

mouse embryonic stem cells in the mechanisms of pluripotency suggest that other factors may be required to achieve similar results with human cells. Further investigation of the factors is needed to elucidate their roles in reprogramming and to ensure that we can avoid any detrimental effects they may have on cells. Transient expression of factors (using vectors that do not integrate into the genome) in fibroblasts or the identification and use of small molecules that mimic the effects of the factors would enable researchers to avoid the possibility of generating mutations in the genome through random insertions and reactivation of transgenes in the retroviral vectors.

Reprogramming of adult cells to generate patient-specific therapies represents the future for stem-cell biologists. Inducing plu-

ripotent stem cells is the first successful way of instructing somatic cells to become pluripotent by introducing defined factors. A recent report on identifying induced pluripotent stem cells on the basis of morphologic criteria alone brings us a step closer to translating this work safely into human cells. Such identification would obviate the need for transgenic reporter genes in the donor fibroblasts.⁵ Despite these encouraging results, research on human embryonic stem cells should not be impeded; such cells remain the gold standard for determining the molecular basis of human tissue development and for developing cell-based therapies for human diseases.

An interview with Dr. Douglas Melton, a scientific director of the Harvard Stem Cell Institute and a professor in the Department of Molecular and Cel-

lular Biology at Harvard University, can be heard at www.nejm.org.

Dr. Gearhart is a professor of gynecology and obstetrics, physiology, comparative medicine, and biochemistry and molecular biology and the director of the Stem Cell Biology Program at the Institute for Cell Engineering, Johns Hopkins Medical Institutions, Baltimore, where Ms. Pashos and Ms. Prasad are Ph.D. candidates in the Human Genetics and Molecular Biology Program.

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663-76.
2. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature* 2007;448:313-7.
3. Wernig M, Meissner A, Foreman R, et al. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 2007;448:318-24.
4. Maherali N, Sridharan R, Xie W, et al. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* 2007;1:55-70.
5. Meissner A, Wernig M, Jaenisch R. Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. *Nat Biotechnol* (in press).

Copyright © 2007 Massachusetts Medical Society.

Hematopoietic-Cell Transplantation at 50

Frederick R. Appelbaum, M.D.

September 12, 2007, marked the 50th anniversary of E. Donnall (Don) Thomas's initial report of a radical new approach to cancer treatment: radiation and chemotherapy followed by the intravenous infusion of bone marrow.¹ That publication represented the beginning of a long series of laboratory and clinical investigations; more than a decade would pass before the procedure achieved its first successes. Yet Thomas's persistence in the face of criticism and clinical failure ultimately paid off in a new form of therapy that was used to treat approximately

50,000 people worldwide in 2006 (see timeline).

Thomas's interest in the possibility of hematopoietic-cell transplantation was sparked in 1949, during his residency at Peter Bent Brigham Hospital in Boston, when he learned of Leon Jacobson's experiment showing that a mouse exposed to otherwise lethal irradiation would survive if its spleen, or in later studies its marrow, was shielded.² That its survival was due to a cellular rather than humoral effect was proven several years later, when researchers showed that irradiated mice giv-

en an infusion of marrow with a chromosome marker recovered with marrow cells exclusively of donor origin. With that experiment, Thomas became convinced of the clinical potential of human marrow transplantation.

In 1955, he moved to the Mary Imogene Bassett Hospital in Cooperstown, New York, and began working with Joseph Ferrebee. Thomas and Ferrebee's 1957 article describes the first experience with allogeneic marrow transplantation in humans: six patients were treated with irradiation and chemotherapy and then intravenous

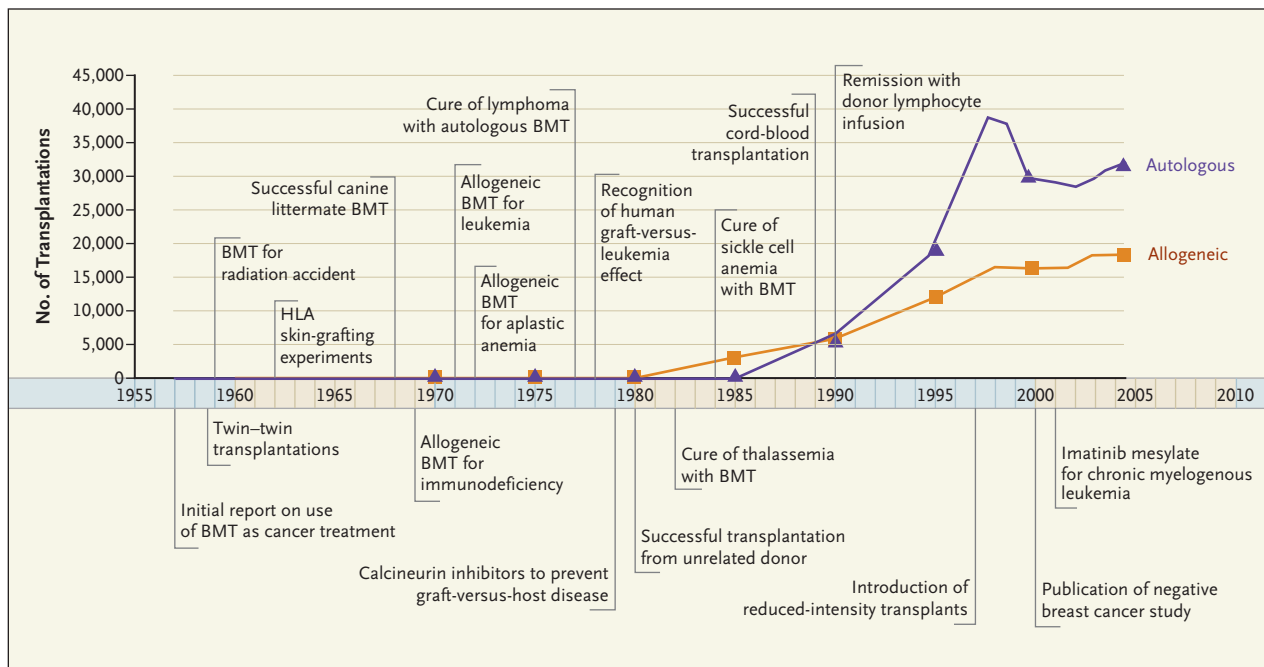
infusion of marrow from a normal donor. Although the infusion was not accompanied by severe adverse effects, only two patients had transiently detectable marrow grafts, and none survived beyond 100 days. Others' attempts at allogeneic marrow transplantation, including some in victims of a nuclear-reactor accident, also failed, and by the early 1960s, many were pessimistic about whether such grafting would ever be possible. With little known about histocompatibility, no one tried to match donors and recipients.

Although many researchers abandoned the field, Thomas remained convinced of its potential and began conducting experiments using an outbred canine model. He found that most dogs that were given total-body irradiation and infusion of littermate marrow had the same problems as humans, in-

cluding graft rejection, graft-versus-host disease (GVHD), and death from opportunistic infection. Occasionally, however, a dog became a healthy long-term survivor with marrow cells of donor origin. Thomas reasoned that appropriate donor selection was the key. In 1963, he moved to Seattle, where he and his colleagues developed rudimentary canine histocompatibility typing. By the mid-1960s, they could show that most dogs given sufficient irradiation followed by grafts from dog leukocyte antigen–matched littermates and a short course of immunosuppression survived long-term. At the same time, the first methods for human leukocyte antigen typing were being developed, and Thomas decided to return to human marrow grafting.

He assembled a research team and in 1969 began clinical trials

of allogeneic marrow transplantation from matched siblings. His first patients had very advanced leukemia, and Thomas went to extraordinary lengths to support them through the procedure, including asking staff members to donate platelets, using patients with chronic myeloid leukemia as granulocyte donors, and working with Robert Hickman to develop a catheter for intravenous alimentation. When GVHD developed in patients, Thomas inoculated horses with human lymphocytes to create antithymocyte globulin as a treatment (and was kicked more than once in the process). Most patients died of progressive leukemia or complications of transplantation, but some entered complete remission. In 1970, the Seattle group published the results of their efforts in patients with leukemia, and in 1972 they reported



Timeline Showing Numbers of Bone Marrow Transplantations and Advances in the Field, 1957–2006.

BMT denotes bone marrow transplantation, and HLA human leukocyte antigen. Data are from the Center for International Blood and Marrow Transplant Research.

the first successful allogeneic transplantations for aplastic anemia. In 1975, Thomas published an article showing a plateau in the survival curve of patients who received transplants for end-stage acute leukemia, suggesting that a cure had been achieved in a minority of patients.³ This cure of even a small percentage of otherwise incurable patients led Thomas to explore transplantation earlier in the course of disease, and in 1979 he and his colleagues reported achieving a cure rate above 50% with transplantation for acute myeloid leukemia that was in remission.⁴

Initially, transplantation was limited to the approximately 25% of patients with a matched sibling or identical-twin donor. In the mid-1970s, autologous marrow transplantation was shown to be potentially useful when some patients with non-Hodgkin's lymphoma were cured with high-dose therapy followed by infusion of their own previously harvested and cryopreserved normal marrow. In the late 1970s, the Seattle group performed the first successful marrow grafting from a matched, unrelated donor in a patient with leukemia, which helped to stimulate the formation of the National Marrow Donor Program and the subsequent registration and typing of more than 11 million volunteer donors. These advances, coupled with the new use of umbilical cord blood as a source of hematopoietic stem cells, mean that today appropriate donors can be found for the large majority of patients. In addition, clinical research has rendered transplantation less dangerous, with the development of safer preparative

regimens to eradicate cancer and achieve engraftment, better prophylaxis against GVHD, and improved measures for preventing death from opportunistic infections.

Hematopoietic-cell transplantation is now available at 500 or more centers in more than 50 countries. Transplantation from a matched sibling is the treatment of choice for severe aplastic anemia and can cure thalassemia and sickle cell anemia. Improved survival with autologous transplantation has been demonstrated for recurrent non-Hodgkin's lymphoma and as initial therapy for multiple myeloma. Studies also show a survival advantage with transplantation from matched siblings for adults with acute myeloid or acute lymphocytic leukemia in first remission. Recurrent Hodgkin's disease and advanced myelodysplasia are also indications for the procedure.

In addition to its therapeutic importance, Thomas's extended experiment has had broad scientific influence on hematology, immunology, and oncology. By showing that it is possible to transplant the entire lymphohematopoietic system from one person to another by transferring a limited number of marrow cells, Thomas elucidated the nature of hematopoiesis and stimulated efforts to identify the hematopoietic stem cell and factors that control its growth and development. He showed that with the correct selection of donor and recipient and the proper use of short-term immunosuppression, lifelong bidirectional immunologic tolerance between adults was achievable — an observation that triggered decades of basic and ap-

plied research in immunogenetics that has deepened our understanding of the immune system.

Perhaps most important, Thomas's work demonstrated the power of the human immune system to eradicate cancer. By showing that relapse rates after transplantation were lowest in patients in whom GVHD developed, higher in allogeneic recipients without such disease, and highest in identical-twin transplant recipients, Thomas illustrated that the genetically non-identical graft and the immune system it gives rise to can recognize and contribute to the elimination of tumors. This observation raised a key question: How much of the antitumor effect of allogeneic transplantation was due to the intense preparative regimen and how much to the immunologic graft-versus-tumor effect? Preparative regimens have since been developed that are markedly less intense but still ensure allogeneic engraftment, and results indicate that at least for the slower-growing hematologic cancers, much of the antitumor effect remains. This advance allows transplantation for selected diseases to be performed safely on an outpatient basis and in patients 50 to 70 years of age. Much more important, the demonstration that the graft-versus-tumor response could eradicate disseminated cancer provided much of the impetus for early efforts to develop effective T-cell and vaccine-based immunotherapeutic approaches to tumors.

In 1990, Thomas and Joseph Murray — who performed the first kidney transplantation between twins — shared the Nobel Prize “for their discoveries concerning

organ and cell transplantation in the treatment of human disease.” Their legacy is a field of clinical research and treatment that has saved tens of thousands of lives. At 87, Thomas continues to work part-time and to watch the unfolding of a story that began 50 years ago with his seminal experiment.

No potential conflict of interest relevant to this article was reported.

Dr. Appelbaum is director of the Clinical Research Division of the Fred Hutchinson Cancer Research Center and head of the Division of Medical Oncology at the University of Washington — both in Seattle.

1. Thomas ED, Lochte HL Jr, Lu WC, Ferree JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. *N Engl J Med* 1957;257:491-6.

2. Jacobson LO, Marks EK, Robson MJ, Gaston EO, Zirkle RE. Effect of spleen protection on mortality following x-irradiation. *J Lab Clin Med* 1949;34:1538-43.

3. Thomas ED, Storb R, Clift RA, et al. Bone-marrow transplantation. *N Engl J Med* 1975;292:832-43, 895-902.

4. Thomas ED, Buckner CD, Clift RA, et al. Marrow transplantation for acute nonlymphoblastic leukemia in first remission. *N Engl J Med* 1979;301:597-9.

Copyright © 2007 Massachusetts Medical Society.