Recent Advances in Stem Cell Biology: Implications for Tropical Haematology Practice

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Abstract

Stem cells have long been defined by their remarkable potential to repopulate tissue systems indefinitely. This ability depends on their capacity for self-renewal, extensive proliferation and differentiation into the mature progeny. These ensure that they maintain the many different cell types in the body during embryogenesis and normal growth. Stem cells are conventionally of two types: embryonic stem cells and somatic or adult stem cells.

Haematopoietic stem cell transplantation (HSCT) has found extensive use in the management of both malignant and non-malignant haematologic diseases ranging from leukemias, lymphomas, and myelomas to sickle cell anaemia and thalassemias. Replacement gene therapy is also becoming increasingly relevant, though ensuring efficient viral transduction of the hematopoietic stem cells remains a challenge. These and similar advances in blood transfusion and supportive care, have significantly improved the outlook for these diseases. However, in the tropics, these gains are either non-existent or largely restricted to patients in the immediate vicinity of specialized healthcare facilities which are far too few.

Keywords: *Stem cells, Tropics, Haematology, Nigeria*

Introduction

Stem cells have long been defined by their remarkable potential to repopulate tissue systems indefinitely. This ability depends on three main characteristics; self-renewal, extensive proliferation and ability to differentiate into the mature progeny. Essentially, when a stem cell divides, each new cell has the capacity to remain a quiescent stem cell, to divide or to mature into a more specialized cell, such as a myocyte, an erythrocyte, or a neuron. These ensure that they maintain the many different cell types in the body during embryogenesis and normal growth. In some organ systems, stem cells regularly divide to repair and replace old or damaged tissues; while in others they only divide under special conditions.

Conventionally, stem cells derived from animals and humans were of two types: embryonic stem cells (ESC) and somatic or adult stem cells (SSC, ASC). In 1981, Martin reported the discovery of a method to isolate ESCs from early mouse embryos[1]. Further studies on mouse ESC soon led to the

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discovery, in 1998, of a method to isolate and grow ESCs from human embryos[2]. These human embryonic stem cells (hESC) were originally created during in-vitro fertilization (IVF) procedures, but later donated for research (with the informed consent of the donor). However, due to the ethical controversies which the use of the hESC attracted, it soon became obvious that sustained research in the field required a more acceptable source of cells. Fortunately, in 2006, scientists identified conditions necessary to induce mature somatic cells to assume a stem cell-like state[3]. These were called induced pluripotent stem cells (iPSCs).

Definitions

Stem cells may be classified according to their ability to differentiate into different cell types, giving rise to several potency definitions:

Totipotent (or omnipotent) stem cells: can differentiate into embryonic and extra-embryonic cell types, i.e. can form a complete, viable, organism. This includes the zygote and cells of the morula (from the first few divisions of the zygote).

Pluripotent stem cells: can differentiate into cells derived from the three germ layers. They are typically descendants of totipotent stem cells.
Multipotent stem cells: can differentiate into a closely-related family of cells, such as the haematopoietic stem cell (HSC) which is the precursor of the myeloid and lymphoid stem cells.

Oligopotent stem cells: can differentiate into a few cell types, such as lymphoid or myeloid stem cells.

Unipotent cells: can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells).

Identification of the stem cell

The realistic definition of a stem cell is a functional one – it must have the potential to regenerate tissue over a lifetime.[4] For example, the hematopoietic stem cell (HSC) is defined by its ability to repopulate the marrow of an individual without HSCs i.e. be able to produce new blood and immune cells over a long term.[4] It should also be possible to isolate stem cells from the transplanted individual, which can themselves be transplanted into another individual without HSCs, demonstrating that the stem cell was able to self-renew. Unfortunately, this implies that stem cells can only be identified after they must have successfully served their purpose or failed to do so.

It therefore became desirable to define parameters for the identification of stem cells before the fact. These efforts led to the current practice of using the in-vitro metabolic, clonogenic and immunophenotypic properties of these cells to identify and characterize them, and essentially serve as surrogates for their ability to differentiate and self-renew. However, in-vitro culture conditions have been known to alter the behavior of cells, making it uncertain whether the cells will behave in a similar manner in-vivo. Here, my discussion is limited to the haematopoietic stem cells, being first to be discovered, best-understood and the only clinically-applicable population of stem cells.[4] For practical purposes, the HSC is identified as $CD34^+$ mononuclear cells in the peripheral blood or bone marrow graft, though they are also known to be c-kit^+, $CD133^+$, Lin^- and $CD38^-$.[5,6]

The role of stem cells in haematology

Haematopoietic stem cells have found extensive use in the management of several malignant and non-malignant haematologic diseases ranging from leukemias, lymphomas, and myelomas to sickle cell anaemia and thalassemias.

In the setting of autologous HSC transplantation (HSCT), the patient's own stem cells are harvested and cryopreserved, to be transfused after the patient has been exposed to myeloablative doses of chemo-radiotherapy. The availability of these HSCs thus eliminates the dose-limiting effect of the bone marrow in the administration of chemo-radiotherapy. This therefore allows the oncologist to use high dose (typically, in excess of ten-fold) chemo-radiotherapy, and thus achieve better reduction of the tumour load and consequently improve the chances of achieving a complete remission. The rationale for the use of similarly high doses of chemo-radiotherapeutic agents in the allogeneic HSCT setting is radically different.[7,8] Most importantly, the regimen kills or renders native immune cells ineffective, thus the recipient is incapable of rejecting donor HSCs. Additionally, it serves to reduce the load of malfunctioning native blood cells or tumour load. Finally, it creates marrow space for the incoming (donor) HSCs.

Haematopoietic stem cells are ordinarily resident in the bone marrow, though smaller numbers are known to be present in peripheral and cord blood.[9] Clinically significant numbers of HSCs can be obtained from bone marrow, peripheral blood or cord blood. However, the yield of HSCs in peripheral blood can be significantly improved by mobilizing them with either growth factors (e.g. G-CSF) or alkylating agents (e.g. cyclophosphamide). In addition to the HSC, the bone marrow also contains the so-called mesenchymal stem cells (MSC), which are the precursors of the various stromal cells.[10,11] Several researchers have found that MSC can be induced to develop into HSC as well as several other types of multipotent stem cells. These cells have undergone trials in a bid to use them for the repair or regeneration of damaged tissues especially in organs with quiescent stem cells.[11] Studies have been conducted in cardiology, nephrology, neurology and even osteology for the management of ischaemic heart diseases, acute tubular necrosis, neurodegenerative disorders and osteogenesis imperfecta respectively.[10,11]

Implications for Tropical haematology practice

The many advances in the field of stem cell biology have brought with them several opportunities for drug development, stem cell research, stem cell gene therapy and clinical HSCT. Patients with sickle cell disease (SCD),[12-20] and other non-malignant haematological diseases[21-30] have benefitted from these advances.

In spite of the advances in blood transfusion and supportive care, which have significantly improved life expectancy in SCD in the tropics, these gains are largely restricted to patients in the immediate vicinity of specialized healthcare facilities. Majority of the SCD patients continue to suffer disabling symptoms and childhood mortality rates remain unacceptably high, due to complications of the disease.

In selected patients with severe SCD, transplanted with stem cells from HLA-identical related donors, overall survival of up to 90%, disease-free survival (DFS) of 82–86% have been achieved; though transplant-related mortality (TRM) of 7–8% and graft rejection of 8% have also been recorded.[17,19,20] All patients that were able to achieve stable engraftment no longer had clinical features of SCD. However, not more than 300 patients have been transplanted worldwide, almost all children and mostly for complicated disease, particularly SCD-related stroke. It would obviously be more desirable if these patients could be transplanted before they develop disabling complications.

The place of HSCT in the management of SCD

The decision in favor of HSCT is obviously not a light one because SCD is a highly heterogeneous disease, which in severe cases, is associated with significant morbidity. It may not be immediately life-threatening, though

long-term survival is significantly reduced. It is therefore pertinent that the physician and patient carefully weigh the potential risk and benefit; such that "the treatment will not be more grievous than the disease itself". It is however, becoming increasingly clear that the benefits of HSCT outweigh its risks, in SCD patients with severe disease. It is equally vital to be able to identify patients with features that may predict for severe disease; because it may be futile or even impossible to transplant SCD patients whose major organs have been damaged or severely compromised or who have developed multiple allo-antibodies. However, it is difficult to define severity before the onset of overt clinical features. This is why a case can be made for severity scoring for SCA patients being considered for HSCT, much like the Pesaro classification developed for thalassemia major.[23] Lucarelli et al have reported a correlation between significant organ dysfunction and increased rates of transplant-related mortality (TRM) and reduced post-HSCT survival, in thalassemic patients.[23,31,32] It is worth emphasizing that the success of HSCT is closely linked to the integrity of the hepatic and renal systems, which are required to process and eliminate the high doses of chemotherapy associated with the procedure.

Non-myeloablative HSCT in the management of SCD

Some of the major challenges of HSCT remain graft-versus-host disease (GvHD), toxicity of the myeloablative conditioning regimens and, to a smaller extent, graft rejection. Several attempts have gone into overcoming these odds in the context of non-malignant diseases. One of the most ambitious is the use of non-myeloablative (or reduced-intensity) conditioning (RIC) regimens. These regimens were conceived to be sufficiently immunosuppressive to prevent graft rejection, but to preserve the myeloid cells long enough to ensure that patients experience fewer or no cytopenic episodes (and consequently fewer infections) before stem cell engraftment. The reduced doses of chemotherapeutic agents also translate to less overall toxicity (and consequently, the potential for use in older patients, or those with compromised hepato-renal function), fewer days of hospital admission, and the possibility of out-patient HSCT. Schleuning et al reported the case of a 22-year-old SCD patient who underwent HLA-matched related RIC allo-HSCT and was alive without disease or GvHD as at day +315.[33] This case exemplifies several of the advantages of RIC-HSCT in SCD, with an encouraging outcome. On the contrary, Iannone et al reported on six patients with SCD who also underwent RIC-HSCT, but in whom it did not result in stable engraftment.[34] This is in spite of the fact that these patients had similarly minimal toxicity and were SCD-free during the transient period of low-level donor chimerism. These and other clinical and animal studies have left us with some salient lessons: that attainment of as little as 20-30% of stable donor chimerism is sufficient to alleviate the clinical features of SCD and that the outcome of HSCT in SCD is not affected when the donor has the sickle trait (HbAS).[33-37]

Replacement gene therapy in the management of SCD

Replacement gene therapy (RGT) takes advantage of the theoretically simple nature of the genetic defects in most SCD, point mutations; and seeks to achieve a cure by replacing the defective gene with a "normal" copy.[38,39] Since gene therapy promises to make changes to the autologous haematopoietic stem cell, scientists hope that it would permanently resolve the problem of GvHD. This replacement is done on the patient's own (autologous) stem cells, which are then transfused back to the patient, following myeloablative or non-myeloablative conditioning. If successful, this should "cure" SCD, and at the same time, eliminate the risks of GvHD and rejection, resulting in a potential increase in DFS and a reduction in TRM. However, some issues have been raised in regards to the propriety of totally eradicating

any chance of GvHD by using autologous stem cells. Most scholars believe that because SCD is a non-malignant genetic disorder, GvHD serves no beneficial effect, as obtains in patients with leukemias or other malignant lesions, in which the graft-versus-leukemia (GvL) component may be a reason to desire some GvHD.[40]

However, the clinical role of replacement gene therapy (RGT) in the management of SCD remains uncertain. This is largely because ensuring efficient viral transduction of the hematopoietic stem cells remains a challenge. Additionally, the ability to reliably produce safe, stable, erythroid-specific replacement gene expression at a clinically significant level is uncertain. Thus intense research is ongoing to improve the outcome of RGT for SCD.[39]

The challenges of stem cell therapy in the Tropics

Locally and in most of the tropics, the hurdles to be overcome to ensure that the benefits of stem cell research and its associated applications do not pass us by, are much more numerous. Most tropical populations have either very few or no HSCT centres. Several centres can hardly sustain regular and standard haematologic and blood transfusion services. Some of the identified challenges have been attributed to hospital-level problems such as manpower shortages, infrastructural deficiencies and limited maintenance capacities. Yet others are due to lack of an effective health insurance system, poor healthcare budgeting practice, and inadequate political support. Several of these challenges are well-documented, and in Nigeria, several specialists, working within the framework of various professional associations, are in the forefront of meeting these challenges.[41-43]

Even though sickle cell disease is one of the uniquely tropical haematologic diseases that stand to benefit the most from advances in this area of research, it is important to emphasize that the management of SCD using HSCT cannot be embarked upon in a hurry. The appropriate equipments and personnel must be in place first. The centre itself should "establish itself" by carrying out "simpler" HSCT procedures, such as autologous, before embarking on the more challenging SCD HSCT. There is no doubt, several more challenges will surface in this journey, and the team should be willing to make it a continuous learning process. Additionally, the management of many other diseases would be significantly impacted as a result of improvements in HSCT. Such conditions include the leukemias, lymphomas, myelomas, and several other chemo- or radio-sensitive malignancies. Many genetic disorders would also benefit from further advances in replacement gene therapy. There is therefore a need to invest more resources to train more skilled personnel, acquire more equipment and maintain them better. It is equally important to improve on health insurance systems, and sensitize policy makers on the need for these services and improved healthcare
funding.

References

1. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proceedings of the National Academy of Sciences of the United States of America. 1981; 78: 7634-7638.
2. Thomson JA, Itskovitz-Eldor J, Shapiro SS, *et al.* Embryonic Stem Cell Lines Derived from Human Blastocysts. Science. 1998; 282: 1145-1147.
3. Takahashi K and Yamanaka S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell. 2006; 126: 663-676.
4. Wodnar-Filipowicz A. Biological properties of haematopoietic stem cells. In: Apperley J, Carreras E, Gluckman E, Gratwohl A, Masszi T, eds. Hematopoietic Stem Cell

Transplantation (ed 5th.). Paris: ESH-EBMT; 2008.

5. Mayani H and Lansdorp PM. Biology of Human Umbilical Cord Blood-Derived Hematopoietic Stem/Progenitor Cells. Vol. 16: John Wiley & Sons, Ltd.; 1998:153-165.
6. Civin CI and Gore SD. Antigenic Analysis of Hematopoiesis: A Review. Journal of Hematotherapy. 1993; 2: 137-144.
7. Apperley J and Gratwohl A. Introduction to the EBMT. In: Apperley J, Carreras E, Gluckman E, Gratwohl A, Masszi T, eds. Hematopoietic Stem Cell Transplantation (ed 2nd rev.). Paris: ESH-EBMT; 2004: 11-19.
8. Apperley J, Carreras E, Gluckman E, Gratwohl A and Masszi T. Hematopoietic Stem Cell Transplantation. (ed 5th.). Paris: ESH-EBMT; 2008.
9. Johnsen HE, Lanza F, Fruehauf S, *et al.* Sources and procurement of HSC. In: Apperley J, Carreras E, Gluckman E, Gratwohl A, Masszi T, eds. Hematopoietic Stem Cell Transplantation (ed 2nd rev.). Paris: ESH-EBMT; 2004: 79-89.
10. Horwitz EM, Prockop DJ, Fitzpatrick LA, *et al.* Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. NatMed. 1999; 5: 309-313.
11. Lange C, Cakiroglu F, Spiess AN, Cappallo-Obermann H, Dierlamm J and Zander AR. Accelerated and safe expansion of human mesenchymal stromal cells in animal serum-free medium for transplantation and regenerative medicine. JCell Physiol. 2007; 213: 18-26.
12. Walters MC, Patience M, Leisenring W, *et al.* Bone marrow transplantation for sickle cell disease. NEngJMed. 1996; 335: 369-376.
13. Bernaudin F, Souillet G, Vannier JP, *et al*. Report of the French experience concerning 26 children transplanted for severe sickle cell disease. Bone Marrow Transplant. 1997; 19: 112–115.
14. Vermylen C, Cornu G, Ferster A, *et al.* Haematopoietic stem cell transplantation for sickle cell anaemia: the first 50 patients transplanted in Belgium. Bone Marrow Transplant. 1998; 22: 1-6.
15. Walters MC, Storb R, Patience M, *et al.* Impact of bone marrow transplantation for symptomatic sickle cell disease: an interim report. Multicenter investigation of bone marrow transplantation for sickle cell disease. Blood. 2000; 95: 1918-1924.
16. Hoppe CC and Walters MC. Bone marrow transplantation in sickle cell anemia. Curr Opin Oncol. 2001; 13: 85–90.
17. Vermylen C. Hematopoietic stem cell transplantation in sickle cell disease. Blood Reviews. 2003; 17: 163-166.
18. Iannone R, Ohene-Frempong K, Fuchs EJ, Casella JF and Chen AR. Bone marrow transplantation for sickle cell anemia: Progress and prospects. Pediatric Blood & Cancer. 2005; 44: 436-440.
19. Bernaudin F, Socie G, Kuentz M, *et al.* Long-term results of related myeloablative stem-cell transplantation to cure sickle cell disease. Blood. 2007;110:2749-2756.
20. Panepinto JA, Walters MC, Carreras J, *et al.* Matched-related donor transplantation for sickle cell disease: report from the Center for International Blood and Transplant Research. British Journal of Haematology. 2007;137:479-485.
21. Lucarelli G, Clift RA, Galimberti M, *et al*. Bone marrow transplantation in adult thalassemic patients. Blood. 1999; 93: 1164-1167.
22. Sullivan KM, Parkman R and Walters MC. Bone Marrow Transplantation for Non-Malignant Disease. Hematology (Am SocHematol EducProgram). 2000: 319-338.

23. Lucarelli G, Andreani M and Angelucci E. The cure of thalassemia by bone marrow transplantation. Blood Reviews. 2002; 16: 81-85.
24. Sodani P, Gaziev D, Polchi P, *et al.* New approach for bone marrow transplantation in patients with class 3 thalassemia aged younger than 17 years. Blood. 2004; 104: 1201-1203.
25. Chandy M, Balasubramanian P, Ramachandran SV, *et al.* Randomized trial of two different conditioning regimens for bone marrow transplantation in thalassemia—the role of busulfan pharmacokinetics in determining outcome. Bone Marrow Transplant. 2005; 36: 839-845.
26. Gaziev J, Sodani P, Polchi P, Andreani M and Lucarelli G. Bone marrow transplantation in adults with thalassemia: Treatment and long-term follow-up. AnnNYAcadSci. 2005; 1054: 196-205.
27. La Nasa G, Argiolu F, Giardini C, *et al.* Unrelated bone marrow transplantation for beta-thalassemia patients: The experience of the Italian Bone Marrow Transplant Group. AnnNYAcadSci. 2005; 1054: 186-195.
28. Hongeng S, Pakakasama S, Chuansumrit A, *et al.* Outcomes of transplantation with related- and unrelated-donor stem cells in children with severe thalassemia. BiolBlood Marrow Transplant. 2006; 12: 683-687.
29. Zhu KE, Gu J and Zhang T. Allogeneic stem cell transplantation from unrelated donor for class 3 beta-thalassemia major using reduced-intensity conditioning regimen. Bone Marrow Transplant. 2006; 37: 111-112.
30. Lucarelli G and Gaziev J. Advances in the allogeneic transplantation for thalassemia. Blood Reviews. 2008; 22: 53-63.
31. Andreani M, Nesci S, Lucarelli G, *et al.* Long-term survival of ex-thalassemic patients with persistent mixed chimerism after bone marrow transplantation. Bone Marrow Transplant. 2000; 25: 401-404.
32. Lucarelli G, Andreani M and Angelucci E. The cure of thalassemia by bone marrow transplantation. Blood Rev. 2002; 16: 81-85.
33. Schleuning M, Stoetzer O, Waterhouse C, Schlemmer M, Ledderose G and Kolb HJ. Hematopoietic stem cell transplantation after reduced-intensity conditioning as treatment of sickle cell disease. ExpHematol. 2002; 30: 7-10.
34. Iannone R, Casella JF, Fuchs EJ, *et al.* Results of minimally toxic nonmyeloablative transplantation in patients with sickle cell anemia and beta-thalassemia. BiolBlood Marrow Transplant. 2003; 9: 519-528.
35. Kean LS, Durham MM, Adams AB, *et al.* A cure for murine sickle cell disease through stable mixed chimerism and tolerance induction after nonmyeloablative conditioning and major histocompatibility complex-mismatched bone marrow transplantation. Blood. 2002; 99: 1840-1849.
36. Iannone R, Luznik L, Engstrom LW, *et al.* Effects of mixed hematopoietic chimerism in a mouse model of bone marrow transplantation for sickle cell anemia. Blood. 2001; 97: 3960-3965.
37. Roberts IAG and de la Fuente J. HSCT in the haemoglobinopathies. *In:* Apperley J, Carreras E, Gluckman E, Gratwohl A, Masszi T, eds. Hematopoietic Stem Cell Transplantation (ed 5th.). Paris: ESH-EBMT; 2008.
38. Sadelain M, May C, Rivella S and Glade BJ. Basic principles of gene transfer in hematopoietic stem cells. Prog Exp Tumor Res. 1999; 36: 1-19.
39. Walters MC. Stem Cell Therapy for Sickle Cell Disease: Transplantation and Gene Therapy. Hematology. 2005; 6-73.

40. Greystoke B, Bonanomi S, Carr TF, *et al.* Treosulfan-containing regimens achieve high rates of engraftment associated with low transplant morbidity and mortality in children with non-malignant disease and significant co-morbidities. British Journal of Haematology. 2008; 142: 257-262.
41. Oyekunle AA. Haematopoietic stem cell transplantation: Prospects and challenges in Nigeria. Ann Ibad Postgraduate Med. 2006; 4: 17-27.
42. Durosinmi MA and Oyekunle AA. Stem cell transplantation. Nig J Health Sciences. 2008; 8: 7-11.
43. Ocheni S. Challenges to overcome in setting up haematopoietic stem cell transplantation in Nigeria. Ifemed. 2009; 15: 55-57.