

Toward a Zero-Intensity Preparation (ZIP) Transplant

Transplanting the immune system from genetically matched donors to patients with hematologic cancers—allogeneic stem cell transplants—are among the first and best examples of cures for cancer. However, the procedure is associated with potentially lethal toxicities, many of which are the direct or indirect result of the preparative regimen administered just prior to transplant. Dan Fowler, M.D., Senior Investigator in CCR’s Experimental Transplantation and Immunology Branch (ETIB), has spent 25 years at NCI in a quest to convert the classical bludgeoning approach of ablating and replacing the entire immune system to a more nuanced approach that selectively transplants key mediators of the therapeutic antitumor effect: cytokine-polarized donor T cells.

Into the Minefield

Bone marrow transplantation was first evaluated in cancer treatment more than 50 years ago as a rescue strategy after high-dose chemotherapy combined with radiation therapy. Well into the 1980s, curing leukemia was thought to rely primarily upon the high-dose preparative regimens that ablated leukemic host stem cells; transplanting normal stem cells from bone marrow or peripheral blood cells was meant to reconstitute the hematopoietic system once the cancer was eradicated. The main causes of lethality with ablative transplants were due to the preparative regimen or to the transplant attack on normal tissues known as Graft versus Host Disease (GVHD, see “Treat the Cure,” *CCR connections* Vol. 8, No. 2).

By 1990, experimental murine models and clinical data demonstrated that donor T cells were critical to the success of transplantation, as they mediated both detrimental effects (GVHD)

and beneficial effects (prevention of graft rejection; mediation of graft-versus-tumor [GVT]). Because of this new understanding of allogeneic transplantation as an immune therapy rather than simply a rescue from high-dose preparation, the transplant field evolved to include alternative preparative regimens that were termed nonablative or reduced-intensity. The argument was: if the curative aspect of the transplant was due in part to T cells in the graft, why are we giving such toxic host preparation?

In 1990, I stepped into this minefield as a Medical Oncology Fellow and then worked in the lab of Ronald Gress, M.D., now Chief of ETIB and a CCR Deputy Director.

Laying the Groundwork

At this time, immunology researchers were just defining the concept of functional subsets of T cells with differential *in vivo* effects based on their pattern of cytokine secretion (initially, the TH1/TH2 subsets). My first project

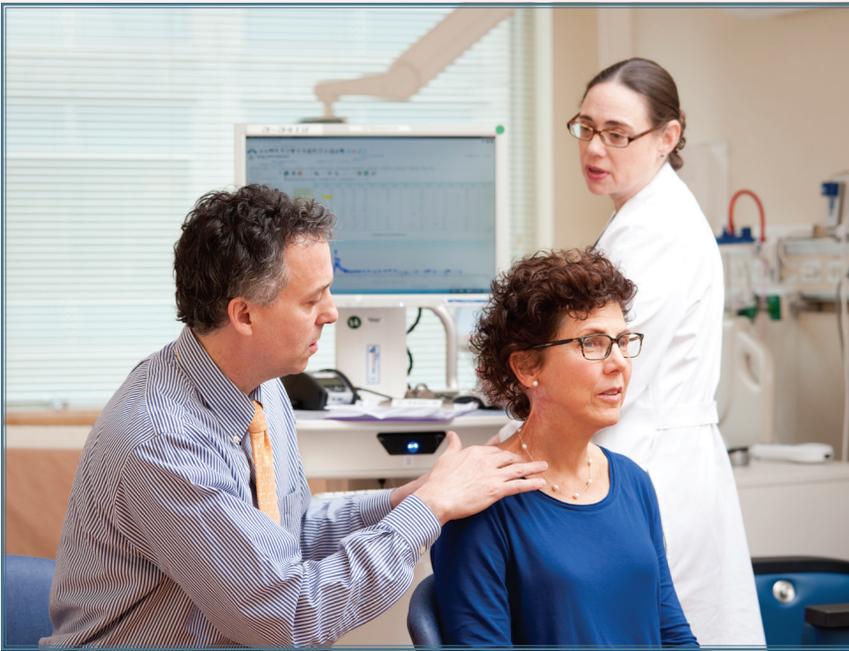


(Photo: R. Brer)

Dan Fowler, M.D., in the lab

was to use mouse models to address the hypothesis that donor TH2 cells, which we manufactured by exposing T cells to the cytokine IL-4, would cause less GVHD. Indeed, IL-4-polarized donor T cells did not induce lethal GVHD in mice, and more importantly, they protected mice against GVHD mediated by donor TH1 cells (TH2/TH1 cross-regulation). We also studied mouse models of graft rejection and found

(Photo: R. Baer)



Dan Fowler, M.D., patient Annette Abrams, and Daniele Avila, M.S., Nurse Practitioner

that IL-4-polarized cells prevented graft rejection by an “infectious tolerance” mechanism. That is, donor TH2 cells produced IL-4, which engaged the IL-4 receptor on host T cells; the host T cells became TH2-like and mediated less graft rejection. Meanwhile, we found that donor TH1 cells were critical for the GVT effect. So although donor TH2 cells could prevent GVHD and graft rejection, we realized early in our research that we did not want a total TH2 phenotype in the transplant; we needed some TH2/TH1 mixture.

Trials and Tribulations

We have been fortunate to translate this research at the NIH Clinical Center. My first clinical trial began in 1999, and we enrolled 49 patients. I submitted an investigational new drug application (IND) to the Food and Drug Administration (FDA) that allowed us to manufacture donor T cells *ex vivo* here at the NIH Clinical Center, Department of Transfusion Medicine, Cell Processing Section (David Stroncek, M.D., is the current Chief and a key collaborator). Before we

performed the routine donor stem cell collection, we collected lymphocytes and purified the CD4+ T-cell population. We cultured these cells with IL-4 and administered them as a T-cell booster at the time of transplant. We hypothesized that the recipients of the IL-4 polarized, TH2-type cells would have less GVHD; however, relative to the control group, such recipients had similar rates of GVHD. In parallel with this initial clinical trial, our lab was evaluating new methods to optimize our approach. Usually, after a transplant, patients receive some type of immunosuppressive drug; at the time, we were using cyclosporine. I reasoned that cyclosporine might be neutralizing

the effect of the infused donor TH2 cells and that it may be advantageous to use an alternative drug such as rapamycin. Indeed, manufactured T cells could not survive in media containing cyclosporine whereas some fraction of T cells always survived in rapamycin. This observation led to our now 10-year effort to characterize and harness a phenomenon known as T-cell rapamycin resistance (T-Rapa cells).

Rapamycin is an inhibitor of a protein kinase known as the Mechanistic Target of Rapamycin (mTOR). When mTOR is blocked, cells are starved and start to digest their internal organelles as a source of fuel in a survival maneuver; with their organelles downsized and cell volumes reduced, T-Rapa cells exist in a relatively quiescent state in order to survive starvation. We did not initially predict that T-Rapa cells would be powerful *in vivo* after their transplantation, but that is the somewhat paradoxical result that we obtained. In side-by-side comparisons of TH2 cells manufactured either with or without rapamycin, the T-Rapa cells were always much more powerful in preventing experimental murine GVHD and graft rejection.

So when we began a new clinical trial in 2004, in which we kept all transplant parameters the same except for the way we manufactured the TH2 cells. This time, we added rapamycin.

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The Importance of Mixed Chimerism

On this new protocol, infusion of rapamycin-resistant T cells resulted in rapid full donor engraftment and toxic side effects due to a “cytokine storm.” This observation indicated that the T-Rapa cells were indeed more powerful than the T-cell population we previously manufactured. However, the protocol needed to be amended to improve the safety of this novel cell therapy product. First, we reduced the fludarabine/cyclophosphamide preparative regimen intensity by 75 percent, which allowed the transplant to be performed on an outpatient basis. We also waited two weeks after the stem cell transplant before administering T-Rapa cells. These two changes to the transplant platform allowed for safe infusion of the T-Rapa cells. In 2013, we published the results of a multicenter, phase 2 study in 40 patients with refractory hematologic malignancies. We had no transplant-related mortality, a low rate of acute GVHD, and 18 of 40 patients remained in complete remission from their malignancy with a minimum follow-up time of 42 months (see “The Art of Living,” *CCR connections* Vol. 9, No. 1).

One key element of this kind of transplant is that patients start out as a mixed chimera. At two weeks after the stem cell transplant, immune T cells and stem cell-derived myeloid cells are each typically at a 50-50

split in terms of donor and patient. The activated T-Rapa cells are thus administered in the mixed chimera state, with subsequent progression towards full donor engraftment. It is during this phase that we will observe clinical antitumor responses. We have also developed an iterative, risk-adjusted approach to allogeneic transplantation. That is, if a patient goes into complete remission, we will keep the patient as a mixed chimera, which offers a huge protection against GVHD (severe GVHD typically only occurs with full donor engraftment). On the other hand, if a complete remission is not attained, we intervene with additional infusions of donor T-cell products to achieve full donor engraftment to potentiate further GVT effects.

Most recently, we have tested the stringency of this mixed chimerism strategy by eliminating the chemotherapy preparative regimen. This represents the final step in our attempt to reduce the intensity of transplant from ablative, to reduced-intensity, to low-intensity, and now to a zero-intensity preparative regimen, or ZIP regimen. At this point, the therapeutic approach is highly focused on the donor T-cell immune therapy rather than any antitumor effect derived from chemotherapy; in the words of David Halverson, M.D., Staff Clinician in ETIB, and lead investigator on this protocol: “We have to trust the immunology.”

We have now performed the zero-intensity prep transplant procedure on 12 patients (see Figure 1, treatment schema). Basically, we allow patients to recover from their prior chemotherapy and ensure that their immune system is somewhat depleted (CD4 count of less than 100). Patients are started on a high dose of rapamycin to prevent graft rejection and then they get the stem cell transplant without any further chemotherapy. Now, when we do our chimerism test at two weeks after transplant, we find a much lower contribution of the donor elements (usually only 10 percent donor T-cell engraftment, less than 1 percent donor stem cell engraftment). This exaggerated state of mixed chimerism is generally considered to be unsustainable as the host immune system can reject the outnumbered donor elements. However, infusion of donor T-Rapa cells causes a marked increase in donor elements within two weeks of cell transfer and associates with clinical antitumor effects (see Figure 2, case study). So with the zero-intensity prep transplant, we have developed a new transplant platform that should further improve the safety of the immunotherapeutic T-Rapa cells by reducing chemotherapy toxicity, reducing infection (absence of peritransplant neutropenia), and preventing GVHD (more patients may achieve remission while remaining in a mixed chimeric state).

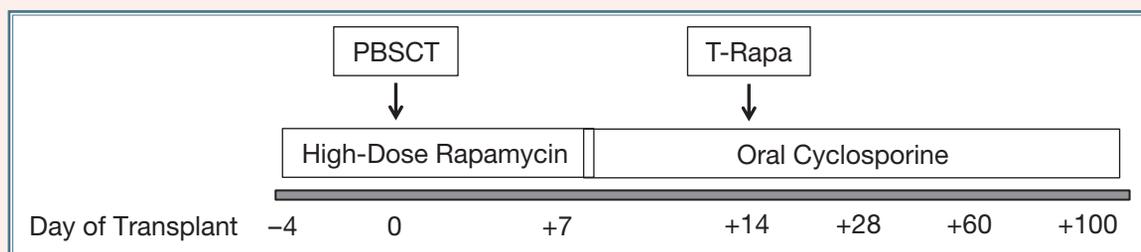


Figure 1. Patients with a hematologic cancer that do not respond to standard chemotherapy regimens proceed to a peripheral blood stem cell transplant. Host immunity is moderately compromised then rapamycin is administered four days prior to a peripheral blood stem cell transplant (PBSCT) with the donor being an HLA-matched sibling. Seven days after transplant, rapamycin is switched to oral cyclosporine therapy. Then at 14 days after transplant, patients receive *ex vivo* manufactured donor T-Rapa cells.

(Figure: D. Fowler, CCR)

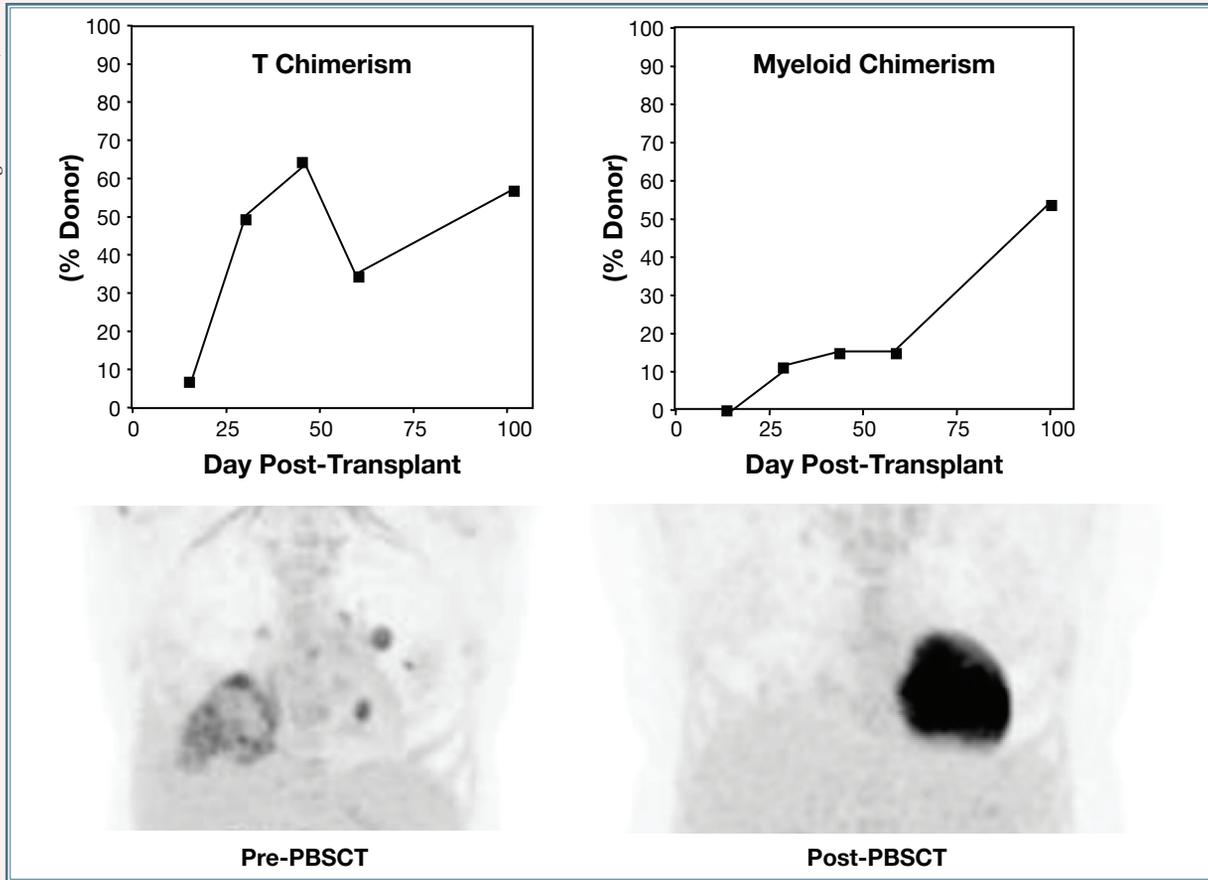


Figure 2. Zero-intensity preparation (ZIP) allogeneic peripheral blood stem cell transplant (PBSCT) yields exaggerated mixed chimerism and generates antitumor responses. Top panels show engraftment results from a representative patient transplanted without a preparative regimen. At day 14 post-transplant, both T cell and myeloid cell populations are overwhelmingly of host origin. However, donor elements increase rapidly after infusion of T-Rapa cells at day 14 post-transplant. Within the first 100 days post-transplant, during the state of mixed chimerism, the patient enters complete remission from diffuse large B-cell non-Hodgkin's lymphoma.

T-cell Immunotherapy and a Zero-Intensity Prep Future

At this point, most of our patients have advanced stages of hematologic malignancy that is refractory to conventional therapy and all have received transplants from HLA-matched siblings. Going forward, we envision that the zero-intensity prep transplant, by increasing the safety of the procedure, would allow transplantation to be used earlier in

disease treatment algorithms, for patients who do not have an HLA-matched sibling, and for patients of advanced age or organ impairment.

We are also continuing our laboratory research to discover new approaches to further improve the safety and efficacy of transplantation through the modulation of T-cell function. I am very fortunate to have an outstanding laboratory team and clinical research team in ETIB. We have also collaborated

with Scott Rowley, M.D., at the Hackensack University Medical Center in New Jersey to treat approximately 30 patients using our T-Rapa cells. This has served as a good proof-of-principle that we can manufacture T-Rapa cells at the NIH and then ship them out to other centers, thus expanding our work into the extramural community. As such, we are on our way to developing safer and more effective transplant approaches that can hopefully soon be implemented in standard practice.

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To learn more about Dr. Fowler's research, please visit his CCR website at <https://ccr.cancer.gov/daniel-h-fowler>.