

Thirty Years of Bone Marrow Transplantation in the Singapore General Hospital

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“For patients transplanted for end-stage leukemia, it became apparent that a plateau was developing on the Kaplan-Meier plot of survival so that it became possible to use the term ‘cure’ for these patients”.

Edward Donnell Thomas, Nobel Lecture, 1990

The first adult haematopoietic stem cell (HSC) transplant (HSCT) in Singapore was performed in July 1985 at the Singapore General Hospital (SGH). A patient with relapsed acute lymphoblastic leukaemia received conditioning with total body irradiation (TBI) followed by infusion of bone marrow from his human leukocyte antigen (HLA)-matched brother. This commentary briefly recounts the major milestones in our HSCT programme which evolved with advances in the field as new scientific knowledge and novel technologies became available.

Conditioning therapy before HSC infusion aims to eradicate tumour cells and immunological memory, create “space” within the marrow niche, and in allogeneic HSCT, reduce the risk of graft rejection. The first conditioning regimen, as used by Edward Donnell Thomas in the 1960s consisted of TBI 10 Gy delivered in a single fraction.¹ This takes advantage of the extreme sensitivity of haematopoietic cells to radiation and the ability of TBI to penetrate “sanctuary” sites. However, despite myeloablative doses of TBI, relapses continued to occur and there were substantial problems with “non-target” organ toxicity when TBI was delivered in this way. Concurrently, there was general acknowledgment of the marked sensitivity of normal tissues to fractionation of TBI dose and rate of delivery and these eventually led to important modifications to initial myeloablative conditioning regimens. The first was the addition of chemotherapy to TBI and development of

chemotherapy-only regimens with cyclophosphamide and busulfan, which is especially toxic to non-dividing early myeloid precursors.² The second was fractionation of TBI to deliver equivalent doses with lower toxicity.³

For many years, conventional myeloablation remained the only available preparative regimen, built upon the delivery of high-doses of ablative chemo-radiotherapy/chemotherapy before rescue of recipient haemopoiesis with either autologous or allogeneic HSCs depending on the disease. Whereas antitumour effects are solely from cytotoxic chemotherapy in autologous transplants, allogeneic HSCs may also have beneficial immune reactivity against tumour/leukaemia, the so-called graft-versus-leukaemia (GvL) effect.^{4,5} This formed the basis for the development of regimens that relied less on ablative intensity and more on GvL for its antitumour effects. Non-myeloablative (NM) regimens act to immunosuppress the recipient permitting engraftment of allogeneic HSCs while reduced intensity conditioning (RIC) regimens employ variable chemotherapy intensities with varying cytotoxic effects, not amounting to conventional myeloablation. We performed our first NM transplant in 1990 using immunoablative-dose cyclophosphamide with antithymocyte globulin for a patient with severe aplastic anaemia. The improved toxicity profile and tolerability of RIC and NM preparative regimens meant that older patients with comorbidities which previously excluded them from transplantation became eligible for HSCT. This contributed significantly to the rise in our transplant activity from the 1990s (Fig. 1).

The rise in the number of HSCTs performed also coincided with the increasing use of peripheral blood (PB) rather than bone marrow as the source of HSCs. Key to the development of peripheral blood stem cell harvesting (PBSCH) was

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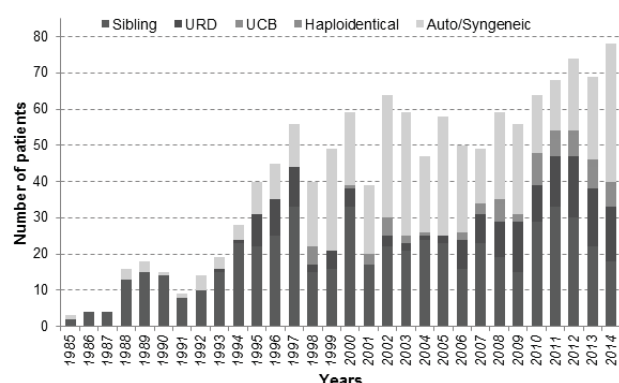


Fig. 1. Graph showing the number and types of transplants per year in Singapore General Hospital.

the observation in mice, of HSCs circulating from areas shielded from irradiation to radio-ablated marrow, followed by cross-circulation studies in primates demonstrating the ability of these circulating HSCs to recapitulate the entire haematopoietic system.^{6,7} Together with improved leukapheresis and cryopreservation techniques, the first PBSCH that resulted in successful engraftment was carried out in London in 1981 for a patient with chronic myeloid leukaemia.⁸ Mainstream application of PBSCH coincided with the use of G-CSF as a means of temporarily expanding PB CD34+ HSCs. The major advantages over bone marrow HSCs include a higher HSC cell dose, more rapid engraftment, lowered graft rejection rate, and easier HSC collection. The first autologous and allogeneic PBSC transplants performed in SGH were in 1990 and 1995, respectively. Worldwide, PBSCs are used much more frequently than bone marrow and in SGH, it accounts for 96.6% of all transplants done in the last 10 years.

One of the vital steps that propelled HSCT into the “modern era” was the characterisation of the HLA system. Prior to this, most transplants had resulted in graft failure or lethal graft-versus-host disease (GvHD). Standard practice currently dictates that HLA-typing is performed at the HLA-A, -B, -C, and -DRB1 loci except with cord blood when HLA-C is not needed for selection of a suitable donor. The best donor is a full “8/8” HLA-matched sibling donor followed by a fully-matched unrelated donor (URD) or “7/8” HLA-matched sibling or URD. The chance of a sibling of same parentage being fully HLA-matched is 25%. In the last decade, about 58% of our allogeneic transplants used a sibling donor, 29% URD, 11% cord blood, and 2% haploidentical donor.

Our first URD transplant was performed in 1994 for a patient with advanced myelodysplastic syndrome. The patient succumbed within 100 days of transplant from

severe sepsis. A year later, we successfully carried out an URD transplant for a patient with thalassaemia major and he remains well and on follow-up today. Refinements in HLA-typing and improved immunosuppressive and preparative regimens (among others) have translated to an increase in URD transplant survival approaching that of matched sibling transplants.⁹

The 1990s also saw the adoption of alternative sources of HSCs. The first umbilical cord blood (UCB) transplant we performed was in 1998. UCB transplantation is an option for patients who are unable to find a suitable sibling or URD or who for medical reasons, require a transplant urgently. In this regard, UCB remains an important source of readily available “off-the-shelf” cryopreserved HSCs which have been screened for infectious diseases and which require less stringent HLA-matching ($\geq 4/6$ HLA-matching for HLA-A, -B, -DRB1). Survival outcomes after UCB transplants in adults are still inferior compared to adult donor transplants and relates to several factors including poorer platelet recovery, delayed immune reconstitution from thymopoietic failure and late memory T-cell skewing, higher rates of graft failure and longer time-to-engraftment (and therefore longer period of neutropaenia).^{10,11} The latter is dependent on the interplay between the degree of HLA match and the total nucleated cell (TNC) dose with higher doses potentially able to overcome greater HLA disparity.¹² To this end, research into cord blood expansion strategies aim to overcome the discrepancy between available TNC dose per UCB unit and that required by an adult recipient. In 2013, our first transplant combining an unmanipulated UCB unit with an ex vivo expanded unit co-cultured with mesenchymal stem cells and an “in-house” cocktail was performed (NCT01624701).

Another alternative donor source is the haploidentical (half-matched) donor. Almost all patients would have a related donor (parent) identical for 1 HLA haplotype and mismatched at HLA-A, -B, -C or -DR of the unshared haplotype. For many years, the central problem with haploidentical transplants was controlling the potent alloreactivity of host T-cells (graft rejection) and donor T-cells which lead to hyperacute GvHD. However, using cyclophosphamide (CY) at immunoablative doses on days 3 and 4 after HSC infusion (post-transplant CY) has diminished the aforementioned problems by promoting tolerance in host and donor T-cells.¹³ The major mechanisms for the induction of tolerance by CY are the destruction of antigen-stimulated, proliferating T-cells in PB and intrathymic clonal deletion of reactive T-cells associated with establishment of mixed chimerism. Even though CY is administered after donor HSC infusion, engraftment consistently occurs because HSCs contain high levels of aldehyde dehydrogenase which render them insensitive to

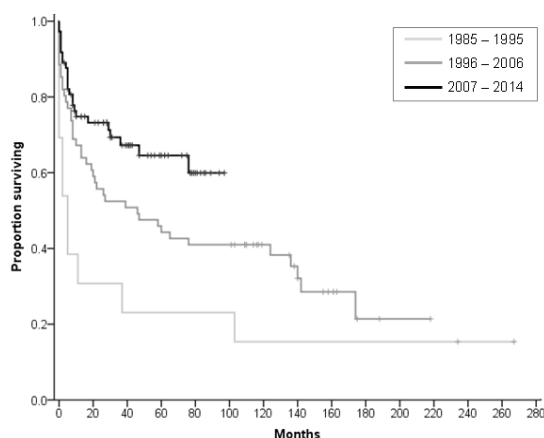


Fig. 2. Chart showing the survival of transplant recipients over 3 decades in Singapore General Hospital.

CY toxicity. Post-transplant CY forms the basis for the T-cell depleting strategy in our current haploidentical transplant protocol, which we first used in 2011.

Apart from refinements in conditioning regimens, harvesting techniques and donor accessibility, improvements in immunosuppressive therapy, antimicrobial agents, infrastructure, and overall medical care have resulted in a significant improvement in survival over 3 decades (Fig. 2). Accordingly, transplant-related mortality has also decreased through the years from 31.7% in the first 10 years to 17.9% in the last decade, consistent with results from other international transplant centres.¹⁴ Although haematological malignancies and marrow failure remain the most frequent indications for adult HSCT, patients with autoimmune diseases like systemic lupus erythematosus and scleroderma as well as neurological conditions like multiple sclerosis who have failed standard immunosuppressive therapy have also benefited from autologous HSC transplantation. Putative mechanisms of action include thymic reprocessing and expansion of regulatory T-cells.

The service that began in 1985 is today, a large multidisciplinary programme comprising an internationally accredited HSC collection facility and processing laboratory, and the clinical programme itself. Over the years, SGH has become a referral centre for HSC transplantation in the region. The number of patients transplanted has steadily increased, surpassing a thousand patients in 2011 and providing a chance for survival in excess of 50%. Yet, there is a continuous need for us to improve on current patient outcomes. Post-HSCT relapse remains a major barrier to long-term survival. To this end, efforts into manipulating the graft to try shift the balance of alloreactivity in favour of graft-versus-tumour and engineering immune cells that preferentially kill tumour have already begun. These,

together with refinements in overall medical care may bring us further in the pursuit of the “cure” we embarked upon many years ago.

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