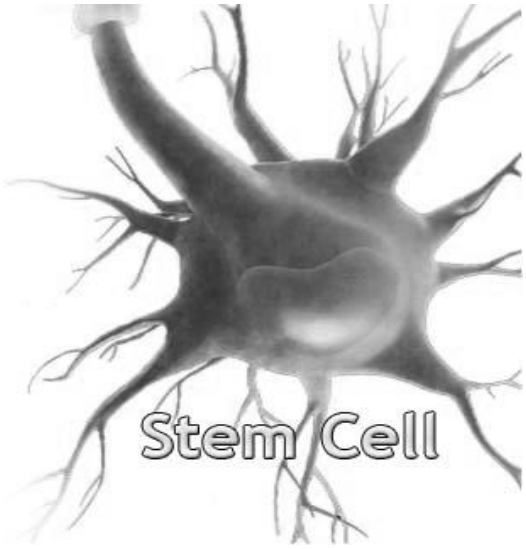
Stem Cell Transplantation: *Timeline and the future*



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This is to acknowledge that Dr. Madhuri Vusirikala, M.D. has disclosed that she does not have any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Vusirikala will be discussing off-label uses in her presentation.

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Dr. Madhuri Vusirikala is an Associate Professor of Medicine at UT Southwestern Medical Center in the Division of Hematology-Oncology. Dr. Vusirikala obtained her medical degree at Lady Hardinge Medical College in New Delhi, India and then moved to the United States. She obtained her graduate training in Internal Medicine at SUNY, Syracuse and Hematology-Oncology at University of Pittsburgh. She did her Bone marrow transplant training at Vanderbilt University Medical Center and joined there as faculty in 2000. She became faculty at UT Southwestern in 2006 in the Division of Hematology-Oncology with focus on Malignant Hematology and Bone marrow transplant. Her particular interests in BMT are allogeneic stem cell transplantation, chronic graft-versus-host-disease and post-transplant survivor issues.

Purpose and Overview:

The purpose of this presentation is to educate the clinicians about the history of stem cell transplantation, how it evolved, the several advances over the decades in its science and outcomes, and where the technology is headed.

Educational Objectives:

1. Educate the audience about the evolution of stem cell transplantation
2. Discuss the different milestones in stem cell transplantation and how they have impacted the management of patients undergoing this procedure
3. Review the recent advances in the technology of stem cell transplantation
4. Describe the current research strategies in the field of stem cell transplantation.

Glossary:

ALLOGENEIC TRANSPLANT- transplant using hematopoietic stem cells from another individual or source (sibling, parent, child, unrelated, cord)

AML- Acute Myeloid Leukemia

AUTOLOGOUS TRANSPLANT- transplant using patient's/recipient's own hematopoietic stem cells

BMT- Bone Marrow Transplant

CBT- Cord Blood Transplant

CMV- Cytomegalovirus

DLI- Donor Lymphocyte Infusion

ECP- Extracorporeal Photopheresis

GVHD- Graft versus Host Disease

GVT- Graft versus Tumor effect

HLA- Human Leucocyte Antigen

MSC- Mesenchymal Stromal Cells

PBSCT- Peripheral Blood Stem Cell Transplant

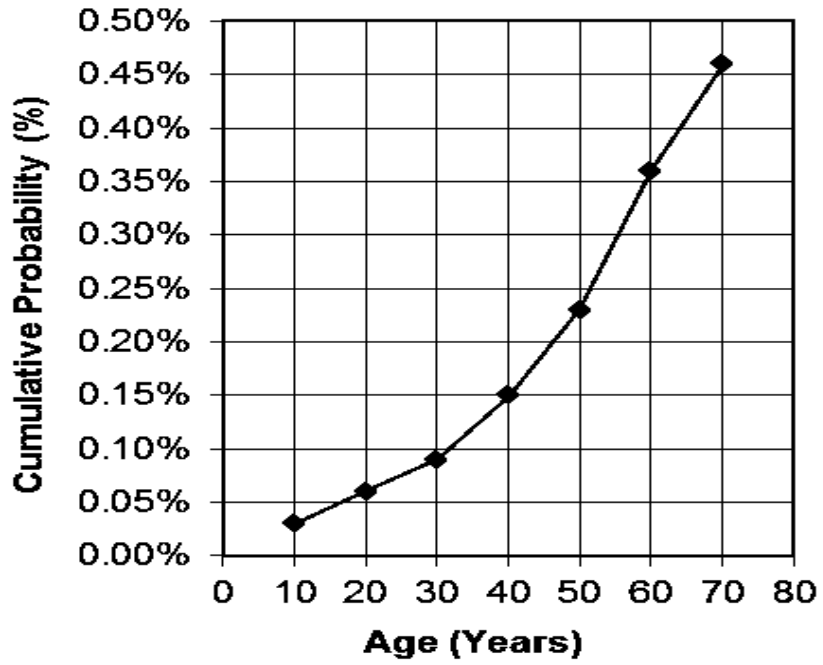
RIC- Reduced Intensity Conditioning

TBI- Total Body Irradiation

Introduction:

Hematopoietic stem cell transplantation (SCT) is a highly effective treatment modality for many malignant and non-malignant hematologic disorders. Previously considered an experimental approach, it is now considered standard of care in the management of several hematologic malignancies. In the US, one in 200 people have an SCT by the age of 70 (Figure 1). Since the onset of clinical transplantation in the late 1950's and early 1960's, there has been enormous progress in all aspects of this exciting science. Due to the advances in the technology, improvements in supportive care and an ever growing donor pool, there continues to be a vast increase in the number of annual stem cell transplants being done across the world.

FIGURE 1



In the USA, 1 in 217 people have a stem cell transplant by age 70 (Nietfeld et al. 2008)

Transplant prehistory:

The earliest mention of the therapeutic use of bone marrow was not in a medical journal. This reference is found in the 8th century Irish epic tale called *Táin Bó Cúailnge* (Cattle Raid of Cooley)[1]. In this epic, an Ulster warrior named Cethern was critically wounded in battle and was treated by a healer Fingin by sleeping him in a bath of bone marrow taken from the

bones of several “beasts”. According to this tale, Cethern’s condition improved to allow him to fight another day and half before he succumbed to his wounds.

In the late 19th century, role of bone marrow in the formation of blood was understood. In 1896, Quine reported the use of oral administration of bone marrow to treat defective blood formation in a patient with leukemia by Brown-Sequard and d' Arsonval[2]. Although a response was not seen in this patient, this was one of the first reported the use of bone marrow for therapeutic purpose.

Early breakthroughs in transplant 1920’s- 1940’s:

Leake & Leake used saline extracts of bone marrow and spleen intravenously for treatment of anemia in animal models and observed responses in 1923[3]. In 1930, Gloor described the cure of a patient with leukemia using stem cell transplantation. Despite this report, this approach was not further investigated until the 1960’s as a treatment modality. In 1937, Schretzenmayr reported intramuscular use of fresh bone marrow to patients with parasitic infections. As we would expect, this approach did not benefit the patients but this was one of the earliest reported approach of using live stem cells.

The true era of modern stem cell transplantation began after the Second World War, in the wake of the first atomic bomb explosions. Leon Orris Jacobson showed that mice could be protected from lethal doses of radiation by shielding either the spleen or the femur. He further showed that a similar effect could be obtained by infusing spleen or bone marrow cells from a litter mate to the mice after treating them with lethal radiation[4]. The mechanism of this effect was attributed to a “humoral” factor in the spleen or bone marrow that stimulated recovery of the marrow.

Advances in the 1950’s- 1960’s:

In 1954, Ford and his colleagues demonstrated using a T6 chromosomal marker that the recovering cells in the recipient mice were indeed derived from the cells from the donor mice[5]. In 1956, firm evidence was presented by Barnes and Loutit that the transplanted cells had a therapeutic immunologic anti-leukemic effect[6]. They also described a “wasting syndrome” in transplanted mice that died despite being leukemia free. This is now recognized as graft-versus-host-disease (GVHD)[7].

In mid-1950's, E. Donnall Thomas and colleagues started studies in leukemia patients by treating them with radiation followed by bone marrow grafting but were unsuccessful due to lack of engraftment/transient engraftment and progressive leukemia.

In 1959, three papers were published which can be considered as landmarks in stem cell transplantation. The first study was published by Dr. Thomas describing two patients with acute lymphoblastic leukemia (ALL) treated with total body irradiation (TBI) (850 rads) followed by infusion of bone marrow from identical twin siblings. The leukemia was transiently under control but progressed eventually in both patients[8].

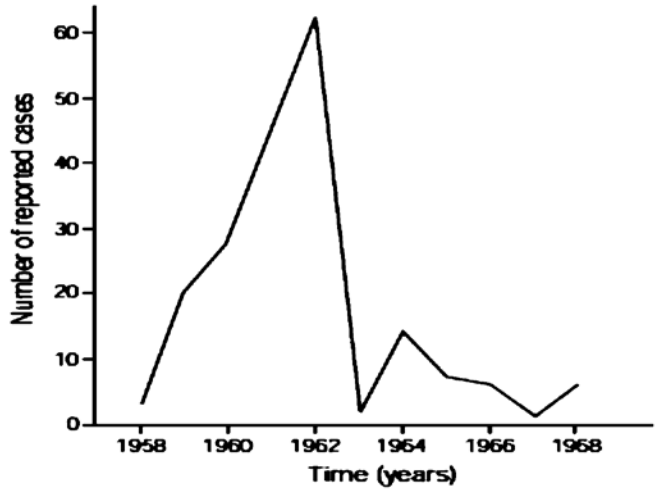
In a second study, Georges Mathé reported use of bone marrow to rescue six physicists who were accidentally exposed to lethal doses of radiation[9]. He treated five of these physicists with multiple bone marrow infusions from several family members. Four of the physicists survived due to transient partial engraftment which was followed by rejection and autologous stem cell recovery. These transplants were done prior to the knowledge of histocompatibility antigens.

In a third study, McGovern and colleagues reported the first autologous bone marrow transplant using cryopreserved cells preserved during a previous remission. The patient had terminal ALL and was conditioned with TBI[10]. Despite achieving a second remission, the leukemia subsequently progressed.

In the 1960's, advances in immunology and elucidation of human histocompatibility antigens helped provide a better understanding of the reasons for failures in stem cell transplantation till then. Reasons for graft failure and graft-versus-host-disease were better appreciated. It was also recognized that graft-versus-host-disease occurred despite well matched recipient-donor pairs. This led to the concept of using post-transplant immunosuppressive agents such as methotrexate.

Despite all the advances, the outcomes of SCT were dismal due to issues with graft failure secondary to lack of adequate HLA typing; disease relapse and progression and finally severe GVHD as a result of lack of appropriate prophylaxis (Figure 2).

FIGURE 2



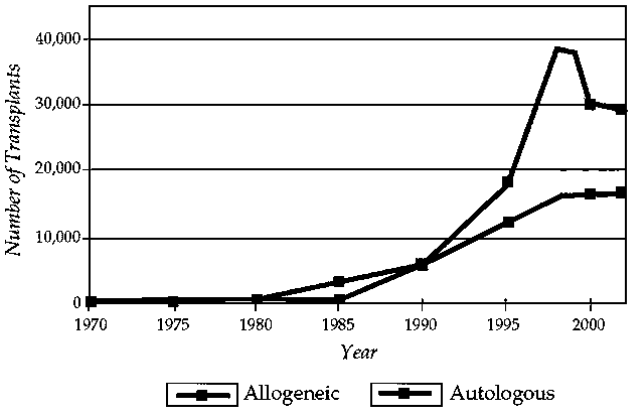
203 cases of transplants carried out between 1958 and 1968. Out of 203 patients, 125 patients experienced graft failure, 49 developed graft-versus-host disease (GVHD) and only 11 achieved long-term engraftment. Only three were alive in 1970.

Strides in 1970's- 1980's:

The several advances in the 1960's laid the foundation for the beginning of clinical transplantation. Newer indications for allogeneic transplant were identified, such as, aplastic anemia, immune deficiency disorders, chronic leukemias.

The International Bone Marrow Transplant Registry was founded in 1972 by Dr. Mortimer M Bortin and the European Bone Marrow Transplant group in 1974. These institutions were established to maintain database from the growing number of transplants being performed at numerous centers worldwide.

FIGURE 3



IBMTR data on transplants done worldwide

Improvements in conditioning regimens were also noted during this time. Prior to the 1970's the only conditioning regimen used was total body irradiation. Combination chemotherapy regimens were introduced with the development of agents such as busulfan and cyclophosphamide. Advances in post-transplant GVHD prophylaxis was noted with introduction of cyclosporine[11]. The combination of low dose methotrexate and cyclosporine remains the standard GVHD prophylaxis even to this date. In addition to improvements in transplant techniques, rapid improvements in supportive care were also noted. Better infection prophylaxis was introduced with availability of acyclovir, amphotericin and newer antibacterials.

There was a renewed interest in autologous bone marrow transplant in the late 1970's due to improved cryopreservation techniques. Barnes and Loutit showed that stem cells had better viability if frozen to -79°C. Glycerol was used until the discovery of dimethyl sulfoxide(DMSO) as a cyropreservant. Due to better use of autologous bone marrow cells, lymphoma and myeloma were added to the indication list for stem cell transplant.

The 1980's continued to see improvements in supportive care in stem cell transplantation leading to better outcomes with reduced transplant-related morbidity and mortality. The introduction of ganciclovir and liposomal amphotericin to the antibiotic armamentarium helped better control cytomegalovirus infections and treat fungal infections with less nephrotoxicity. This era also showed improvement in blood banking leading to improved transplant outcomes.

Further improvements in the understanding of HLA-system led to better use of unrelated donor stem cells and subsequently, a need for larger number of donors. This prompted establishment of unrelated volunteer donor registries such as the Anthony Nolan Research group in London, European registry in Leiden, Holland and the North American Marrow Donor Pool in the US[12].

In the mid-late 1980's, first attempts at stem cell engineering were made. Manipulation of the stem cells to cause T-cell depletion was performed with the knowledge that the T-cells were responsible for causing GVHD. Randomized clinical trials of T-cell depleted transplants clearly showed lower GVHD rates in the T-cell depleted transplants but also noted was a

higher rate of relapse post-transplant due to lack of the graft-versus-tumor effect[13].

Developments since 1990's:

Major advances in the field of BMT have been seen since the 1990s. Some of the highlights I review are use of peripheral blood stem cells instead of bone marrow, both in the autologous and allogeneic setting; availability of recombinant growth factors (G-CSF, GM-CSF) allowing for faster engraftment; introduction of reduced intensity transplants which helped extend allogeneic SCT to older patients; cord blood transplantation; donor lymphocyte infusions; newer modalities for prevention and treatment of GVHD with better understanding of the pathogenesis of GVHD; selective T cell depletion and suicide gene therapy.

Peripheral blood stem cell transplants and recombinant growth factors:

The availability of recombinant growth factors such was granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) impacted stem cell transplant in two ways. The use of these growth factors post-transplant helped hasten white count recovery as a result of which, duration of hospitalization was reduced. Peripheral blood stem cells (PBSC) could be mobilized using higher doses of these growth factors. This helped abrogate need for bone marrow harvest and associated general anesthesia.

Subsequent clinical trials in both autologous and allogeneic setting showed faster white count and platelet recovery in the PBSC transplanted patients[14]. As a result of these trials, PBSC transplant has become standard of care in the autologous setting in adults.

In the allogeneic transplant setting, the use of PBSCs is associated with a greater incidence of chronic graft-versus-host-disease[15]. This is due to 10-fold greater number of CD3+T cells in the PBSC product which contributes to the higher rates of chronic GVHD. This further translated into GVT and improved disease free survival in the high risk diseases.

Reduced Intensity Transplants:

The median age at diagnosis of most hematologic malignancies (acute and chronic leukemia, myelodysplastic syndrome, myeloproliferative disorders, and lymphoma) is around 65 years. Due to this factor, most patients would be ineligible for conventional myeloablative SCT. As the saying goes,

"Necessity is the mother of Invention", several investigators developed less intensive SCTs to circumvent the age restriction.

This led to the era of the reduced intensity stem cell transplantation, also known as "mini" transplants or "RIC" transplants or non-myeloablative transplants[16]. The conditioning regimen in these type of transplants is much less intensive than the conventional transplants and relies on the graft-versus-tumor effect to cure the underlying host malignancy (Figure 4). Due to the markedly reduced toxicity of these regimens, "mini" transplants can be performed in the outpatient setting.

FIGURE 4

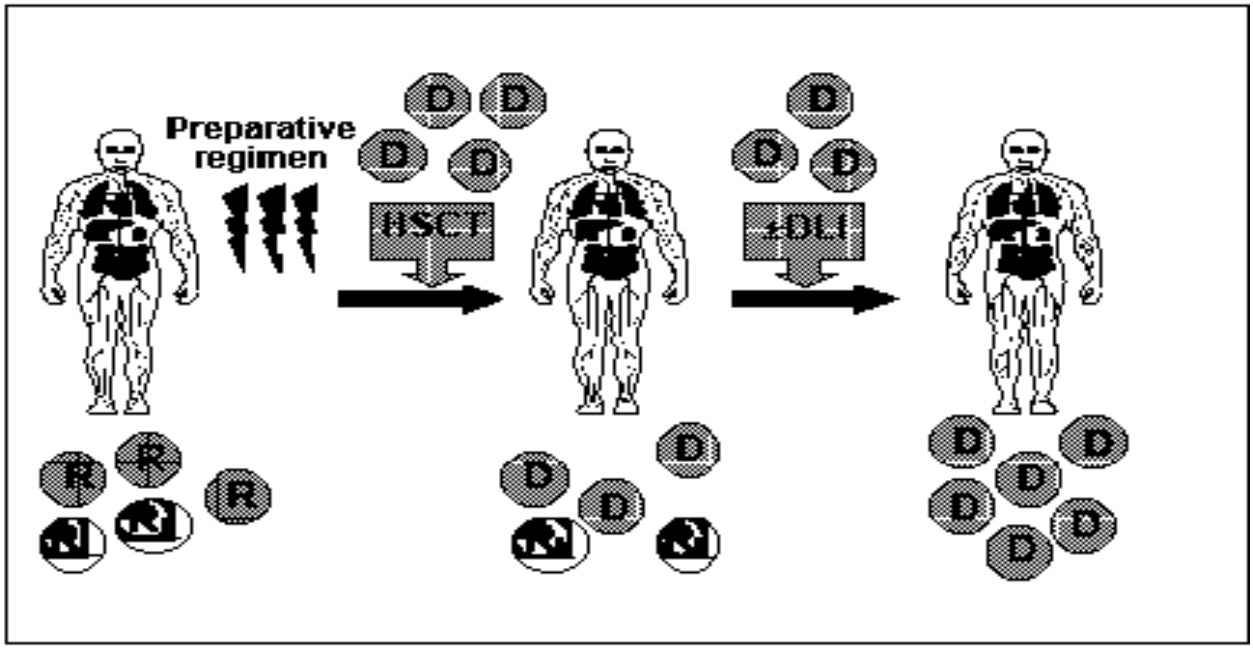


Figure 2: Nonablative Hematopoietic Transplantation for Hematologic Malignancy—Recipients (R) receive a nonmyeloablative preparative regimen and an allogeneic hematopoietic stem cell transplant (HSCT). Initially, mixed chimerism is present with the coexistence of donor (D) cells and recipient-derived normal and leukemia/lymphoma (R_L) cells. Donor-derived T-cells mediate a graft-vs-host hematopoietic effect eradicating residual recipient-derived normal and malignant hematopoietic cells. Donor lymphocyte infusions (DLI) may be administered to enhance graft-vs-malignancy effects.

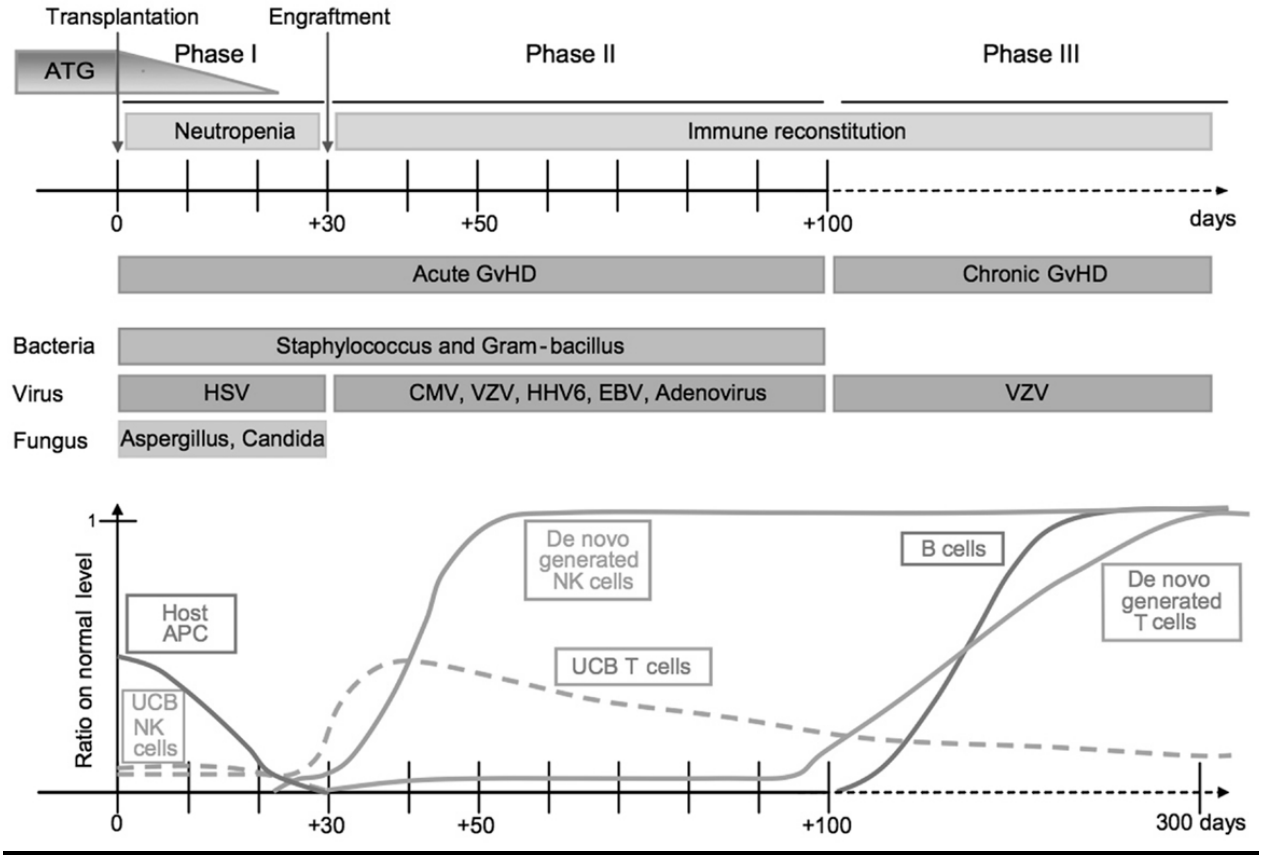
Cord Blood Transplantation:

With improvements in SCT technology, supportive care and better understanding of the immunology, more patients were found to be eligible for SCT. Unfortunately, only 30% of all eligible patients are able to find an acceptable related sibling donor; another 15% are able to receive a

transplant using an unrelated donor. This leaves over half of eligible patients without a donor. The option of cord blood transplant (CBT) and haplo-identical SCT exists for such patients.

The first successful CBT was performed in 1988 in a child with Fanconi's anemia. The concept of CBT was suggested by Hal Broxmeyer, Edward Boyse and Judith Bard. They collaborated with several others and started a company called Biocyte Corporation which funded a 2-year grant to study the biology of cord blood cells. Since then, more than 30,000 CBT's have been performed to date. Despite improvements in the CBT technology, this treatment is associated with limitations in adults. These include slow, delayed and poor count recovery post-transplant[17]. Other issues are delayed immune-reconstitution resulting is multiple infections (Figure 5).

FIGURE 5



A number of strategies are being investigated to overcome the limitations of CBT. The biggest hurdle for single unit CBT is delayed count recovery which is associated with poor overall survival. One of the strategies being used to improve the stem cell dose is ex-vivo expansion of cord blood cells

using several different cytokine cocktails. Another way of improving cell dose is to transplant using a combination of cord blood unit and stem cells from a haplo-identical family donor. This strategy in phase I studies has been found to be feasible as well as successful and is being further investigated. Another popular technique is to use more than one cord blood units (double cord or triple cord). The limitation of this has been finding multiple cord units that compatible with each other as well as the patient.

The second limitation of CBT is prolonged immune-suppression leading to life threatening infections. This is being accomplished by infusion pooled T-regulatory cells, and expansion of antigen specific T cells.

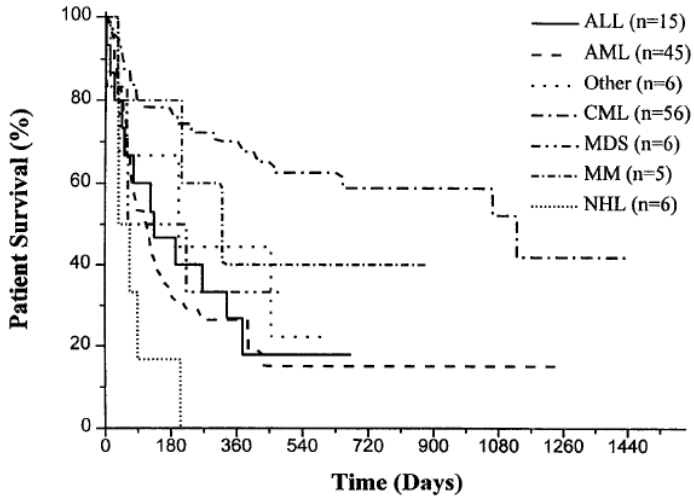
Donor Lymphocyte Infusions:

Another big hurdle to a positive outcome after allogeneic SCT is relapse of the underlying malignancy. Anywhere between 20-60% of patients relapse after SCT and outcome of such patients remains poor. Donor lymphocyte infusion (DLI) therapy is one of the most effective therapies available for such patients. DLI is effective due to the graft versus tumor effect. Kolb et al. provided first evidence of GVT effect of DLI in relapsed chronic myeloid leukemia (CML) patients after allogeneic SCT[18]. Responses to DLI are not uniform across diseases (Figure 6)[19]. CML appears to be the disease where DLI is most effective with molecular remissions being reported after DLI. The drawback of DLI is GVHD which can be seen in 40%-60% patients.

Since the first use of DLI in 1990, several advances have been made in this field too. DLI is now used in different clinical scenarios, treatment of relapsed disease being the most common one. The other situation where DLI is used is treatment of minimal residual disease after allogeneic SCT to induce GVT effect and reduce chances of florid relapse. Another use of DLI is prophylactically, in patients with high risk hematologic malignancy when there is significant concern for relapse. Pre-emptive infusion of escalating doses of DLI given at intervals of 4-6 weeks have been studied in several patients and dose levels have been defined.

Investigators have determined ways to increase the efficacy of DLI by increasing tumor response. This has been attempted by use of adjuvant interleukin-2, interferon alpha in combination with GM-CSF after the DLI. Another technique that has been studied to improve efficacy of DLI is to give a DLI product that has been depleted of regulatory T cells (regulatory T cells reduce alloreactivity and hence reduce GVHD)[20].

FIGURE 6



Outcome of different relapsed hematologic malignancies treated with Donor lymphocyte Infusions

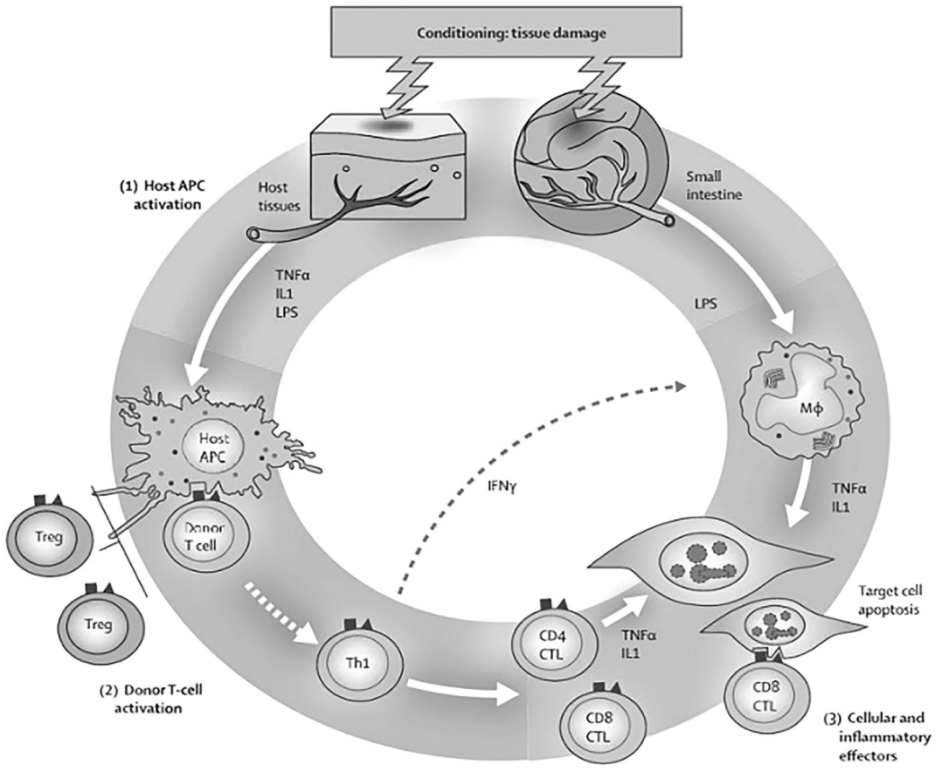
Improvements in GVHD management:

Graft-versus-host-disease remains the most common cause of non-relapse mortality after allogeneic SCT. GVHD can be acute or chronic. Over the past decade, several consensus statements have been published by the NIH GVHD consortium. One of the statements has changed the definition of acute and chronic GVHD. As opposed to previous definition of acute GVHD which was defined as GVHD that occurred during the first 100 days of transplant, the newer system divides acute GVHD into two types, classic (<100 days post-transplant) and persistent/recurrent (beyond 100 days after transplant). Similarly, chronic GVHD is divided into two subtypes, classic and overlap (has features of both acute and chronic GVHD). The subtype of GVHD has prognostic significance.

We also have a better understanding of the pathogenesis of GVHD (Figure 7) which has helped identify targets to develop newer strategies for both prevention as well as treatment of this complication[21]. Some of the newer treatments that have been developed in the past decade for the management of steroid refractory GVHD are TNF alpha (tumor necrosis factor) inhibitors such as infliximab and etanercept, sirolimus which is an mTOR inhibitor and interleukin 2 receptor antagonists (daclizumab).

The two treatments that can be considered as landmarks in the management of GVHD are extracorporeal photopheresis (ECP) and use of mesenchymal stromal cells.

FIGURE 7



Three phase model of pathogenesis of acute graft-versus-host-disease

ECP is an apheresis-based immunomodulatory therapy that has been used for several decades in the management of cutaneous T cell lymphomas. Exposure of the buffy coat to ultraviolet A light causes apoptosis of the mononuclear cells in the peripheral blood which in turn causes a cytokine response and generation of regulatory T cells (Tregs). These Tregs suppress the effector T cells that are causing GVHD. This treatment has been shown to be effective in both acute and chronic GVHD in several clinical trials.

Mesenchymal stromal cells (MSCs) are multipotent progenitors found in the bone marrow and several other tissues[22]. They have several immunoregulatory properties including inhibition of proliferation of T lymphocytes, promotion of Tregs and modulation of response of B cells, NK cells and dendritic cells which are all involved in the pathogenesis of GVHD. They do not need to be HLA matched to the recipient and hence

can be obtained from a third-party donor. The use of MSCs for management of acute GVHD was pioneered by Le Blanc in 2004. Now several commercial preparations are available for use in management of acute steroid refractory GVHD.

Suicide gene therapy:

Gene therapy is promising therapeutic modality for both genetic and acquired hematologic conditions. With improvement in safety and efficacy of gene transfer techniques, this technology was used in SCT by infusing donor lymphocytes transduced (gene transfer) to express HSV-TK suicide gene. The suicide gene was used to selectively control GVHD. Once the patient developed GVHD, ganciclovir was initiated to selectively kill the HSV-TK containing allo-reactive T cells without any effect on the GVT[23]. Several clinical trials are being conducted using this method to selectively eliminate allo-reactive T cells without effecting the anti-viral donor T cells and potentially anti-tumor specific T cells.

Future Directions:

Stem cell transplantation has come a long way since the 1960's when researchers almost abandoned allogeneic SCT due to recurrent failures caused by disease relapse, GVHD and graft rejection. There is no doubt that SCT is a very effective treatment modality for several hematologic disorders.

The future of this science is directed towards further improving the efficacy and reducing the toxicity of this highly sophisticated treatment. Under investigation are techniques to minimize GVHD while maintaining the GVT effect by selective depletion of T cells. Several investigators are studying the use of vaccines and adoptive transfer of T cells to induce anti-tumor responses. The use of gene therapy is being further investigated to cause selective elimination of designated lymphocyte populations which are responsible for GVHD while sparing the non-alloreactive T cells.

References:

1. Kinsella T. The Tain. Oxford University Press, Oxford, 1970, 212
2. Quine WE. The remedial application of bone marrow. JAMA 1896; 26:1012-1013
3. Leake CD, Leake BW. The erythropoietic action of red bone marrow and spleen extracts. J Pharmacol Exper Ther 1923;22:75-88

4. Jacobson LO, Simmons EL, Bethard WF. Studies on hematopoietic recovery from radiation injury. J Clin Invest 1950;29:825
5. Ford CE, Hamerton JL, Barnes DWH, Loutit JF. Cytological identification of radiation chimeras. Nature 1956;177:452-454
6. Barnes DWH, Loutit JF. What is the recovery factor in spleen? Nucleonics 1954;12:68-71
7. Barnes DW, Loutit JF, Micklem HS. 'Secondary disease' of radiation chimeras: a syndrome due to lymphoid aplasia. Ann NY Acad Sci 1962;99:374-385
8. Thomas ED, Lochte HL, Cannon JH, Sahler OD, Ferrebee JW. Supralethal whole body irradiation and isologous marrow transplantation in man. J Clin Invest 1959;38:1709-1716
9. Mathé G, Amiel JL, Schwarzenberg L et al. Adoptive immunotherapy of acute leukemia: experimental and clinical results. Cancer Res 1965;25:1525-1531
10. McGovern JJ, Russel PS, Atkins L, Webster EW. Treatment of terminal leukemic relapse by total body irradiation and intravenous infusion of stored autologous bone marrow obtained during remission. N Engl J Med 1959;260:675-683
11. Powles RL, Clink HM, Spence D et al. Cyclosporin A to prevent graft-versus-host-disease in man after allogeneic bone-marrow transplantation. Lancet 1980;1:327-329
12. Goldman JM, Cleaver S, Warren P. World Marrow Donor Association: a progress report. Bone Marrow Transplant 1994;13:689-691
13. Goldman JM, Gale RP, Horowitz MM et al. Bone marrow transplantation for chronic myelogenous leukaemia in chronic phase: increased risk of relapse associated with T-cell depletion. Ann Intern Med 1988;108:806-814
14. Schmitz N, Dreger P, Linch DC et al. Randomised trial of filgrastim-mobilised peripheral blood progenitor cell transplantation versus autologous bone-marrow transplantation in lymphoma patients. Lancet 1996; 347:353-357
15. Ringdén O, Labopin M, Bacigalupo A et al. Transplantation of Peripheral Blood Stem Cells as Compared With Bone Marrow From HLA-Identical Siblings in Adult Patients With Acute Myeloid Leukemia and Acute Lymphoblastic Leukemia. J Clin Onc 2002; 20:4655-4664
16. Barrett J, Childs R. Non-myeloablative stem cell transplants. Br J Haematol 2000;111:6-17

17. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. Blood 2013;122:491-498
18. Kolb HJ, Mittermuller J, Clemm C et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. Blood. 1990;76:2462-2465
19. Collins RH Jr, Shpilberg O, Drobyski WR et al. Donor Leukocyte Infusions in 140 Patients With Relapsed Malignancy After Allogeneic Bone Marrow Transplantation. J Clin Onc 1997;15:433-44
20. Maury S, Redjoul R, Cabanne L et al. Regulatory T-cell depletion in donor lymphocyte infusions for haematological malignancies: long-term outcomes from a prospective study. Br J Haemat 2014;166:452-455
21. Dhir S, Slatter M, Skinner R. Recent advances in the management of graft-versus-host-disease. Arch Dis Child 2014;0:1-8
22. Ringdén O, Uzunel M, Rasmusson I et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host-disease. Transplantation 2006;81:1388-1389
23. Traversari C, Marktel S, Magnani Z, et al. The potential immunogenicity of the TK suicide gene does not prevent full clinical benefit associated with the use of TK-transduced donor lymphocytes in HSCT for hematologic malignancies. Blood 2007;109:4708-4715

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