development of gene therapy, the only curative option for those with inherited disorders of haematopoiesis was allo-HSCT. Trials using genetically modified autologous products in HSCT have been conducted in patients with thalassaemia, sickle cell disease and primary immunodeficiency disorders [19–23]. Transplantation of genetically modified autologus HSPCs requires myeloablative doses of conditioning to enable significant engraftment, a conditioning agent that provides potent but targeted ablation of HSPCs would be ideal in this scenario [24].

The ideal target molecule would be expressed on HSPC and not outside of the haemopoietic system. Attempts to develop mAbs to deplete HSPC have been limited by the lack of sustained depletion using the cytotoxic and biological properties of naked antibodies, but new approaches with CD47 blockade, antibody-drug conjugates (ADC) and chimeric antibody receptor (CAR) T cells have opened the possibility of achieving targeted conditioning regimens (Fig. 1). To date, only four targets have been studied as outlined in Table 1.

Naked antibodies

CD45

CD45 (leucocyte common antigen) is expressed on all leucocytes and not outside of the haemopoietic system [25, 26]. CD45 is expressed as multiple isoforms, all of which are found on $CD34^+$ cells but the earliest progenitors are $CD45RA^-$ [27]. Despite the isoforms, mAbs directed at pan CD45 epitopes target all leucocytes. The effect of an anti-CD45 mAb on reduction of engraftment was demonstrated using an anti-CD45 ($RT7^a$ allotype) mAb in a rat heart transplant model where passenger donor leucocytes were reduced to <0.1% in peripheral blood compared to 4–5% for those not treated with the mAb [28]. The anti-CD45 $RT7^a$ antibody prevented engraftment of $RT7^a$ allotype HSPC in a competitive engraftment experiment, as well as causing lethal bone marrow failure in $RT7^a$ rats. The mAb depleted HSPC, mature myeloid and T cells while allowing for stable engraftment by donor cells from CD45 $RT7^b$ rats [29]. B cell progenitors were depleted but mature B cells were resistant despite being coated with the antibody. The anti-CD45 $RT7^a$ mAb facilitated high haploidentical donor chimerism when used at myeloablative doses [30]. The rat anti-mouse CD45 mAb 30-F11 transiently depleted mature myeloid and lymphoid cells but not HSPC in immunocompetent mice [31]. 30-F11 conditioning alone did not allow for donor engraftment in syngeneic or allogeneic models but did increase donor chimerism when combined with radiation.

Unconjugated rat anti-human CD45 mAb depleted human lymphoid and myeloid populations prior to HSCT. The rat anti-human CD45 mAbs YTH25.4 and YTH54.12 were administered in equal combination two to ten days prior to traditional chemo-radiotherapy conditioning for

Table 1 Targeted conditioning studies to date

Target	Construct	Findings	References
CD45	Anti-rat mAb (RT7[a] allotype)	Depletes mature lymphocytes and HSPC in rats, conditioning allows for haploidentical HSCT	[29, 30]
	Anti-mouse mAb (30-F11)	Depletes mature lymphocytes and myeloid cells but not HSPC in mice, increases donor chimerism when used with radiation	[31]
	Anti-human mAbs YTH25.4 and YTH54.12	Depletes mature lymphocytes and myeloid cells but not HSPC, reduction in peripheral AML blasts in 2/3 cases. Incorporated into minimal intensity conditioning regimen with 11/16 patients achieving full or high level chimerism	[32–34]
	Anti-mouse ADC (clone 104-Saporin)	Depletes mature lymphoid cells and HSPC, conditioning allows for high level sustained multilineage engraftment of congenic mice	[49]
CD117	Anti-mouse mAb (clone ACK-2)	Causes a transient neutropenia, anaemia and thrombocytopenia with a reduction of progenitor subsets in immunocompetent mice. Can deplete HSPC when combined with radiation. Only leads to significant donor engraftment in immunocompromised mice and not immunocompetent mice	[4, 40, 41]
	Anti-human mAb (clone SR-1)	Conditioning allows for long term engraftment in 1 of 5 macaques with non-myeloablative irradiation or busulfan in an autologous gene modified model. Depletes normal and MDS human HSPC in an mouse xenograft model	[42, 43]
	Anti-human mAb (clone AMG 191)	Depletes normal and MDS human HSPC in an mouse xenograft model, clinical trial (NCT02963064) for patients with primary immunodeficiencies to receive AMG191 conditioning prior to allo-HSCT is currently recruiting	[43]
	Anti-mouse ADC (CD117-saporin)	Combined with T cell depleting agents allowed for significant and durable engraftment in an immunocompetent mouse allo-HSCT model	[50]
	Anti-human CD117- ADC (conjugate unspecified)	Depletes human HSPC in vitro and in mouse xenografts	[51]
	Anti-mouse CD117 CAR T cells	Depletes mouse HSPC in vitro and in vivo, allow durable limited chimerism in congenic and CGD models	[57]
CD47	Anti-mouse CD47 FAB	Depletes immunocompetent mouse HSPC when combined with anti-CD117 mAb and allows for durable chimerism in a congenic HSCT and allo-HSCT (with added T cell depletion)	[47]
CD123	Anti-human CD123 CAR T cells	Causes ablation of healthy human haematopoiesis in a mouse xenograft, after depleation of the CAR T cells a second sex mismatch healthy human graft can be performed. Depletes co-engrafted healthy human HSPC and AML	[58, 59]

HSPC haemopoietic stem and progenitor cells, *HSCT* haemopoietic stem cell transplant, *allo-HSCT* allogeneic haemopoietic stem cell transplant, *AML* acute myeloid leukaemia, *MDS* myelodysplastic syndrome, *CGD* chronic granulomatous disease

allo-HSCT in 14 patients with myelodysplastic syndrome (MDS) or acute leukaemia [32, 33]. Doses of 400 µg/kg (maximum tolerated dose) over four days depleted mature lymphoid and myeloid cells [32]. Even at this maximum dose myeloid progenitor cells were spared. The half-life of the mAb was 12 h and 12 of 14 patients engrafted. Two of the three patients with measurable leukaemic blasts at the time of mAb administration had reductions in blast percentage. In a subsequent trial in children less than one year of age receiving allo-HCT for primary immunodeficiency, the combination of YTH25.4 and YTH54.12 was incorporated into a reduced intensity conditioning regimen including alemtuzumab, fludarabine and low dose cyclophosphamide [34]. Fifteen of 16 patients engrafted and 11 achieved full or high percentage donor chimerism. Three patients achieved T lineage engraftment only and one patient required a second transplant. 81% of the patients survived with immune restoration observed at a median of 40 months.

CD117

CD117 (c-Kit) is highly expressed on HSPC and with its ligand, stem cell factor (SCF), is essential for haemopoiesis [35–37]. A concern about targeting CD117 is its expression outside of the haemopoietic system; CD117 is expressed on melanocytes and epidermal cells, as well as in the CNS and GI tract [38, 39]. The rat anti-mouse CD117 antibody, ACK-2, transiently reduced HSC in the RAG2$^{-/-}$ γc$^{-/-}$ immunodeficient mouse, and when used as the only conditioning modality, facilitated stable engraftment [4]. ACK-2 also inhibited HSPC proliferation when exposed to SCF in vitro confirming a non Fc dependant mechanism. ACK-2 could only induce significant long term reduction of HSPC in immunocompetent mice when combined with low dose radiation [40]. This combination facilitated the transplant of a lentivirus vector transduced autologous HSC in mice with X-linked chronic granulomatous disease (CGD) as a model for mAb conditioning with gene therapy. ACK-2 conditioning allowed for long term donor engraftment in immunocompetent mice when administered in utero with HSCT performed on day one after birth [41].

The mouse anti-human CD117 mAB, SR-1, produced long term engraftment in one of five subjects when combined with non-myeloablative irradiation or busulfan in an autologous gene modified macaque model [42]. Autologous CD34 cells were transfected with a lentiviral vector to allow for long term tracking and reinfused after administration of reduced dose total body irradiation (TBI) and SR-1 (3 subjects) or reduced dose busulfan and SR-1 (2 subjects). The only subject that demonstrated long term engraftment (<1% at 300 days) was in the TBI cohort . SR-1 reduced both human and macaque colony forming units in the absence of effector cells.

SR-1 and a humanised CD117 mAb, AMG 191, deplete HSPC derived from patients with MDS in an NOD/SCID-γ (NSG) model [43]. The anti-CD117 mAbs cleared MDS derived cells from low and intermediate risk MDS for greater than 8 weeks, whilst high risk and very high risk MDS cells would rebound to near baseline levels within 8 weeks. The rebound of MDS derived cells was prevented with a second transplant of healthy HSPC performed one week after the mAb administration. A clinical trial is currently recruiting (NCT02963064) patients with severe combined immunodeficency to receive AMG 191 conditioning prior to allo-HSCT. The trial design does not incorporate other conditioning agents. To avoid the loss of donor HSPC, pharmacokinetic levels of the AMG191 will be monitored and patients will receive the graft only after antibody has cleared from the circulation. The long half-life of mAbs in circulation may limit their utility unless modifications that increase their clearance (such as removing the FcRn region) are made to ensure timely and safe delivery of the donor cells.

CD47

CD47 is an immunoglobulin like protein that interacts with its ligand SIRPα to inhibit phagocytosis of CD47 expressing cells in what is known as a “don’t eat me” signal [44, 45]. Blocking CD47 with mAb has been shown to potentiate the antibody dependent cell mediated cytotoxicity (ADCC) effect of different mAb directed at other surface antigens [46]. Combining the anti-CD117 antibody ACK-2 with an anti-CD47 Fab increased ADCC in vitro using immunocompetent mouse HSC [47]. This combination facilitated engraftment in an immunocompetent CD45 congenic mouse model. ACK-2 was then combined with an anti-CD47 Fab and recipient T cell depletion in an allo-HSCT minor MHC mismatch model. Durable but variable engraftment of myeloid, T, B, NK and HSPC populations was achieved. Naked antibodies have demonstrated the potential to target HSPC but lack the potency to play a key role in conditioning for most types of HSCT. Blocking CD47 overcomes the limited efficacy of naked antibodies. The limitations of naked antibodies can be further overcome using the ability of mAb targeting in more potent therapeutics.

Antibody drug conjugates

ADC are a rapidly emerging class of therapeutic in which a mAb is bound to a drug or toxin. The appeal of this design rests with the targeted potent toxins leading to a wide therapeutic window. The key elements of ADC are the choice of target for the mAb, the design and function of the

antibody, the linker and toxic payload chosen. Most current payloads are small molecules with sub-nanomolar inhibitory concentration 50 (IC_{50}) values, which led to improved efficacy compared to early generation ADC that often used conventional chemotherapy payloads [48].

CD45 ADC in HSCT conditioning was demonstrated with a rat anti-mouse mAb conjugated to a ribosomal inhibitory saporin (SAP) payload [49]. Anti CD45.2, CD49d, CD84, CD90, CD133, CD135 and CD184 mAbs were evaluated using a SAP payload. CD45.2-SAP caused the greatest depletion of HSPC in immunocompetent C57BL/6 mice (CD45.2 allotype). The cytotoxicity of the ADC was dependant on the anti-CD45 mAb clone. Clone 104-SAP depleted 98% of HSPC at the highest dose level but colony forming progenitors were less effected. A 3mg/kg dose of CD45.2-SAP produced chimerism levels of 75–90% using a CD45.1 donor. At 8 months, normal numbers of donor myeloid, B and T cells were recorded suggesting unbiased stem cell engraftment. CD45.2-SAP preserved normal bone marrow architecture compared to TBI, which reduced vascular integrity and bone marrow cellularity. Mice conditioned with CD45-SAP had a quicker recovery of their peripheral myeloid cells and had a survival advantage when exposed to *C. albicans* compared to mice conditioned with TBI. Conditioning with CD45.2-SAP resulted in significant chimerism when recipient mice with a knock-in human sickle cell gene were transplanted with wild type donors, leading to normalisation in haemoglobin, reduction of splenomegaly and an absence of sickle cells on blood films.

An anti-CD117-saporin immunotoxin combined with T cell depleting agents allowed for significant and durable engraftment in an immunocompetent mouse allo-HSCT model [50]. Multi-lineage chimerism developed with B cell chimerism being most pronounced. The transplanted mice tolerated same donor skin transplants without rejection. A human anti-human CD117 mAb ADC has been developed that kills >95% of human HSPC in vitro [51]. In vivo studies using humanised NSG mice demonstrated that the CD117-ADC depleted >98% of HSPC. B and T cells were spared in the in vivo testing. The mAb has been engineered to have a half-life of less than 12 h to allow for donor cell infusion without real time pharmacokinetic monitoring. The increased potency of ADC allows for highly efficient stem cell depletion, but also increases the risk of off target effects.

CAR T cells

Chimeric antigen receptor (CAR) T cells are genetically modified T cells that use the variable regions of mAb to direct the T cells to the chosen antigen. The extracellular CAR region activates a signal independently of the T cell receptor [52]. Success in the treatment of B cell acute lymphoblastic leukaemia, non-Hodgkin lymphoma and chronic lymphocytic leukaemia with CAR T cells demonstrate their powerful cytotoxic ability [53–56]. Murine CAR T cells directed against CD117 deplete mouse HSPC in vitro and in vivo and facilitate long term, but limited engraftment when used as conditioning in a CGD mouse model [57]. The murine derived CAR T cells initially had limited expansion and migration into the bone marrow in vivo but this was overcome by co-transducing mouse CXCR4 DNA (with the anti-CD117 CAR) and pre-treating with cyclophosphamide. CAR T cells were derived from $Thy1.1^+$ mice while recipients were $Thy1.2^+$, this divergence allowed the CAR T cells to be depleted with an anti-Thy1.1 antibody prior to donor cell infusion. In a mouse model using CD45.1 recipients CD45.2 donors, donor chimerism ranged from 20–30% at 36 weeks. In a CGD mouse model, the activation of reactive oxygen species (ROS) in neutrophils was assessed, the neutrophils of CGD mice do not produce ROS, but a partial ROS response was obtained at 12 weeks after engraftment with wild type donor cells using CAR T cell conditioning.

CAR T cells directed against the CD123 (IL-3 Receptor α subunit) to target acute myeloid leukaemia (AML) have been trialled in xenograft models, but their utility is limited by significant myelotoxicity [58]. A potential way to circumvent myelotoxicity is to use CD123 directed CAR T cells as part of allogeneic conditioning. In a humanised mouse model, subsequently engrafted with an AML cell line, anti-CD123 CAR T cells eliminated both the AML cell line and human graft [59]. The CAR T cells were depleted with alemtuzumab or were co-transfected at the time of production with the CD20 gene and could be eliminated with rituximab. Elimination was evaluated with flow cytometry and further confirmed with AML rechallenge. Mice engrafted with an AML cell line cleared the disease with anti-CD123 CAR T cell administration and exhibited a large T cell expansion as well preventing engraftment on AML rechallenge. Mice who had CAR T cell depleting therapy after initial AML clearance did not undergo T cell expansion and died with AML progression after the second presentation. Administration of CD20 expressing CAR T cells in NSG mice depleted healthy human xenografts, subsequently rituximab eradicated the CAR T cells and allowed for a second sex mismatch healthy human graft, which was confirmed using FISH on myeloid cells. Total CAR T cell depletion is required for graft safety. Targeting surface molecules as a method to deplete CAR T cells carries the risk of increased immunosuppression with T cell depletion using native targets or potential failure of CAR T cells to express a transfected target.

Conclusions

Early attempts to target HSPC for conditioning with mAb were met by limited efficacy using naked antibodies. New methods with antibody combinations, ADC and CAR T cells have now put targeted conditioning within reach but several questions remain. With highly potent cytotoxic strategies available, the concern for off target effects of new conditioning agents increases. Stringent evaluation of therapeutics with targets expressed outside of the haemopoietic system will be required prior to human trials. Another major safety concern is ensuring that the depleting agent is no longer active at the time of donor infusion. Many of the animal models used, circumvent this concern by targeting recipient specific isoforms. Real time drug monitoring may prevent primary graft failure but if the agent has an extended half-life this would prolong neutropenia. A robust and predictable elimination of ADC or CAR T cells prior to donor cell infusion is required.

The choice of target and modality is critical, with different antibody clones towards a single target having a range of cytotoxicity even when matched with identical toxins [49]. Implementation of targeted conditioning therapy will most likely be initially in the genetically modified autologous transplant setting or those with primary immunodeficiency, as only a single conditioning agent would be required. Targeted conditioning in allo-HSCT would be more complex as T cell depletion and possibly NK depletion is required to avoid graft loss [60, 61]. If the targeted conditioning regimen did not reduce these populations, conventional chemotherapies (e.g., purine analogues such as fludarabine) or other targeted therapies would need to be incorporated. Traditional conditioning regimens lead to tissue inflammation and the activation of host antigen presenting cells which influence graft-versus-host and graft-versus-tumour effects [62, 63]. How targeted conditioning may alter these factors has not been explored and requires further study. Targeted conditioning agents may have a role in reducing tumour burden (including minimal residual disease) at the time of transplant by binding to surface molecules shared in both HSPC and malignant cells, a number of CD45 radioisotopes have been trailed in conditioning regimens in high risk AML to reduce relapse risk [64].

Targeting the populations of the stem cell niche may be able to reduce the toxic effects of conditioning thereby expanding the therapeutic window of HSCT. Significant challenges remain but renewed interest with the accessibility of new modalities has increased the potential that targeted conditioning may become a reality.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. EBMT. EBMT Annual Report. 2017. https://www.ebmt.org/ebmt/documents/ebmt-annual-report-2017.
2. CIMBTR. CIMBTR Transplant Data. 2018. https://bloodcell.transplant.hrsa.gov/RESEARCH/Transplant_Data/US_Tx_Data/.
3. Micklem H, Clarke C, Evans E, Ford C. Fate of chromosome-marked mouse bone marrow cells tranfused into normal syngeneic recipients. Transplantation. 1968;6:299–302.
4. Czechowicz A, Kraft D, Weissman IL, Bhattacharya D. Efficient transplantation via antibody-based clearance of hematopoietic stem cell niches. Science. 2007;318:1296–9.
5. Storb R, Yu C, Barnett T, Wagner JL, Deeg HJ, Nash RA, et al. Stable mixed hematopoietic chimerism in dog leukocyte antigen–identical littermate dogs given lymph node irradiation before and pharmacologic immunosuppression after marrow transplantation. Blood. 1999;94:1131–6.
6. Bhattacharya D, Rossi DJ, Bryder D, Weissman IL. Purified hematopoietic stem cell engraftment of rare niches corrects severe lymphoid deficiencies without host conditioning. J Exp Med. 2006;203:73–85.
7. Tjonnfjord G, Steen R, Veiby O, Friedrich W, Egeland T. Evidence for engraftment of donor-type multipotent CD34+ cells in a patient with selective T-lymphocyte reconstitution after bone marrow transplantation for B-SCID. Blood. 1994;84:3584–9.
8. Müller SM, Kohn T, Schulz AS, Debatin K-M, Friedrich W. Similar pattern of thymic-dependent T-cell reconstitution in infants with severe combined immunodeficiency after human leukocyte antigen (HLA)–identical and HLA-nonidentical stem cell transplantation. Blood. 2000;96:4344–9.
9. Atilla E, Atilla PA, Demirer T. A review of myeloablative vs reduced intensity/non-myeloablative regimens in allogeneic hematopoietic stem cell transplantations. Balk Med J. 2017;34:1.
10. Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood. 1998;91:756–63.
11. Shouval R, Labopin M, Bondi O, Mishan-Shamay H, Ciceri F, Esteve J, et al. Prediction of allogeneic hematopoietic stem-cell transplantation mortality 100 days after transplantation using a machine learning algorithm: a European Group for Blood and Marrow Transplantation Acute Leukemia Working Party Retrospective Data Mining Study. J Clin Oncol. 2015;33:3144–52.
12. Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. Blood. 2014;124:344–53.
13. Bhatia S. Long-term health impacts of hematopoietic stem cell transplantation inform recommendations for follow-up. Expert Rev Hematol. 2011;4:437–54.
14. Brennan B, Shalet SM. Endocrine late effects after bone marrow transplant. Br J Haematol. 2002;118:58–66.
15. Sanders JE, Hawley J, Levy W, Gooley T, Buckner CD, Deeg HJ, et al. Pregnancies following high-dose cyclophosphamide with or

without high-dose busulfan or total-body irradiation and bone marrow transplantation. Blood. 1996;87:3045–52.
16. Krishnan A, Bhatia S, Slovak ML, Arber DA, Niland JC, Nademanee A, et al. Predictors of therapy-related leukemia and myelodysplasia following autologous transplantation for lymphoma: an assessment of risk factors. Blood. 2000;95:1588–93.
17. Rizzo JD, Curtis RE, Socié G, Sobocinski KA, Gilbert E, Landgren O, et al. Solid cancers after allogeneic hematopoietic cell transplantation. Blood. 2009;113:1175–83.
18. Martin PJ, Counts GW Jr, Appelbaum FR, Lee SJ, Sanders JE, et al. Life expectancy in patients surviving more than 5 years after hematopoietic cell transplantation. J Clin Oncol. 2010; 28:1011–6.
19. Boztug K, Schmidt M, Schwarzer A, Banerjee PP, Díez IA, Dewey RA, et al. Stem-cell gene therapy for the Wiskott–Aldrich syndrome. New Engl J Med. 2010;363:1918–27.
20. Shaw KL, Garabedian E, Mishra S, Barman P, Davila A, Carbonaro D, et al. Clinical efficacy of gene-modified stem cells in adenosine deaminase–deficient immunodeficiency. J Clin Invest. 2017;127:1689–99.
21. Kang EM, Malech HL. Gene therapy for chronic granulomatous disease. Methods in enzymology. Vol. 507. Elsevier; USA. 2012. p. 125–54.
22. Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, et al. Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. Nature. 2010;467:318–22.
23. Thompson AA, Walters MC, Kwiatkowski J, Rasko JE, Ribeil J-A, Hongeng S, et al. Gene therapy in patients with transfusion-dependent β-thalassemia. New Engl J Med. 2018;378:1479–93.
24. Uchida N, Weitzel RP, Platner C, Ballantine J, Bonifacino AC, Price SD, et al. Myeloablative conditioning is required for efficient engraftment of gene-modified cells and prevention of antibody production against transgene products in a rhesus stem cell gene therapy model. Mol Ther. 2015;23:S118–S9.
25. Shah VO, Civin CI, Loken MR. Flow cytometric analysis of human bone marrow. IV. Differential quantitative expression of T-200 common leukocyte antigen during normal hemopoiesis. J Immunol. 1988;140:1861–7.
26. Thomas ML, Lefrançois L. Differential expression of the leucocyte-common antigen family. Immunol Today. 1988;9:320–6.
27. Craig W, Poppema S, Little MT, Dragowska W, Lansdorp PM. CD45 isoform expression on human haemopoietic cells at different stages of development. Br J Haematol. 1994;88:24–30.
28. Ko S, Deiwick A, Jäger MD, Dinkel A, Rohde F, Fischer R, et al. The functional relevance of passenger leukocytes and microchimerism for heart allograft acceptance in the rat. Nat Med. 1999;5:1292–97.
29. Dahlke MH, Lauth OS, Jäger MD, Roeseler T, Timrott K, Jackobs S, et al. In vivo depletion of hematopoietic stem cells in the rat by an anti-CD45 (RT7) antibody. Blood. 2002;99:3566–72.
30. Jäger MD, Vondran FW, Ramackers W, Röseler T, Schlitt HJ, Bektas H, et al. A depleting anti-CD45 monoclonal antibody as isolated conditioning for bone marrow transplantation in the rat. PLoS One. 2016;11:e0154682.
31. Wulf GG, Luo K-L, Goodell MA, Brenner MK. Anti-CD45–mediated cytoreduction to facilitate allogeneic stem cell transplantation. Blood. 2003;101:2434–9.
32. Krance RA, Kuehnle I, Rill DR, Mei Z, Pinetta C, Evans W, et al. Hematopoietic and immunomodulatory effects of lytic CD45 monoclonal antibodies in patients with hematologic malignancy. Biol Blood Marrow Transplant. 2003;9:273–81.
33. Brenner MK, Wulf GG, Rill DR, LUO KL, Goodell MA, Mei Z, et al. Complement-Fixing CD45 Monoclonal Antibodies to Facilitate Stem Cell Transplantation in Mouse and Man. Ann NY Acad Sci. 2003;996:80–8.
34. Straathof KC, Rao K, Eyrich M, Hale G, Bird P, Berrie E, et al. Haemopoietic stem-cell transplantation with antibody-based minimal-intensity conditioning: a phase 1/2 study. Lancet. 2009; 374:912–20.
35. McKinney-Freeman SL, Naveiras O, Yates F, Loewer S, Philitas M, Curran M, et al. Surface antigen phenotypes of hematopoietic stem cells from embryos and murine embryonic stem cells. Blood. 2009;114:268–78.
36. Shin JY, Hu W, Naramura M, Park CY. High c-Kit expression identifies hematopoietic stem cells with impaired self-renewal and megakaryocytic bias. J Exp Med. 2014;211:217–31.
37. Russell ES. Hereditary anemias of the mouse: a review for geneticists. Advances in genetics. Vol. 20: Elsevier; USA. 1979. p. 357–459.
38. Lammie A, Drobnjak M, Gerald W, Saad A, Cote R, Cordon-Cardo C. Expression of c-kit and kit ligand proteins in normal human tissues. J Histochem Cytochem. 1994;42:1417–25.
39. Miettinen M, Lasota J. KIT (CD117): a review on expression in normal and neoplastic tissues, and mutations and their clinicopathologic correlation. Appl Immunohistochem Mol Morphol. 2005;13:205–20.
40. Xue X, Pech NK, Shelley WC, Srour EF, Yoder MC, Dinauer MC. Antibody targeting KIT as pretransplantation conditioning in immunocompetent mice. Blood. 2010;116:5419–22.
41. Derderian SC, Togarrati PP, King C, Moradi PW, Reynaud D, Czechowicz A, et al. In utero depletion of fetal hematopoietic stem cells improves engraftment after neonatal transplantation in mice. Blood. 2014;124:973–80.
42. Chandrasekaran D, Nakamoto B, Watts KL, Kiem H-P, Papayannopoulou T. Modeling promising nonmyeloablative conditioning regimens in nonhuman primates. Hum Gene Ther. 2014;25:1013–22.
43. Pang WW, Czechowicz A, Poyser J, Park CY, Weissman IL, Shizuru JA. Anti-Human CD117 antibodies mediate clearance of myelodysplastic syndrome hematopoietic stem cells and facilitate establishment of normal hematopoiesis in transplantation. Biol Blood Marrow Transplant. 2018;24:S230–S1. (Abstract 313)
44. Chao MP, Majeti R, Weissman IL. Programmed cell removal: a new obstacle in the road to developing cancer. Nat Rev Cancer. 2012;12:58–67.
45. Jaiswal S, Jamieson CH, Pang WW, Park CY, Chao MP, Majeti R, et al. CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. Cell . 2009;138:271–85.
46. Willingham SB, Volkmer J-P, Gentles AJ, Sahoo D, Dalerba P, Mitra SS, et al. The CD47-signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors. Proc Natl Acad Sci. 2012;109:6662–7.
47. Chhabra A, Ring AM, Weiskopf K, Schnorr PJ, Gordon S, Le AC, et al. Hematopoietic stem cell transplantation in immunocompetent hosts without radiation or chemotherapy. Sci Transl Med. 2016;8:351ra105–351ra105.
48. Beck A, Goetsch L, Dumontet C, Corvaïa N. Strategies and challenges for the next generation of antibody–drug conjugates. Nat Rev Drug Discov. 2017;16:315–37.
49. Palchaudhuri R, Saez B, Hoggatt J, Schajnovitz A, Sykes DB, Tate TA, et al. Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin. Nat Biotechnol. 2016;34:738–45.
50. Li Z, Czechowicz A, Scheck A, Rossi D, Murphy P. Robust Hematopoietic Mixed Chimerism and Donor-Specific Skin Allograft Tolerance after Non-Genotoxic Anti-CD117 Immunotoxin Conditioning and Donor Bone Marrow Allotransplantation. Am J Transplant. 2018;18:399. (Abstract 398)

51. Hartigan AJ, Pearse BR, McDonough SM, Proctor JL, Adams HL, McShea MA, et al. A Non-Genotoxic Antibody Drug Conjugate Targeting C-Kit for Hematopoietic Stem Cell Transplant Conditioning. Biol Blood Marrow Transplant. 2018;24:S47–S8. (Abstract 23)
52. Rivière I, Sadelain M. Chimeric antigen receptors: a cell and gene therapy perspective. Mol Ther. 2017;25:1117–24.
53. Brentjens RJ, Rivière I, Park JH, Davila ML, Wang X, Stefanski J, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood. 2011;118:4817–28.
54. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor–modified T cells in chronic lymphoid leukemia. New Engl J Med. 2011;365:725–33.
55. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric antigen receptor–modified T cells for acute lymphoid leukemia. New Engl J Med. 2013;368:1509–18.
56. Kochenderfer JN, Dudley ME, Kassim SH, Somerville RP, Carpenter RO, Stetler-Stevenson M, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol. 2015;33:540–9.
57. Arai Y, Choi U, Corsino CI, Koontz SM, Tajima M, Sweeney CL, et al. Myeloid conditioning with c-kit-targeted CAR-T cells enables donor stem cell engraftment. Mol Ther. 2018;26:1181–97.
58. Gill S, Tasian SK, Ruella M, Shestova O, Li Y, Porter DL, et al. Preclinical targeting of human acute myeloid leukemia and myeloablation using chimeric antigen receptor–modified T cells. Blood. 2014;123:2343–54.
59. Tasian SK, Kenderian SS, Shen F, Ruella M, Shestova O, Kozlowski M, et al. Optimized depletion of chimeric antigen receptor T cells in murine xenograft models of human acute myeloid leukemia. Blood. 2017;129:2395–407.
60. Gill S, Olson JA, Negrin RS. Natural killer cells in allogeneic transplantation: effect on engraftment, graft-versus-tumor, and graft-versus-host responses. Biol Blood Marrow Transplant. 2009; 15:765–76.
61. Mattsson J, Ringdén O, Storb R. Graft failure after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2008;14:165–70.
62. Hill GR. Inflammation and bone marrow transplantation. Biol Blood Marrow Transplant. 2009;15:139–41.
63. Stenger EO, Turnquist HR, Mapara MY, Thomson AW. Dendritic cells and regulation of graft-versus-host disease and graft-versus-leukemia activity. Blood. 2012;119:5088–103.
64. Gyurkocza B, Lazarus H, Giralt S. Allogeneic hematopoietic cell transplantation in patients with AML not achieving remission: potentially curative therapy. Bone Marrow Transplant. 2017;52: 1083–90.