Chemo-radiotherapy free conditioning regimen: immunotoxin at its magical best

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In the June, 2016 issue of *Nature Biotechnology*, Palchaudhury and colleagues report a novel immunotoxin-based preparative regimen that efficiently conditions immunocompetent mice for autologous hematopoietic stem cell transplantation (HSCT) (1). In comparison with total body irradiation (TBI), there was equivalent endogenous HSC depletion with similar donor chimerism, avoided neutropenia and anemia, enabled rapid immune reconstitution, better preservation of bone marrow architecture and had minimal overall toxicity.

Professor E. Donnall Thomas [1920–2012] got the Nobel Prize for Medicine in the year 1990 for his pioneering work in the field of bone marrow transplantation (BMT), now called allogeneic haematopoietic stem cell transplantation (HSCT). Currently, allogeneic HSCT is being done for the treatment of hematological malignancies (leukemias, lymphomas, myelodysplastic syndrome etc.) and non-haematological conditions e.g., severe aplastic anemia, haemoglobinopathies and immunodeficiency and childhood congenital metabolic disorders etc. Over past four decades procedure has been well established with study of long term effects. This procedure involves conditioning of recipient by high-dose chemotherapy with or without radiotherapy (preparative regimen) followed by stem cell infusion obtained from an HLA-identical donor. This results in engraftment of donor hematopoietic cells following ablation of the recipient's bone marrow (myeloablation) and immune system (immune-ablation). The haematological malignancy is eradicated both by myeloablation and more importantly by the allo-immune reaction (graft-versus-leukaemia or GVL effect) of the engrafted donor cells against residual cells in the recipient. This procedure is not easy to control every time and can lead to conditioning regimen-related organ toxicity (RRT) and graft-versus-host disease (GVHD) where non-haemopoietic cells (e.g., gut, skin, liver, and lung) are targeted. The later remain the major cause of morbidity and mortality. Following success of transplant in a case of Wiskott–Aldrich syndrome in 1978, it was hypothesized that HSCT can potentially cure inherited hematologic disorders. From gene therapy point of view, allogeneic HSCT as a procedure replaces mutant hematopoietic stem cells (HSCs) with functional HSCs from a healthy donor resulting in normalization of the hematopoietic system. Thus, allogeneic HSCT is potentially curative option for most patients with malignant and non-malignant hematological disorders, transplant related morbidity and mortality remains a major deterrent. In addition, a number of long-term complications (an effect of conditioning regimen) e.g., infertility and gonadal failure, growth disturbance, chronic GVHD, and a risk for secondary malignancies are other important limitations (2).

In search for better conditioning regimen, reduced-intensity-conditioning (RIC) regimen was developed in early 2000. This uses lower doses of chemotherapy and radiation than the standard myeloablative conditioning (MAC) but more of immune-ablation with use of drugs e.g., ATG, fludarabine. This novel approach results in less or limited myeloablation but sufficient lymphoablation, which is enough to achieve donor chimerism (mostly mixed in initial days). This approach was associated with reduction in early regimen-related toxicities and is considered as an option for

patients with hematologic malignancies who are not eligible for standard allograft either because of age or major co-morbidities. There was further support for this approach when it was observed that mixed chimerism remained stable in patients transplanted for hemoglobinopathies (3). For such patients, mixed chimerism even with a low fraction of donor marrow progenitors (20–30%), have significant enrichment of erythrocyte over leukocyte chimerism which improves their hematological parameters (4). In addition, the presence of residual host cells during the mixed chimerism state also offer an advantage of reducing the incidence and severity of GVHD due to central immune tolerance (5).

Three main objectives of conditioning prior to allogeneic HSCT are (I) to create space for the donor HSCs to home in; (II) to provide immunosuppression for favorable engraftment; and (III) eradication of the residual disease. Conventional MAC regimens (including TBI and cytotoxic agents) not only play a role in the creation of space for donor HSC but also act as immune suppressants to enhance the engraftment further. In immunodeficient mice, an omission of conditioning resulted in very low donor HSC chimerism as the endogenous HSC niches were too few for the purified donor HSCs to access (6). The low-level mixed donor chimerism though sufficient to restore immune function as evidenced in clinical transplantation, at times result in rejection of graft (4,7). So, the existing host stem cells must be eradicated by the conditioning regimen in order to create space for the donor HSCs to access these niches to obtain the necessary support for proliferation and differentiation and result in favorable engraftment as proposed by several studies (8,9).

The principle behind allogeneic HSCT for non-malignant disorder is to create a favorable niche for the donor HSCs to engraft as well as to prevent rejection of donor HSCs achieving a chimerism sufficient enough to produce a functional cure of the underlying disease. HSCT using genetically corrected autologous stem cells after its initial success (10) is emerging as a valid alternative to allogeneic HSCT and will avoid complications like GVHD. But the conditioning regimen may still have to eradicate the defective endogenous HSCs from the niche for these genetically corrected autologous HSCs to home in with utmost safety to the patient.

Modern approaches to anticancer drug development are based on Paul Ehrlich's concept of 'Magic Bullet' in the early 1900s. Since then, polyclonal and later monoclonal antibodies have been developed for targeting drugs, toxins etc. with the aim of developing targeted therapies for cancer. These have the advantage of being highly cytotoxic to the targets and easy to be manipulated by genetic engineering methods. This concept has been evaluated to specifically target the HSCs in the BM niche and spare the non-hematopoietic cells, thereby decreasing the morbidity as well as mortality due to off-target toxicity. The targets that have been evaluated in mice are monoclonal antibodies against HSC antigens like CD117 (C-Kit) and CD45 as summarized in *Tables 1,2*.

C-Kit interacts with its ligand, stem cell factor (SCF), which is a key signaling pathway involved in homing, adhesion, maintenance, and survival of HSCs in the hematopoietic niche (24,25). HSCs from all stages of development express the same levels of the C-Kit receptor or CD117 on the cell surface and as HSC differentiates to mature progeny expression of C-Kit is down regulated (24,25). Therefore, an antibody against the C-Kit receptor will deplete endogenous HSCs selectively than the mature progenitors. C-Kit signaling inhibition seems a feasible approach in immunodeficient mice (11) and to some extent in fetal immunocompetent mice (in-utero transplant) (12). However, in immunocompetent adult mice, it failed when used alone (13) and required additional radiation or chemotherapy or CD47 blockade (which enhance antibody-dependent cellular phagocytosis) to achieve desirable chimerism (*Table 1*) (14,15).

It was observed that there was a lower hematological relapse with a higher dose of TBI in conditioning (16 *vs.* 12 Gy) but at a cost of higher mortality because of higher radiation dose to important organs like liver and lungs (26). So, further improvement in allogeneic HSCT may still be possible if the radiation dose could still be increased at the level of bone marrow without any additional toxicity.

CD45 is a tyrosine phosphatase stably expressed on virtually all hematopoietic cells and absent in non-hematopoietic tissues and was tagged with high energy emitting radioisotope in one study (27). Subjects with high-risk hematological malignancies were focus of studies using anti-CD45 antibody with (22,23) or without (18-21) radioisotope in addition to DNA damaging agents (*Table 2*). This approach was associated with modest success in mice (18-21) and dogs (22,23).

In the present study, Palchaudhuri *et al*. (1) have taken one step further; an anti-CD45 antibody was coupled with saporin (SAP), which is a ricin-family toxin without a general cell entry domain and halts protein synthesis only upon receptor-mediated internalization into the cytoplasm.

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Table 1 Monoclonal Ab based HSC depletion strategy targeting CD 117 (C-Kit)

Author/year	Conditioning	Recipient	Engraftment	Comment
Targeting CD 117: anti-C-KIT naked antibody (ACK2)				
Czechowicz et al. [2007] (11)	ACK2 alone	Immunocompromised mice	Yes, ~90%	Administration of ACK2 led to the transient removal of >98% of endogenous HSCs in immunodeficient mice and up to 90% chimerism of donor HSCs
Derderian et al. [2014] (12)	ACK2 alone	In utero (fetal) immunocompetent mice	Yes, 15–20%	ACK2 conditioning alone is sufficient to enhance engraftment in this setting
Kraft et al. [2004] (13)	High dose ACK2	Immunocompetent mice	No	ACK2 does not enhance donor engraftment in immunocompetent adult as a single isolated regimen
Xue et al. [2010] (14)	ACK2 + SLR	Immunocompetent adult mice	Yes, ~75%	Engraftment was very low in ACK2 alone vs. ACK2 + SLR (5.0%±2.5% vs. 76.5%±5.5%)
Chhabra et al. [2014] (15)	ACK2 + CV1	Immunocompetent mice	Yes, ~70%	CV1 is CD47 antagonist, which enhance the antibody-dependent cellular phagocytosis (ADCP) activity of ACK2. Engraftment was very low in ACK2 only conditioning vs. ACK2 + CV1 (0 % vs. ~70%)
Chandrasekaran et al. [2014] (16)	ACK2 + SLR or busulfan	Non-human primate (juvenile pigtailed macaques)	Yes, ~20%	SLR seems a better partner than busulfan. The engraftment levels were low and dependent on competitive advantage of gene corrected cells over endogenous cells
Targeting CD 117: anti-human-ckit monoclonal antibody SR-1				
Czechowicz et al. [2011] (17)	SLR→SR1 mAb	Xenotransplantation in immunocompromised mice	Yes, ~90%	SR-1 did not induce human HSC cell death via apoptosis, but rather shifted the differentiation profile

HSC, hematopoietic stem cell; SLR, sub-lethal radiation; mAb, monoclonal antibody.

Table 2 Monoclonal Ab based HSC depletion strategy targeting CD 45

Author/year	Conditioning	Recipient	Engraftment	Comment
Wulf et al. [2002] (18)	Non-radioactive CD45 mAb + SLR	Immunocompetent mice	Yes, ~80%	There was non-significant increase in the chimerism as compared with SLR alone (P= NS)
Brenner et al. [2003] (19)	Complement-fixing CD45 mAb + SLR	Immunocompetent mice	Mixed chimera	Treatment with anti-CD45 alone did not enhance syngeneic engraftment
Dahlke et al. [2002] (20)	Anti-CD45 (RT7) antibody	Immunocompetent rat	Stable mixed chimeras	Anti-RT7(a) mAb effectively depletes mature T cells as well as BM precursor cells of myeloid, B-/T-cell, and thrombocytic lineage but spares mature B cells
Jäger et al. [2016] (21)	Anti-CD45 (RT7) antibody	Immunocompetent rat	Stable mixed chimeras	This successfully conditions for engraftment of MHC syngeneic BM as well as MHC haplo-identical BM after MAC
Chen et al. [2012] (22)	Astatine-211-labeled anti-CD45 antibody	Immunocompetent dog	Yes, 19–58%	Safe and efficacious non-myeloablative conditioning in dog leukocyte antigen-identical BM followed by a short course of cyclosporine and mycophenolate mofetil immunosuppression
Burtner et al. [2015] (23)	Astatine-211-labeled anti-CD45 antibody	Immunocompetent canine model	Low level engraftment	Cell dose equal to or exceeding 1.0×10^6 cells/kg is required for long-term engraftment

HSC, hematopoietic stem cell; SLR, sub-lethal radiation; mAb, monoclonal antibody; NS, not significant; BM, bone marrow; MHC, major histocompatability complex.

Among several cell-surface markers tested (like CD 45, CD49D, CD84, CD90, CD133, CD135 and CD184), only the immunotoxin targeting CD45 was capable of depleting endogenous HSCs. This was probably related to the amount of expression and the extent of internalization. The internalization frequency of the present immunotoxin was 12% over a 24-hour period, which was sufficient for potent HSC depletion in immunocompetent mice and also enables sufficient and durable donor-cell engraftment (75–90% at 4 months post-HSCT) and was comparable to 5 Gy TBI and a lot better than ACK2 alone (<3%). ACK2, failed as an isolated agent in this immunocompetent mice setting even when an immunotoxin was coupled (ACK2-SAP) (13). Apart from this, there was also significant synergy between 3 mg/kg CD45-SAP and 5 Gy TBI with donor chimerism increasing up to 90%.

In contrast to 5 Gy TBI, CD45-SAP also preserved the bone marrow architecture, as it was less detrimental to bone marrow cellularity, hematopoietic progenitors, and bone marrow microenvironment. It also preserves innate immunity, avoids anemia and facilitates rapid B- and T-lymphocyte recovery as compared to TBI. They also demonstrated that CD45-SAP induces rapid and transient myeloid and lymphoid cell depletion and a stronger, long lasting depletion of HSCs in mice. When used alone as a conditioning agent for HSCT in immunocompetent mice, the treatment is nearly as effective as TBI at establishing long-term stable donor cell chimerism but with substantially less toxicity, as shown by the faster recovery of myeloid, B cells and T cells in the blood with better preservation of bone marrow and thymus architecture.

And finally in a sickle cell disease (SCD) mouse model this innovative conditioning at a higher dose of 4–6 mg/kg was able to achieve >90% donor myeloid chimerism at 4 months with complete normalization of RBC parameters as well as non-sickled morphology in peripheral smear and normal haemoglobin protein by native-PAGE analysis. More importantly, the spleen size also returned to normal size as compared to wild-type, the information about the normalization of splenic haematopoiesis was not detailed in the present study (1). Spleen in mice serves as the normal site of hematopoiesis like bone marrow in humans. Spleen in SCD mouse model is enlarged because of high erythropoietic rate due to hemolysis because of size-constrained limits on bone marrow hematopoiesis (4). Therefore, murine splenic hematopoiesis likely serves as a model of human bone marrow hematopoiesis (4) and in the present study has possibly improved as evidenced by their corresponding reduction in splenomegaly. But despite all the above inferences, the question still remains about the minimum level of modification required from conditioning to derive curative outcome in SCD model.

This innovative immunotoxin-based conditioning regimen of Palchaudhuri *et al.* (1) will require further research to determine how far these results in mice can be extrapolated in humans. A potential limitation of this method of conditioning is the failure to deplete endogenous HSCs completely to establish a complete donor cell chimerism. But in the SCD mouse model, it was observed that increasing the dose from to 3 to 4–6 mg/kg is feasible and possibly achieves a better chimerism status in the higher dose subset (75–90% in 3 mg/kg *vs.* >90% in 4–6 mg/kg). Thus, it seems there is still a lot of scope to improve upon the current results. Again, mice and humans differ in many aspects including size, environmental conditions (e.g., controlled environment conditions in a lab for mice) and lifespan, which impose different selective proliferative pressures on HSCs from each species (28). As a result, the functional aspect of human HSCs is different with respect to self-renewal or quiescence as compared to mouse HSCs. For instance, competitive transplantation studies in mice have estimated that murine HSCs divide every 2.5 weeks, which is significantly faster than human HSCs (every 40–45 weeks) (28). Again, the number of red blood cells (RBCs) produced by mice in a 2-year lifespan is comparable to the number of RBCs produced daily by a healthy adult individual (28). So, these results need to be interpreted with caution and possibly needs replication in non-human primate models. Team led by Prof. David T. Scadden must be congratulated for this outstanding effort in this direction. These results came at a time when lot of development is happening in the era of gene therapy and gene editing in haemoglobinopathies.

Footnote

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