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Expansion of Haematopoietic Stem and Progenitor Cells: Paving the Way for Next-Generation Haematopoietic Stem Cell Transplantation

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Abstract

Haematopoietic stem cell transplantation (HSCT) is now an established practice with over 70,000 transplants performed annually, and over 1.5 million around the world so far. The practice of HSCT has improved over the years due to advances in conditioning regiments, preparatory practices for patients leading up to the transplant, graft versus host disease (GVHD) and infection prophylaxis, as well as a better selection of patients. However, in many instances, the stem cells supplied to the patient may not be adequate for optimal transplantation outcomes. This may be seen in a few areas including umbilical cord blood transplantation, inadequate bone marrow, peripheral blood stem cell harvest, or gene therapy. Growing and expanding HSCs in culture would provide an increase in cell numbers prior to stem cell infusion and accelerate haematopoietic recovery, resulting in improved outcomes. Several new technologies have emerged in recent years, which have facilitated the expansion of haematopoietic stem and progenitor cells (HSPCs) in culture with good outcomes *in vitro*, *in vivo*, and in clinical trials. In this review, we will outline some of the reasons for the expansion of HSPCs as well as the new technologies facilitating the advances in HSCT.

Key words: Haematopoietic Stem & Progenitor Cell, Haematopoietic Stem Cell Transplants, Umbililcal Cord Blood Transplants, Gene Therapy, Bone Marrow & Peripheral Blood Transplants

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Evolution of haematopoietic stem cell transplantation (HSCT)

Haematopoietic stem cell transplantation (HSCT) is now an established clinical practice with over 70,000 transplants performed annually, and over 1.5 million around the world so far[1]. Since the first HSCT was carried out in identical twins[2,3], several important milestones that allow HSCT to be carried out across international borders and between unrelated individuals[4]have been achieved. These include the identification of the human leukocyte antigen (HLA) system[5,6]; development of effective immunosuppressive and anti-microbial drugs[7]; discovery of stem cell mobilizing agents[8]; and establishment of donor registries[9]. In recent years, HSCT has been enhanced by the advent of reduced intensity conditioning regimens especially for elderly patients (>55 years)[10]; innovative graft manipulations to mitigate graft-versus-host-disease (GVHD) and maximize graft-versus-leukaemia (GVL)[11]; and advanced anti-microbial cellular therapeutics to provide effective infection control[12]. In addition, the use of donor lymphocyte infusion (DLI) to treat minimal residual disease (MRD) and post-transplant relapses[13] along with an improved algorithm for patient selection[14] has contributed to the advances in the use of HSCT[15].

Amongst other factors, the outcome of HSCT strongly correlates with the quality and quantity of the hematopoietic stem and progenitor cells (HSPCs) that are delivered to the recipient[16,17]. In many instances, there could be inadequate number of HSPCs or HSPCs that are of sub-optimal quality being supplied to the patients, resulting in poor outcomes measured through parameters such as neutrophil recovery and transplant-related mortality

(TRM)[18,19]. Growing and expanding HSPCs in culture increase the number of desired potent cell populations (for example, cells that express surface markers such as CD34 and CD90) prior to infusion, which could potentially accelerate haematopoietic recovery and result in improved outcomes[16,17]. Several new technologies have emerged in recent years, which allow the expansion of primitive HSPCs in culture as demonstrated through effective *in vitro* phenotypic and *in vivo* functional assays followed by outstanding outcomes in pivotal early-phase clinical trials[16-20]. In this review, we will outline the major reasons for HSPC expansion as well as describe a selected group of new technologies facilitating the advances in HSCT and that will serve as a backbone of the new cellular therapy-based industry.

Clinical relevance of expanding HSPC to enable next-generation HSCT

Enabling umbilical cord blood (UCB) transplantation (UCBT)

UCB is now an FDA-approved HSPC source for HSCT[21]. The strong stem cell potential and immune pliability of UCB cells facilitate their use in HSCT despite requiring lower starting cell numbers than bone marrow (BM) or mobilized peripheral blood (mPB) along with the ability to cross histocompatibility barriers without *in vitro* or *in vivo* immune manipulation[18,19]. The immune naivety of UCB facilitates mismatched donor HSCT without an increase in GVHD and with retained GVT effects[22,23]. UCBT has been able to achieve equivalent outcomes, despite having one to two antigen mismatches when compared to fully matched BM or PB stem cell from unrelated donor[24]. Furthermore, a study conducted by Milano *et al.* showed that UCBT had better outcomes than bone marrow transplants (BMT) or mobilized peripheral blood stem cell transplants (PBSCT) due to a decrease in the relapse rates[25]. However, UCBT is fraught with the problem of inadequate or suboptimal cell dosage for transplantation as over 70% of the over 700,000 publicly banked cryo-preserved grafts fail to meet the minimal total nucleated cell (TNC) dosage of 25 million cells/kg of recipient's body weight for most adults[16,17]. When UCBT is carried out with suboptimal cell dosage, TRM could exceed 30% but when a sufficient cell dose of UCB graft is infused, TRM could fall below 10%[26]. The infusion of a UCB graft with suboptimal cell dose causes delayed recovery of neutrophils and platelets, resulting in an increased risk of infections and bleeding compared to BMT or PBSCT[27]. Even with an optimal cell dose in a single or dual graft approach, neutrophil and platelet engraftment is slower compared to BMT or PBSCT by at least 1-2 weeks, increasing the chances of contracting life-threatening infection along with prolonged period of hospitalization[28]. The advent of more recent technologies in UCB expansion has resulted in accelerated haematopoietic recovery, with neutrophil engraftment being reduced from a median of 27 days to 11 days post-transplant[16-20]. Several other studies have also shown promising results that represent a significant potential advancement for UCBT which could facilitate its use as an important stem cell source to benefit at least more than 20,000 patients per annum worldwide[16-20].

Improving BM and PBSC harvest

Inadequate HSPC dosage is a barrier to clinical usage of not only UCB grafts[18,19]; but also BM and mPB stem cell products which may fail to meet the optimal stem cell collection criteria to facilitate a standard HSCT[29,30]. BM and PBSC transplantations involve the harvest of HSPCs from a patient (autologous transplants) or from a donor (allogeneic transplants)[31]. BM harvests typically target $2\text{-}3 \times 10^8$ nucleated cells/kg of recipient bodyweight, and BM are extracted through multiple punctures carried out in the operating theatre for the extraction of approximately 1L of BM[29]. PBSC harvests are conducted after the injection of a HSPC mobilising agent such as G-CSF or plerixafor, followed by the collection of the cells through apheresis[32,33]. These harvests usually result in an adequate number of HSPC for transplantation; however, in many instances, stem cell harvests may not be adequate for the best transplant outcomes. For example, a recent study for mPB-based autologous HSCT demonstrated that a 5-day long subcutaneous administration of 15 μg/kg of filgrastim resulted in 17%, 78%, and 5% of patients ($n=102$), achieving optimal ($\geq 5.0 \times 10^6$ cells/kg), sub-optimal ($\geq 2.0\text{-}< 5.0 \times 10^6$ cells/kg), and poor ($< 2.0 \times 10^6$ cells/kg) stem cell harvest for subsequent transplantation[34,35]. In allogeneic transplants, the problem of suboptimal cell dose of BM or mPB graft occurs particularly when the full or half HLA-matched donor: (1) is of a much smaller body size than the patient; (2) is of an older age than the patient; and (3) may have difficult venous access or some other health conditions. Autologous transplants patients, who are subjected to strong myelotoxic therapies to treat the primary disease preceding the stem cell harvest, are most likely to have insufficient collection of HSPC for subsequent infusion[36]. HSPC expansion could help us overcome the problem of inadequate stem cell harvest and, if established, may facilitate successful HSCT in these situations without inflicting unnecessary and excessive repeated harvests on the donor or patient. Furthermore, a reduced time required for haematopoietic recovery was observed with expanded UCB as described in subsequent sections; therefore, a similar expansion of BM or mPB grafts could further reduce the time to neutrophil engraftment from a median of 16 days to approximately 7 days (equivalent

drop of 9 days when comparing median time to neutrophil engraftment of non-expanded versus expanded UCB-25 days versus 13 days), thus enabling safer outpatient HSCT procedures with minimal post-transplant neutropenia[16-20].

Facilitating gene therapy (GT) for inherited blood disorders

GT allows the correction of genetic disorders in haematopoietic cells and the treatment of patients with otherwise fatal or debilitating haematopoietic disorders such as thalassemia[37] and sickle cell disease[38]. Despite the extensive amount of scientific studies that have been carried out in the field of GT over the past few decades, it has seen minimal clinical progress primarily due to problems such as (1) leukemogenesis caused by viral integration into unexpected sites[39,40]; and (2) poor transduction efficiency resulting in low number of transduced gene-corrected cells that does not meet the criteria of minimal cell dosage to carry out a successful HSCT[41]. However, in recent years, the regulatory approval and marketing of genetically engineered chimeric antigen receptor (CAR) T-cells primarily to treat CD19+diseases such as acute lymphoblastic leukaemia (ALL)[42,43] and diffuse large B-cell lymphoma[44] has re-ignited the hope of establishing GT as a clinical practice to improve patients' lives. Newly established gene editing technologies using evolved and safer lenti-viral vectors[45]; clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9)[46]; zinc finger nucleases[47]; and transcription activator-like effector nucleases[48] could allow targeting and integration of specific gene sequences at precise locations of the genome, thus overcoming the problem of unexpected site integration and associated oncogenicity[49]. *Ex vivo* expansion of the gene-corrected HSPCs could facilitate the development of HSCT grafts that have sufficient therapeutic cell dosage of the gene-corrected healthy cells to enable a successful transplantation through engraftment and subsequent reconstitution of the healthy blood cells.

Providing transient haematopoietic recovery after chemotherapy

Patients suffering from chemotherapy-associated neutropenia could also benefit from the infusion of late hematopoietic progenitor cells (HPCs) to minimize neutropenia and its associated complications through transient increase in infection-fighting white blood cells (WBCs)[50]. While most chemotherapy results in mild or negligible neutropenia, there are some, for example, induction chemotherapy for acute myeloid leukaemia (AML) that may result in profound neutropenia for more than 2 weeks[51]. Furthermore, cytotoxic antineoplastic therapy administered as conditioning regimen prior to HSCT adversely impacts both myelopoiesis and the integrity of the gastrointestinal mucosa, thus exponentially increasing the risk of life-threatening infections in these patients who are unable to mount an inflammatory response[52]. Current clinical management of high-risk chemotherapy-associated neutropenia (absolute neutrophil count, ANC$<$500cells/μL for$>$7 days) and its associated opportunistic infections involve prophylactic mono- or combination therapy of antimicrobial drug/s along with recombinant human hematopoietic growth factors such as G-CSF to stimulate the production of neutrophils[53,54]. Although G-CSF has shown proven efficacy in shortening the period of chemotherapy-induced neutropenia, it is ineffective in very early HSPCs but only effective in committed/late hematopoietic progenitor cells (HPCs) once the BM has partially recovered[55]. Based on epidemiological studies carried out by a pharmaceutical company (Pfizer, Bristol-Myers Squibb, Novartis), the number of HSCTs performed is expected to rise worldwide in the next decade or so due to the increasing incidence of leukaemia and other BM disorders, improved transplant outcomes, and growth in the number of transplant centres and donor registries especially in densely populated regions of Asia. To reduce post-transplant complication rates associated with the rising trend, there is an urgent need to develop therapeutic strategies to mitigate neutropenia that occurs after chemotherapy and during the pre-engraftment phase following HSCT. Because the expansion and infusion of late/committed HPCs, preferably obtained from on-demand, off-the-shelf, and non-HLA matched donors, do not retain the ability to impart life-long haematopoiesis and could only provide a wave of early and late myeloid cells to impart innate immunity while awaiting for the patient' s endogenous BM recovery, they act as a lucrative alternative to recombinant G-CSF therapy. Such non-engrafting expanded HSCT grafts that could tackle neutropenia could potentially have applicability in areas of solid tumours and organ transplantation, and they are being developed by several groups[56] (**Table 1**).

Methods for expanding early HSPCs and late HPCs to provide long-term and transient hematopoiesis

Majority of the HSPC expansion studies were performed using UCB grafts given that its clinical usage is primarily limited by low cell dosage[16-20]. Over the past two decades, up to 15 different clinical trials have explored various methods of expanding UCB HSPC which have resulted in the recruitment of over 350 patients[57]. All *ex vivo* HSPC expansion cultures involve the use of various early-acting cytokines such as stem cell factor (SCF), thrombopoietin (TPO), and Flt-3 ligand

Table 1. Clinical relevance of expanding HSPC to enable next-generation HSCT

Enabling Umbilical Cord Blood Transplantation	Improving Bone Marrow & Peripheral Blood Stem Cell Harvest
➢ Increases availability and utility of UCB grafts. ➢ Reduces time to neutrophil & platelet engraftment. ➢ Reduces chance of graft failure, infection and transplant related mortality.	➢ Minimizes multiple harvest from donor. ➢ Potentially reduces time to neutrophil & platelet engraftment. ➢ Potentially moderates immune related complications
Facilitating Gene Therapy for Inherited Blood Disorders	**Providing Transient Haematopoietic Recovery After Chemotherapy**
➢ Efficient transduction with advanced genetic manipulation tools. ➢ Increases dosage of gene-corrected autologous cells. ➢ Overcomes complications of allogeneic transplantation.	➢ Non-engrafting late HPCs that acts as an alternate to G-CSF to overcome chemotherapy associated neutropenia. ➢ Confers innate immunity that reduces opportunistic infection.

G-CSF, Granulocyte-colony stimulating factor; HPC, Haematopoietic Progenitor Cell; HSPC, Haematopoietic Stem & Progenitor Cell; HSCT, Haematopoietic Stem Cell Transplantation.

(Flt-3L) for maintaining stemness, whilst late-acting cytokines such as interleukin (IL)-3, IL-6, IL-11, and granulocyte-colony stimulating factor (G-CSF) are added to support rapid proliferation accompanied by differentiation[16-20]. In recent years, a number of novel growth factors such as Notch ligand[58], insulin-like growth factor-binding protein-2 (IGFBP-2)[59], angiopoietin-like (Angptl) proteins[60] and pleiotrophin[61] have been discovered and used in various expansion technologies. In addition to a cytokine cocktail, clinical trials have involved the use of mesenchymal stromal cell (MSC) co-culture system that mimics the BM niche to expand non-enriched UCB grafts[62] and bio-reactors to enable up-scaled culturing of CD34-selected HSPCs[63]. Furthermore, they have involved the addition of several small molecules associated with HSPC maintenance and proliferation such as tetraethylenepentamine (copper chelator that modulates differentiation)[64,65], nicotinamide (sirtuin-1 inhibitors)[66,67], StemRegenin-1 (aryl hydrocarbon receptor antagonist)[68], and UM171 (mechanism of action not known)[69]. Recent pre-clinical studies have demonstrated the roles of a range of new small molecules in the expansion of HSPCs. They include P18IN003, P18IN011, and XIE18-6 (cyclin-dependent kinase inhibitors)[70]; NR101 (non-peptidyl small molecule agonist of c-MPL)[71]; eltrombopag (a human specific thrombopoietin receptor agonist)[72]; CHIR99021 and rapamycin (Wnt and β catenin pathway modulators)[73]; and 5-azacytidine, trichostatin A, garcinol, and valproic acid (epigenetic modulators)[74,75]. Others such as resveratrol (a naturally occurring polyphenol)[76]; serotonin (a monoamine neurotransmitter)[77]; GW9662 (a PPARγ antagonist)[78]; SB203580, Vx702, BIRB-796 and Ly2228820 (potent inhibitors of p38 mitogen-activated protein kinases)[79]; and C7 (a new structural analogue of SB203580)[80] have also been used in HSPC expansion studies. Amongst these compounds, only C7 has been shown to be able to expand HSPC from both non-enriched and CD34-enriched grafts that retain both *in vitro* primitive HSPC phenotype and long-term *in vivo* functionality in xenotransplantation model[80]. While some of the methods have met with limited success, we would like to highlight a few that have had effective clinical outcomes in recent years and could potentially change the field of HSCT.

Omidubicel/NiCord®, MGTA-456, and ECT-001 expanded HSPC grafts to enable UCBT

Gamida Cell (Israel) has established nicotinamide (NAM), a form of vitamin B3, as a novel agent to expand CD34/CD133 selected UCB cells[81], and the manufactured cell therapy product is referred to as Omidubicel/NiCord®. High-throughput screening of over 100,000 heterocycles compounds identified a purine derivative, StemRegenin-1 (SR-1), that could increase the absolute

number of $CD34^{+}$ cells by inhibiting aryl hydrocarbon receptor in *ex vivo* expansion cultures[82] resulting in an investigational product, MGTA-456/HSC835[68], by Novartis (Switzerland) and Magenta Therapeutics (MA, USA). Similarly, another manipulated UCB graft known as ECT-001 was developed by ExCellThera (Canada) using UM171, a pyrimidoindole derivative[83] discovered via high-throughput screening of over 5,300 low molecular weight compounds.

Omidubicel (single centre pilot study, n = 11, with ClinicalTrials.gov identifier: NCT01221857[66] and multicentre phase Ⅰ/Ⅱ study, n = 36, with ClinicalTrials.gov identifier: NCT01816230[67]), MGTA-456 (single centre pilot study, n = 17, with ClinicalTrials.gov identifier: NCT01474681[68]), and ECT-001 (single centre pilot study for n = 21 with ClinicalTrials.gov identifier: NCT0266831569) underwent clinical trial for patients (total n = 85) suffering from high-risk haematological malignancies who received myeloablative conditioning regimen and standard GvHD prophylaxis. The production process for Omidubicel requires up to 27 days and involves the culturing of $CD133^{+}$/$CD34^{+}$UCB cells in Minimum Essential Medium α supplemented with 10% FBS, 50 ng/mL of SCF, 50 ng/mL of TPO, 50 ng/mL of Flt-3L, 50 ng/mL of IL-6 and 2.5 mM of NAM[66,67]. Compared to Omidubicel, the manufacturing process of MGTA-456 takes a shorter duration of up to 14 days and involves the culturing of the $CD34^{+}$ UCB cells in serum-free expansion media supplemented with 50 ng/mL of SCF, 50 ng/mL of TPO, 50 ng/mL of Flt-3L, 50 ng/mL of IL-6, and 750 nM of SR-1[68]. ECT-001 manufacturing requires only 7 days and involves the culturing of purified $CD34^{+}$ UCB cells using a fed-batch closed bioreactor with serum-free expansion medium supplemented with 100 ng/mL of SCF and 100 ng/mL of Flt-3L, 50 ng/mL of TPO, 10 μg/mL of low density lipo-proteins, and 35-50 nM of UM171[69]. The $CD133^{-}$/$CD34^{-}$ fraction of Omidubicel, MGTA-456, and ECT-001 were cryopreserved for infusion into the patients along with the manipulated $CD34^{+}$ fraction thus enabling the infusion of minimally manipulated T, B, and other immune cells that potentially supports engraftment after transplantation[66-69]. Production failure rates of approximately 10% and 22% was reported for Omidubicel and MGTA-456, respectively[66-68]. Majority of the patients (71%) in these trials received a second unmanipulated UCB graft along with expanded UCB product as a measure of clinical safety.

All the stated clinical trials reported significant expansion of CD34 cells (as outlined in **Table 2**) which resulted in patients receiving significantly higher cell dose (TNC and CD34) that resulted in faster hematopoietic recovery[66-69] for graft-derived neutrophils and platelets (**Table 2**). Post-transplant acute GvHD (aGvHD) for Omidubicel recipients manifested primarily as grade Ⅱ to Ⅳ whereas MGTA-456 and ECT-001 exhibited very low risk of aGvHD[66-69]. Immune cell reconstitution of patients receiving these investigational cell therapy products did not exhibit any abnormalities compared to conventional UCBT[66-69]. Transplantation of Omidubicel, MGTA-456, and ECT-001 did not cause any acute infusional toxicities (＜24 hours) or adverse events (up to 30 days post-transplant), and no patient experienced graft failure[66-69]. Furthermore, both *in vitro* experiments (telomere length, proliferation rates, and pathology-related tests including cytogenetics)[82-83] and long-term follow up of patients[66-69] enrolled in these studies did not indicate any possible mutagenesis or leukemogenesis of the culture expanded CD34 cells. A major advantage of Omidubicel, MGTA-456, and ECT-001 recipients manifested in a shorter hospital stay by at least 2 weeks compared to historical patients receiving conventional single or double UCBT[66-69] suggesting a significant drop in hospitalization associated costs. Another advantage of *ex vivo* HSPC expansion as demonstrated by the ECT-001 study is that it allowed the selection of UCB units with a better HLA-matching rather than with a higher cell dosage[69] which resulted in the clinical team having access to almost half of the UCBs in the banks instead of the typical 5% that is available for a standard 70 kg patient[69].

Currently Omidubicel is being evaluated in a multicentre, randomized, phase Ⅲ registration trial (ClinicalTrials.gov identifier: NCT02730299) that aims to recruit close to 120 patients suffering from haematological malignancies. Stand-alone MGTA-456 graft is being evaluated in ongoing phase Ⅱ studies for patients suffering from haematological malignancies (ClinicalTrials.gov identifier: NCT03674411) and inherited metabolic disorders (ClinicalTrials.gov identifier: NCT03406962) with plans to extend its application to the treatment of sickle cell anaemia. Similarly, the clinical application for ECT-001 is being broadened through a single centre phase Ⅰ/Ⅱ trial (ClinicalTrials.gov identifier: NCT03441958) involving high risk multiple myeloma patients.

NLA101-Notch Ligand-based expansion of late HPC by NOHLA Therapeutics (USA) to overcome prolonged chemotherapy-associated neutropenia

Initial laboratory studies demonstrated the role of Notch signalling pathway in controlling the fate of $CD34^{+}$ HSPC, for example, the overexpression of Notch-1 gene in $CD34^{+}$ cells allowed enhanced self-renewal capacity[84]. The NLA-101 expansion process by NOHLA Therapeutics (CA, USA) requires up to 16 days where purified UCB $CD34^{+}$ cells (from frozen or fresh grafts) are cultured in serum-free expansion medium supplemented with 300 ng/mL of SCF, 300 ng/mL of FLT-3L, 100 ng/mL of TPO, 100 ng/mL of IL-6, 10 ng/

Table 2. Pre-clinical and clinical outcomes of expanding HSPC grafts to enable UCBT in patients suffering from haematological malignancies

Expansion Technology to Enable UCBT	Critical Pre-Clinical Outcomes *In vitro* CD34+ expansion and *in vivo* SRC		Critical Clinical Outcomes In conventional UCBT, median time to: Neutrophil recovery: >25 days; and Platelet recovery: >50 days			Economic Outcomes
	CD34+ Expansion	SCID-repopulating capacity (SRC)	CD34+ Expansion	Neutrophil Engraftment	Platelet Engraftment	Amount and Source of Funds
NiCord®/ Omidubicel (Gamida Cell, Israel)	80–fold compared to input cells	7.6–fold compared to control	• 33–fold (Ph I/II study) • 72–fold (Pilot study)	• 11.5 days (n=36, Ph I/II study) • 13 days (n=11, Pilot study)	• 34 days (n=36, Ph I/II study) • 33 days (n=11, Pilot study)	• Raised $60 million from private & government funds. • IPO worth $69 million.
MGTA-456 (Magenta Therapeutics, USA)	>400–fold compared to input cells	17.0–fold compared to control	• 330–fold (Ph I/II study)	• 15 days (n=17, Ph I/II study)	• 49 days (n=17, Ph I/II study)	• Raised $150 million from private funds. • IPO worth $100 million.
ECT-001 (ExCellThera, Canada)	Close to 100–fold compared to input cells	13.0–fold compared to control	• 35–fold (Ph I/II study)	• 18 days (n=21, Ph I/II study)	• 42 days (n=21, Ph I/II study)	• Not disclosed

IPO, Initial Public Offering; Ph, Phase; SCID, Severe Combined Immunodeficiency; SRC, SCID-Repopulating Cells.
Funding and IPO information for each company obtained from their respective website (press releases) as of 22-Mar-2019. All clinical trials stated above were open to patients suffering from high-risk haematological malignancies. ClinicalTrials.gov identifier for (a) NiCord®: NCT01221857[66] and NCT01816230[67]; (b) MGTA-456: NCT01474681[68]; and (c) ECT-001: NCT02668315[69]. The pre-clinical data were retrieved from prior manuscripts for NiCord®/Omidubicel[81], MGTA-456/HSC835[82] and ECT-001[83].

mL of IL-3, and the required density of Delta 1 (Ext-IgG)[56,85]. The $CD34^-$ fraction containing the lymphoid cells is discarded and not infused into the patient unlike Omidubicel, MGTA-456, and ECT-001[66-69]. In an early phase pilot study (ClinicalTrials.gov identifier: NCT00343798), NLA-101 was administered to ten myeloablated patients suffering from haematological malignancies along with a second unmanipulated graft[85]. The expansion process resulted in 164-fold expansion of the absolute $CD34^+$ cell dosage and resulted in a faster median time to neutrophil recovery at 16 days compared to 26 days in the control cohort[85]. Majority of the patients exhibited the engraftment of NLA-101 at early time points, whereas only a couple of the patients retained long-term hematopoiesis from the expanded graft while the rest had stable engraftment from the second unmanipulated graft. Although NLA-101 proved to be safe, with no adverse outcomes with regards to graft failure, GvHD or relapse but non-persistence of the expanded unit raised concerns that the *ex vivo* expansion process was depleting true HSPCs or could be producing cells that merely facilitated the engraftment of the unmanipulated UCB graft[85].

Based on the outcomes of the pilot clinical study, the clinical use of NLA-101 was repurposed as an off-the-shelf (i.e. no matching on HLA is required) cellular therapy product to overcome neutropenia and reduce infections in patients receiving high-intensity chemotherapy[56]. Twenty-nine patients suffering from AML were recruited for a phase I study (ClinicalTrials.gov identifier: NCT01031368) as infection is a major cause of post-treatment mortality and morbidity[56]. The UCB grafts that underwent expansion resulted in a median expansion of 129- and 973-fold for $CD34^+$ cells and TNC, respectively[56]. Patients received NLA-101 at the end of the first induction cycle and were eligible for a second infusion of NLA-101 with consolidation therapy if they lacked residual leukaemia, toxicity associated with first NLA-101 infusion, or uncontrolled infections[56]. GvHD was not observed in any patient and routine chimerism analysis showed that NLA-101 provided transient engraftment up to 14 days post-infusion[56]. The median time to autologous neutrophil and platelet recovery were 19 and 27 days, respectively, which were similar to control cohort[56]. In conclusion, the study demonstrated that AML patients receiving NLA-101 had a decrease in infection incidence and the time to infection without any noted adverse outcomes[56].

Conclusion

HSPC expansion, particularly from UCB, is now a reality. The impact on the efficacy, safety, and utilisation of UCB for HSCT cannot be underestimated. Improving the safety and results of expanded UCBT would encourage more physicians to perform UCBT for their patients. Furthermore, with the potential significant reduction in hospitalisation, more centres would be keen to adopt this approach. The cost reduction in hospitalisation could also be attractive to insurance companies and other payers even if there are some costs associated with the *ex vivo* $CD34^+$ expansion process. The use of HSPC expansion platforms to mitigate the problem of inadequate harvest of BM or mPB would also significantly increase the market for the expanded HSPC products. Whilst clinical trials are still underway, the use of expanded HSCT grafts in post-chemotherapy or GT settings could further increase the impact of HSCT.

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Authors' Contributions

William YK Hwang conceived, reviewed, and revised the manuscript; Paul Chong transcribed the original draft and added new text and references; Sudipto Bari contributed substantially to the revision of the manuscript.

Conflict of Interest

William YK Hwang and Sudipto Bari have patents in hematopoietic stem cell expansion using small molecule-based platform. Disclosure forms provided by the authors are available here.

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