CLINICAL TRIALS AND OBSERVATIONS

Phase 2 clinical trial of rapamycin-resistant donor CD4\(^+\) Th2/Th1 (T-Rapa) cells after low-intensity allogeneic hematopoietic cell transplantation


1Experimental Transplantation and Immunology Branch, National Cancer Institute, 2Biostatistics and Data Management, National Cancer Institute, and 3Department of Transfusion Medicine, National Institutes of Health, Bethesda, MD; 4The John Theurer Cancer Center, Hackensack University Medical Center, Hackensack, NJ; and 5University of Pennsylvania, Abramson Family Cancer Research Center, Philadelphia, PA

Key Points

- Donor T-Rapa cells were composed of Th1 and Th2 effectors with a reproducible gene expression profile.
- Preemptive T-Rapa donor lymphocyte infusion was safe and associated with donor engraftment without excessive GVHD.

Introduction

Allogeneic hematopoietic cell transplantation (HCT) using non-myeloablative host conditioning\(^1,2\) has reduced transplant-related mortality\(^3\) but is associated with increased tumor progression\(^4\) and graft rejection\(^5\) and remains limited by graft-versus-host disease (GVHD).\(^6\) Competing immune T-cell reactions underlie these clinical events. Donor T-cell–mediated GVHD and host T-cell–mediated rejection are reciprocally related,\(^7\) whereas donor T-cell–mediated graft-versus-tumor (GVT) effects and GVHD are intertwined.\(^8\) New approaches to modulate allogeneic T-cell immunity are therefore required. Imbalance between T helper 1 (Th1), T helper 2 (Th2), and other CD4\(^+\) T-cell subsets predisposes to human disease,\(^9\) including GVHD, which is primarily Th1 driven.\(^10\) As such, we hypothesized that allograft augmentation with T cells of mixed Th2 and Th1 phenotype may beneficially balance immunity after allogeneic HCT.

In murine models, we have evaluated the novel ex vivo application of rapamycin to control the Th2/Th1 balance posttransplant as an alternative to in vivo rapamycin drug therapy, which in various models has been found to prevent graft rejection and GVHD but abrogate antitumor effects through inhibition of Th1-type cells and preservation of Th2-type cells.\(^11,12\) Prevent GVHD through promotion of regulatory T (TReg) cells,\(^13\) or modulation of host antigen-presenting cell,\(^14\) and improve antiviral immunity mediated by CD8\(^+\) T cells.\(^15\) The ex vivo approach that we developed allows one to dissect these seemingly disparate potential in vivo drug effects on a purified T-cell subset under defined polarizing cytokine micro-environments. In our studies, we found that ex vivo rapamycin increased the capacity of interleukin (IL)-4 polarized donor Th2 cells to promote a balanced pattern of Th2/Th1 immune reconstitution for promotion of GVT effects and allograftment with reduced GVHD.\(^16,17\) Ex vivo rapamycin creates a state of T-cell starvation that induces autophagy,\(^20\) thereby resulting in an antiapoptotic T-cell phenotype that dictates persistent T-cell engraftment in


The data reported in this article have been deposited in the Gene Expression Omnibus database (accession number GSE34911).

The online version of this article contains a data supplement.

© 2013 by The American Society of Hematology
mouse-into-mouse\textsuperscript{18} or human-into-mouse\textsuperscript{21} transplantation models. Rapamycin-resistant Th2 cells inhibited GVHD by multiple mechanisms, including IL-4 and IL-10 secretion, consumption of IL-2 required for propagation of pathogenic effector T cells, and modulation of host antigen-presenting cell.\textsuperscript{17} Furthermore, delayed administration of rapamycin-resistant Th2 cells after an initial donor Th1-type response optimized the balance of GVT effects and GVHD,\textsuperscript{16} thereby indicating that a mixed pattern of Th2 and Th1 immune reconstitution was desirable in the setting of cancer therapy. And finally, rapamycin-resistant Th2 cells prevented graft rejection through host T-cell conversion to a Th2-type profile,\textsuperscript{19} thus illustrating that this novel donor T-cell population may have particular application in transplant settings associated with increased graft rejection, such as the use of low-intensity host conditioning.

Building on these data, we transitioned from a phase 1 clinical trial of IL-4 polarized donor CD4\textsuperscript{+} T cells not manufactured in rapamycin\textsuperscript{22} to the current trial that incorporated ex vivo rapamycin during IL-4 polarization to produce donor “T-Rapa” cells. To improve the safety of our transplantation method and to incorporate an engraftment end point into the clinical trial (conversion of mixed chimerism), we developed an outpatient treatment platform consisting of low-intensity host conditioning (75% reduction in chemotherapy intensity relative to our previous studies of reduced-intensity transplantation).\textsuperscript{22} And, in an attempt to tailor posttransplant immune suppression to favor the manufactured T-Rapa cells rather than the unmanipulated T cells contained in the T-cell–replete hematopoietic cell allograft, we administered double-agent GVHD prophylaxis (cyclosporine plus Sirolimus) in the early posttransplant period and subsequent single-agent cyclosporine prophylaxis after T-Rapa cell adoptive transfer at day 14 posttransplant. This latter aspect of the protocol design was informed by our observation that ex vivo manufactured rapamycin-resistant allogeneic murine T cells, in particular the Th1 subset, were susceptible to the in vivo immune suppressive effects of rapamycin drug therapy.\textsuperscript{23}

Methods

Clinical trial design, implementation, and end points

This phase 2 multi-institution protocol (Figure 1) was approved by the National Cancer Institute (NCI) and Hackensack University Medical Center (HUMC) institutional review boards and implemented according to an Investigational New Drug Application accepted by the Food and Drug Administration. Dates of transplant for this protocol ranged from December 13, 2005, to June 29, 2009; 35 patients were transplanted at the National Institutes of Health (NIH) Clinical Center, and 5 patients were transplanted at HUMC. Subjects provided written informed consent in accordance with the Declaration of Helsinki; enrollment was based on age (between 19 and 75), availability of 6/6 HLA-matched sibling donor, organ function, and hematologic malignancy diagnosis (acute and chronic myelogenous or lymphocytic leukemia, myelodysplastic syndrome, multiple myeloma, and Hodgkin and non-Hodgkin lymphoma [NHL]). Patients were eligible independent of their response to prior chemotherapy regimens; acute leukemia patients were eligible if blast frequency was <10%. Patients who had previously received autologous transplantation were eligible. Prior to transplant, patients received 1 to 3 cycles of etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, fludarabine, rituximab (EPOCH-FR) chemotherapy\textsuperscript{24} to achieve a predetermined level of host immune depletion (CD4 count <200/\mu L), to assess chemotherapy sensitivity, and to attempt to reduce disease burden. Fludarabine (30 mg/m\textsuperscript{2} per day) and cyclophosphamide (300 mg/m\textsuperscript{2} per day) were administered on days −6 to −3 prior to the granulocyte-colony-stimulating factor mobilized peripheral blood allograft; this dose of cyclophosphamide was 75% reduced relative to the regimen we previously evaluated.\textsuperscript{22} GVHD prophylaxis was cyclosporine (200-250 mg/m\textsuperscript{2} per day; day −1 to +100, tapered at day +100 or earlier for progressive disease) and Sirolimus (3-12 mg/L; day −2 to +14). T-Rapa cells were infused at day +14 post-HCT (2.5 × 10\textsuperscript{7} cells/kg). The composite primary study objectives were to determine the safety and feasibility of preemptive T-Rapa cell donor lymphocyte infusion (DLI) and to characterize alloengraftment, antitumor effects, GVHD effects, and the Th2/Th1 balance post-HCT. The sample size of n = 40 was selected to give a reasonable estimation of the rate of acute GVHD: with this sample size, the maximum confidence interval width for the fraction of patients with grade 2 to 4 acute GVHD will not exceed ±15%. Organ toxicity was evaluated by NCI Common Toxicity Criteria (version 2.0); GVHD was evaluated using acute\textsuperscript{25} and chronic\textsuperscript{26} grading. Disease responses were evaluated by computed tomographic measurements and marrow examinations, with lymphoma responses measured by standard criteria\textsuperscript{27}; progressive disease was treated with chemotherapy and/or unmanipulated DLI. Alloengraftment was monitored using variable N-terminal repeat polymerase chain reaction assays on total, CD3-enriched, or CD15-enriched cells.

T-Rapa cell manufacturing

Donor lymphocytes were collected by a 10-L steady-state apheresis performed prior to stem cell mobilization. CD4 cells were positively selected (CliniMACS device; Miltenyi) and costimulated (tosylated magnetic beads [Dynal] conjugated with anti-CD3 [OKT3; Ortho] and anti-CD28 9.3 antibodies [3.1 bead/cell ratio]). Purified CD4\textsuperscript{+} T cells (900 × 10\textsuperscript{6} cells at culture initiation) were propagated in polyolefin bags (Baxter) using X-VIVO 20 media (Lonza), 5% donor plasma, recombinant human IL-4 (1000 IU/mL; Schering), recombinant human IL-2 (20 IU/mL; Chiron), and Sirolimus oral solution (1 μM; Wyeth); cytokine- and rapamycin-replete media were added every 2 to 3 days to maintain cell concentrations at <1 × 10\textsuperscript{6} cells per mL. After 12 days, beads were removed; T cells were washed to remove cytokines and Sirolimus, and then cryopreserved. All infused T-Rapa products met release criteria, which included the following: CD4 cell purity >70% (median CD4 purity was 99%), CD8 cell content <5% (median CD8 content was <0.1%), viability >70% (median viability was 95%), absence of bacterial and fungal growth, absence of endotoxin content by limulus assay, negative mycoplasma test, and <100 magnetic beads per 3 × 10\textsuperscript{6} cells. T-Rapa products were manufactured centrally (NIH Clinical Center Department of Transfusion Medicine).

Gene expression profiling

Total RNA was extracted (mRNA Easy Kits; Qiagen) and quantified (ND-1000 Spectrophotometer; NanoDrop), and quality was verified (2100
late acute forms, including liver transaminase elevation) and the cumulative incidence of acute GHVD (combining both classical acute and multiple comparison adjustment, as this is an exploratory analysis. The GATA-3:T-bet ratio was tested to determine if it was different from one distribution. Differences were tested to determine if they differed from zero. Changes in laboratory parameters between stated time points were analyzed by gene expression microarray. The heat map illustrates that genes were consistently differentially expressed, with 18.1% of genes (6147 of 34,051) being differentially expressed in T-Rapa products relative to input CD4+ cells (P < .001); the number of upregulated genes in T-Rapa cells was relatively equal to the number of downregulated genes.

Results

Th2/Th1 phenotype of T-Rapa cells

During the 12-day culture interval, median CD4+ T-cell number was 6.4-fold increased relative to day 0 culture input values (range, 4.5- to 19.1-fold increased). Relative to culture input CD4+ T cells, T-Rapa cells differentially expressed 18.1% (6147 of 34,051) of messenger RNA species, with similar numbers of genes upregulated (3185) or downregulated (2962) (illustrated in heat map, Figure 2). By gene ontology analysis, the 5 gene families most significantly upregulated in T-Rapa cells relative to day 0 culture input CD4+ T cells were cell cycle, DNA metabolism, stress response, glucose catabolism, and oxidative reduction; representative gene members in these families were upregulated 23- to 92-fold above values in the day 0 culture input cells (supplemental Table 1; see the Blood Web site). On the other hand, the 5 gene families most significantly downregulated in T-Rapa cells relative to the day 0 culture input CD4+ T cells were apoptosis, transcription, inflammation, cytokine production, and immune response; representative gene members in these families were downregulated 45- to 341-fold below values in the day 0 culture input cells (supplemental Table 1). In spite of this evidence that cytokine and immune response genes were dramatically downregulated in T-Rapa cell products, the limited number of Th2 and Th1 genes were significantly upregulated in T-Rapa cells: most notably, the Th2 cytokine IL-13 and the Th1 cytokine IL-12Rβ2 were upregulated 21.5- and 18.4-fold, respectively. The gene expression pattern of T-Rapa cells was remarkably reproducible: in an evaluation of n = 21 T-Rapa cell clinical products, the interproduct gene expression variability by intraclass correlation coefficient analysis was 0.93.

T-Rapa products had minimal contamination with CD4+ Foxp3+ cells (<1%) and preferentially expressed GATA-3 (median CD4+ GATA-3+, 11.8%) relative to T-bet (median CD4+ T-bet+, 5.1%); the median intraproduct GATA-3/T-bet ratio was 2.1 (Figure 3A). T-Rapa products secreted low levels of Th2 cytokines, which increased after extended culture without polarizing cytokines and rapamycin (Figure 3B; median values; day 12 to day 18 of culture: IL-4 [1-41 pg/mL], IL-5 [21-363 pg/mL], IL-10 [10-159 pg/mL], and IL-13 [24-725 pg/mL]). T-Rapa products secreted low levels of IFN-γ and TNF-α, which increased after extended culture (IFN-γ [10-418 pg/mL]; TNF-α [1-41 pg/mL]). T-Rapa cell IL-2 secretion actually decreased after extended culture, whereas IL-17 secretion...
increased from undetectable levels to <10 pg/mL after extended culture.

Conversion of mixed chimerism after T-Rapa infusion

Although each patient engrafted with donor cells, the low-intensity conditioning yielded mixed donor/host T lymphoid chimerism at day 14 post-HCT (Figure 4A; median donor chimerism, 61%). After T-Rapa cell infusion at day 14 post-HCT, median values increased to 89% and 94% at days 28 and 100 post-HCT, respectively. Median estimated absolute number of donor CD4\(^+\) T cells increased from 89/μL (day 14) to 198/μL and 250/μL (days 28 and 100 post-HCT, respectively). Reciprocally, median estimated numbers of host CD4\(^+\) T cells decreased from 67/μL (day 14) to 24/μL and 8/μL (days 28 and 100 post-HCT, respectively). Median estimated numbers of donor CD8\(^+\) T cells did not increase significantly after T-Rapa cell infusion (values at days 14, 28, and 100 post-HCT: 89, 92, and 158/μL, respectively); in contrast, median estimated numbers of host CD8\(^+\) T cells decreased from 51/μL (day 14) to 13/μL and 6/μL (days 28 and 100 post-HCT, respectively). As detailed in supplemental Table 2, the T-Rapa cell products were composed primarily of central memory CD4\(^+\) T cells (mean value, 66.4%). Immune reconstitution post-HCT was characterized by relatively balanced numbers of naive, central memory, and effector memory cells in the CD4 compartment through day 180 post-HCT; by comparison, CD8\(^+\) cell immune reconstitution was biased toward the effector memory subsets, including both CD45RA\(^-\) and CD45RA\(^+\) populations (supplemental Table 2). At 1 year post-HCT, median values for CD4, CD8, and B-cell counts were 423, 297, and 151/μL, respectively (n = 22 evaluated); at 1 year post-HCT, median values for serum immunoglobulins IgG, IgM, and IgA were 502, 46, and 40 mg/dL, respectively (n = 14 evaluated). Donor myeloid chimerism was mixed at day 14 post-HCT (Figure 4B; median, 37%); after T-Rapa cell infusion, median values increased to 81% and 99% at days 28 and 100 post-HCT, respectively.

T-Rapa recipients express a balanced Th2/Th1 profile

Low frequencies of Th2 and Th1 cells were detected at day 14 post-HCT just prior to T-Rapa cell infusion (Figure 5A, median values; CD4\(^+\)GATA-3\(^+\), 0.6%; CD4\(^+\)T-bet\(^+\), 1.1%). After T-Rapa cell infusion, median frequencies of CD4\(^+\)GATA-3\(^+\) cells increased to 20.2% and 13.9% at 1 and 3 months post-HCT, respectively; median frequencies of CD4\(^+\)T-bet\(^+\) cells modestly increased to 4.0% and 1.8% (1 and 3 months post-HCT). The median intrapatient GATA-3/T-bet ratio was 0.6 at day 14 post-HCT and increased to 5.4 and 8.1 at 1 and 3 months post-HCT. Median frequencies of CD4\(^+\)FoxP3\(^+\) cells were low at day 14 post-HCT (0.3%) and increased at 1 and 3 months post-HCT (2.5% and 2.4%, respectively).

Post-HCT T cells secreted low levels of Th2 cytokines at days 7 and 14 post-HCT (Figure 5B; IL-4, IL-5, IL-10, and IL-13 values were typically <10 pg/mL); after T-Rapa cell infusion, these values were generally increased at 1 and 3 months post-HCT. By comparison, post-HCT T cells secreted higher levels of Th1 cytokines at days 7 and 14 post-HCT (IL-2, INF-γ, and TNF-α levels ranging from 100 to 10,000 pg/mL); after T-Rapa cell infusion, these values were either stable or increased at 1 and 3 months post-HCT. Post-HCT T-cell secretion of IL-17 was not detected prior to T-Rapa infusion and modestly increased at 1 and 3 months post-HCT.
Rapa infusion (at day 14 post-HCT), low frequencies of post-HCT CD4⁺ and CD8⁺ T cells secreted cytokines by cytokine capture flow cytometry analysis; by comparison, at day 28 post-HCT, increased frequencies of both CD4⁺ and CD8⁺ T cells secreted the type I cytokines IL-2 and IFN-γ and the type II cytokines IL-4 and IL-10 (supplemental Figure 1).

Figure 4. T-Rapa cell infusion results in predominately donor CD4⁺ T-cell reconstitution. (A) Percent donor T lymphoid chimerism for each patient at days 14, 28, and 100 after allogeneic hematopoietic stem cell transplant (SCT) (top; ***, day 28 > day 14 and day 100 > day 28; P < .0001). Post-SCT numbers of donor vs host CD4⁺ or CD8⁺ T cells were estimated by multiplying CD4 and CD8 cell absolute numbers by percent CD3 chimerism values. The figure shows median estimated values for absolute numbers of donor and host CD4⁺ T cells (left) and CD8⁺ T cells (right) at days 14, 28, and 100 post-SCT (comparisons are day 28 vs day 14 and day 100 vs day 28; ***P < .001; **P < .01; *P < .05; between n = 23 and n = 33 evaluated for each paired analysis). (B) Percent donor myeloid chimerism for each patient at days 14, 28, and 100 post-SCT (***, day 28 > day 14 and day 100 > day 28; P < .0001). NS, not significant.
To evaluate antigen-specific T-cell responses post-HCT, transplant recipients were evaluated for immune responses against cytomegalovirus (CMV). In 3 of the 4 HLA-A02\textsuperscript{1} recipients who developed CMV viremia post-HCT, there was an increased frequency of CMV-specific Tc cells by flow cytometry analysis (supplemental Figure 2); by comparison, each of the 6 HLA-A02\textsuperscript{1} recipients who did not develop CMV viremia post-HCT had frequencies of CMV-specific T cells near background levels. In addition, in 13 transplant cases where the donor or host (or both) was CMV seropositive, we observed increased secretion of IFN-\(\gamma\) and IL-4 in response to overlapping CMV peptides at day 60 post-HCT (supplemental Table 3). Consistent with their minimally differentiated effector state, the T-Rapa cell products secreted minimal cytokines in response to either overlapping CMV peptides or a superantigen-like positive control stimulation. Finally, we evaluated the T-cell receptor V-\(\beta\) repertoire of both the T-Rapa cell products and day 60 post-HCT CD4\textsuperscript{+} T cells: the T-Rapa products had a diverse T-cell repertoire similar to normal donor CD4 cells, whereas the post-HCT CD4\textsuperscript{+} T cells tended to have a more skewed T-cell receptor repertoire (supplemental Figure 3).

Patient characteristics and post-HCT outcome
Median patient age was 51 years (range, 23-69; 17 females, 23 males; Tables 1 and 2). To assess risk of disease progression after reduced-intensity HCT,\textsuperscript{4} patients were classified as having low-risk (\(n = 9, 22.5\%\)), standard-risk (\(n = 7, 17.5\%\)), or high-risk (\(n = 24, 60\%\)) diagnoses. Median number of prior regimens was 3 (range, 1-5).
incidence probability of acute GVHD of 20% at day 100 post-HCT (in some patients), there was a cumulative GVHD (including liver transaminase elevation, which occurred prior to day 100 post-HCT in 37.8%), consisted primarily of liver transaminitis without disease progression. Late acute GVHD, which occurred in 14 of 37 patients (39.4%), was observed in 4 of 40 patients (10%; each case steroid responsive); late, nonrelapse mortality was due to postsurgical pulmonary embolus (10%). There were no deaths directly related to transplantation. There were no infectious toxicities or serious adverse events attributable to T-Rapa cell therapy. There were no cases of veno-occlusive disease, engraftment syndrome, or transplant-associated microangiopathy. CMV viremia by DNA–polymerase chain reaction test was detected in 3 of 21 (14.3%) CMV-negative recipients and 6 of 19 (31.6%) CMV-positive recipients. One of 40 recipients (2.5%) developed CMV infection (gastroitis); 1 patient developed disseminated adenoviral infection after intensive therapy of progressive acute myelogenous leukemia (AML). Sixteen of 40 patients (40%) did not develop any form of GVHD. Twenty-one (52.5%) had classical chronic GVHD, with global severity scores of mild (n = 9), moderate (n = 6), or severe (n = 2); the median number of organs involved was 2 (range, 1–4), with tissue distribution of skin (n = 15), oral (n = 10), ocular (n = 7), vulvo-vaginal (n = 3), and lung (n = 2); the cumulative incidence probability of chronic GVHD at 2 years post-HCT was 42.5%. Ten of 40 patients (25%) developed overlap GVHD (classical chronic in combination with classical or late acute); 16 of 40 patients (40%) did not develop any form of GVHD. Each patient with low-risk diagnoses achieved complete remission, although 1 patient died of isolated central nervous system disease; 6 of 9 low-risk diagnosis recipients (66.6%) are in sustained complete remission (median follow-up, 1647 days post-HCT; range, 1312–2566). Three of 7 patients (42.9%) with standard-risk diagnoses are in sustained complete remission at days 1326, 1356, and 2468 posttransplant. Nine of 24 recipients (37.5%) with high-risk diagnoses are in sustained complete remission (median follow-up, 1402 days post-HCT; range, 1285–1669). Posttransplant chemotherapy and/or unmanipulated DLI contributed to sustained complete remission in 5 of 18 (27.8%) patients; a total of 22 patients received chemotherapy and/or DLI for management of progressive disease. The cumulative probability of disease progression was 32.5% at 6 months post-HCT, 50% at 12 months post-HCT, 57.5% at 24 months post-HCT, and 57.5% at 36 months post-HCT. In total, 18 of 40 patients (45%) had classical chronic GVHD, with global severity scores of mild (n = 9), moderate (n = 6), or severe (n = 2); the median number of organs involved was 2 (range, 1–4), with tissue distribution of skin (n = 15), oral (n = 10), ocular (n = 7), vulvo-vaginal (n = 3), and lung (n = 2); the cumulative incidence probability of chronic GVHD at 2 years post-HCT was 42.5%. Ten of 40 patients (25%) developed overlap GVHD (classical chronic in combination with classical or late acute); 16 of 40 patients (40%) did not develop any form of GVHD. Each patient with low-risk diagnoses achieved complete remission, although 1 patient died of isolated central nervous system disease; 6 of 9 low-risk diagnosis recipients (66.6%) are in sustained complete remission (median follow-up, 1647 days post-HCT; range, 1312–2566). Three of 7 patients (42.9%) with standard-risk diagnoses are in sustained complete remission at days 1326, 1356, and 2468 posttransplant. Nine of 24 recipients (37.5%) with high-risk diagnoses are in sustained complete remission (median follow-up, 1402 days post-HCT; range, 1285–1669). Posttransplant chemotherapy and/or unmanipulated DLI contributed to sustained complete remission in 5 of 18 (27.8%) patients; a total of 22 patients received chemotherapy and/or DLI for management of progressive disease. The cumulative probability of disease progression was 32.5% at 6 months post-HCT, 50% at 12 months post-HCT, 57.5% at 24 months post-HCT, and 57.5% at 36 months post-HCT. In total, 18 of 40 patients (45%) remain in sustained complete remission (range of follow-up: 42-84 months).
favor donor immunity rather than residual host immunity and (2) balance donor T-cell effects once they predominate. As a result, transplant outcome can be variably limited by graft rejection, persistent mixed chimerism with associated reduced GVT effects, and GVHD. These obstacles would be anticipated to be particularly relevant with the low intensity of chemotherapy that we used in this study, which is 75% lower in alkylator dose than the reduced-intensity transplant: Res, response to EPOCH-F(R): PR, partial response; CR, complete response; SD, stable disease; hCR, hematologic CR; and PD, progressive disease.

Ex vivo application of rapamycin to the T-cell manufacturing process yielded CD4+ effector T cells that expressed a mixed Th2/Th1 phenotype. Microarray analysis of such T-Rapa cells revealed a broad-based gene expression identity that was remarkably reproducible during clinical trial implementation; such reproducibility was achieved with a manufacturing method that incorporated clinical product cryopreservation and shipment to a multicenter site, thereby demonstrating that subsequent, definitive trials using T-Rapa cells will be feasible. Further studies will be required to evaluate if this gene expression identity dictates functional characteristics of the T-Rapa cell product; in subsequent clinical trials, it may be advantageous to use the gene expression identity as a release criterion for the T-Rapa cell product. Consistent with our results in experimental models,21 the clinical T-Rapa cell products had downregulation of apoptosis genes and were minimally differentiated on the basis of global downregulation of inflammation, cytokine production, and immune response genes. T-Rapa products were minimally contaminated with FoxP3+ cells and thus distinct from TREG-enriched population36 observed in other experimental systems likely due to 2 aspects of the current platform: (1) a 75% reduction in the concentration of rapamycin (thus demonstrating their effector potential) and (2) an ex vivo cytokine addition and in the absence of rapamycin (thus illustrating their limited polarization plasticity34). The T-Rapa cell products were therefore composed of minimally differentiated Th2/Th1 effector T cells rather than an anergic T-cell population35 or a T REG-enriched population36 observed in other experimental systems that also evaluated ex vivo rapamycin.

The clinical treatment platform we evaluated can be considered a low-intensity regimen as it can be administered in the outpatient setting and universally resulted in mixed donor/host T-cell chimerism, with a median of only 61% donor chimerism at day 14 post-HCT. Clinical trials are ongoing, in days post-HCT (+1) Death, day post-HCT; cause due to PD.

| Risk level§ | UPN | Age/sex | Dx | # | Res | Res | NED | s | g | l | Gr | Late acute | Chronic | Tumor | Survival | Death |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 4 | 4/F | AML | 4 | S | HCR | N | 0 | 0 | 0 | 0 | tr | Y (o, s, v) | PD | N | 748 (PD) |
| 4 | 47/M | AML | 2 | S | PD | N | 2 | 0 | 0 | 1 | (n.e.) | (n.e.) | PD | N | 119 (PD) |
| 5 | 6/M | DLC | 5 | R | PD | N | 1 | 0 | 1 | 0 | tr | Y (o, s, v, e) | PD | N | 91 (PD) |
| 6 | 24/M | AML | 3 | S | SD | N | 0 | 0 | 0 | 0 | 0 | N | PD | N | 94 (PD) |
| 7 | 32/M | HD | 5 | R | SD | N | 0 | 0 | 0 | 0 | 0 | N | PD | N | 1004 (PD) |
| 8 | 53/F | A-TCL | 4 | 1 | R | PD | N | 0 | 0 | 0 | 0 | tr | Y (o, s, v, e) | PD | N | 1004 (PD) |
| 9 | 30/M | DLC-EVB | 2 | 1 | R | SD | N | 0 | 0 | 0 | 0 | 0 | N | PD | N | 328 (PD) |
| 10 | 59/M | AML | 3 | S | hCR | N | 0 | 0 | 0 | 0 | 0 | N | PD | N | 386 (PD) |
| 11 | 42/F | HD | 3 | 1 | R | SD | N | 0 | 0 | 0 | 0 | tr | Y (s) | CR | + 1563 | n.a. |
| 12 | 41/M | DLC | 2 | 1 | R | SD | N | 0 | 0 | 0 | 0 | tr | Y (s) | CR | + 1514 | n.a. |
| 13 | 60/F | DLC | 5 | R | PD | N | 0 | 0 | 0 | 1 | tr, g, s | N | PD | N | 192 (PD) |
| 14 | 43/M | DLC | 5 | R | PD | N | 0 | 0 | 0 | 0 | N | PD | N | 170 (PD) |
| 15 | 33/M | NHL-GZ | 3 | R | PD | N | 0 | 0 | 0 | 0 | 0 | N | PD | N | 268 (PD) |
| 16 | 23/M | HD | 4 | R | SD | N | 0 | 0 | 0 | 0 | 0 | Y (s) | PD | N | 1024 (PD) |
| 17 | 39/F | CML | 4 | R | hCR | N | 0 | 0 | 0 | 0 | tr | N | mCR | + 1457 | n.a. |
| 18 | 66/M | DLC | 3 | R | SD | N | 0 | 0 | 0 | 0 | 0 | N | PD | N | 92 (PD) |
| 19 | 47/M | A-TCL | 5 | S | CR | Y | 1 | 0 | 0 | 1 | 0 | Y (s, l) | CR | + 1402 | n.a. |
| 20 | 27/F | HD | 2 | S | SD | N | 0 | 0 | 0 | 0 | g | Y (s, o) | CR | + 1355 | n.a. |
| 21 | 45/M | NHL-GZ | 4 | S | CR | Y | 0 | 0 | 0 | 0 | 0 | N | CR | + 1325 | n.a. |
| 22 | 49/F | DLC | 2 | S | PR | N | 0 | 0 | 0 | 0 | 0 | N | PD | N | 234 (PD) |
| 23 | 52/M | DLC | 4 | S | CR | Y | 0 | 0 | 0 | 0 | 0 | N | CR | + 1299 | n.a. |
| 24 | 58/M | NHL-pDC | 1 | 1 | R | CR | Y | 0 | 0 | 0 | 0 | 0 | Y (o, s, e) | PD | N | 525 (PD) |
| 25 | 55/F | DLC | 3 | S | CR | Y | 0 | 0 | 0 | 0 | 0 | N | CR | + 1285 | n.a. |
| 26 | 49/M | CML | 4 | S | CR | Y | 0 | 0 | 0 | 0 | 0 | N | SD | N | 1272 (PD) |

A-TCL, anaplastic T-cell NHL; CLL, chronic lymphocytic leukemia; DLC, diffuse large cell; DLC-EVB, Epstein-Barr virus–driven DCL; DLC (trCLL), CLL transformed to DLC; DLC (trFL), transformed follicular NHL to DLC; Dx, diagnoses; F, female; HD, Hodgkin disease; M, male; N, no; n.a., not applicable; n.e., not evaluable; NED, no evidence of disease status; NHL-GZ, gray-zone NHL; NHL-pDC, plasmablastic dendritic cell NHL; UPN, unique patient number; Y, yes.

*# number of prior regimens. Res, response to last prior regimen; S, sensitive; R, refractory; 1 R, primary refractory. Pre-HCT, indicates disease status at time of low-intensity transplant; Res, response to EPOCH-F(R): PR, partial response; CR, complete response; SD, stable disease; hCR, hematologic CR; and PD, progressive disease.

†Acute GVHD score of skin (s), gut (g), and liver (l); Gr, overall grade (0-4). Late acute GVHD, manifested as elevated liver transaminase levels (tr), skin (s), or gut involvement (g). Chronic GVHD sites of involvement: oral (o), skin (s), eye (e), vaginal (v), and lung (l).

‡Overall outcome for last tumor staging: PD, progressive disease; CR, complete remission; mCR, molecular CR; and SD, stable disease. Ongoing survival, in days post-HCT (+1). Death, day post-HCT; cause due to PD.

§Risk of progressive disease post-HCT: level 3, high risk.
in cyclophosphamide intensity during host conditioning; and (2) a requirement that the host CD4 count be <200 cells per μL prior to transplantation rather than the more stringent value that we previously used (<50 cells per μL). Preemptive DLI with T-Rapa cells in this setting was associated with the conversion of mixed chimerism toward predominant donor chimerism; no graft rejection occurred in T-Rapa recipients, which included many patients with <50% donor T-cell chimerism at day 14 post-HCT (n = 14). Previously, this level of mixed chimerism at the day 14 post-HCT time point was associated with increased graft rejection.3 It is difficult to compare engraftment results across clinical trials because of differences in patient selection and pretransplant treatment history; nonetheless, the current results indicate that the T-Rapa cell DLI did not promote donor-host tolerance or impair donor T-cell responses posttransplant. Although the T-Rapa cell DLI likely contributed to the rather rapid increase in donor chimerism, definitive clinical trials evaluating this transplant regimen without a DLI or with a control DLI consisting of unmanipulated donor T cells would be required to better address this question. Of note, previous investigations have evaluated CD4-enriched DLI, including in the following settings: for decreasing chimerism after T-cell–depleted transplantation38; for treatment of posttransplant relapse39; or, when used in combination with IL-2 therapy, for the treatment of chronic GVHD.40

In contrast to autologous adoptive T-cell therapy approaches that maximize host immune depletion through high-dose conditioning and immediate T-cell transfer,41 T-Rapa cells were administered remote from conditioning, in a state of host immunity that was relatively T-cell replete, and during calcineurin inhibitor therapy. Nonetheless, the CD4-purified donor T-Rapa cells appeared to break immune tolerance and predominate in vivo, as indicated by the following: (1) a rapid post-HCT increase in donor CD4+ T cells; (2) restricted expansion of donor CD8+ T cells; (3) concomitant reductions in host CD4+ and CD8+ T cells; (4) balanced CD4+ Th2/Th1 immune reconstitution with modest reconstitution of TREG cells; and (5) conversion of early mixed myeloid chimerism through an apparent graft-versus-myeloid lineage effect. The donor CD4 predominance observed after T-Rapa cell DLI suggests that the infused product expanded in vivo; however, the T-Rapa cells were not labeled for cell tracking, and as such, it is possible that CD4 cells contained in the mobilized allograft may have also contributed to the observed pattern of immune reconstitution. Indeed, our finding that both CD4+ and CD8+ T cells had balanced secretion of Th1 and Th2 cytokines post-HCT suggests that the CD4-purified T-Rapa cell DLI may have resulted in the in vivo modulation of T cells emanating from the mobilized allograft. Nonetheless, the preferential expansion of donor CD4 cells rather than donor CD8 cells after T-Rapa cell DLI stands in marked contrast to other clinical results in myeloablative or nonmyeloablative transplantation, in which a predominant CD8 cell reconstitution has been observed.42 Taken together, these results suggest that T-Rapa cells may represent a particularly potent effector T-cell population, as an array of biological effects were observed after adoptive transfer in the face of systemic immune suppression.

Only 4 of 40 patients (10%) developed classical acute grade 2 to 4 GVHD through day 100 post-HCT, which compares favorably with our first-generation clinical trial of ex vivo expanded CD4 cells where we observed a rate of 64% (18 of 28 cases).22 The low rate of classical acute GVHD that we observed is similar to results obtained using host conditioning with total lymphoid irradiation,43 which also promoted Th2 cytokines. We did observe a significant incidence of late acute GVHD, in particular liver transaminase elevation (which occurred in several patients before day 100 post-HCT), which resulted in a cumulative overall incidence probability of all forms of acute GVHD of 20% and 40% at days 100 and 180 post-HCT, respectively. Several factors may have influenced the incidence, type, and severity of acute GVHD in our study, including balanced CD4+ Th2/Th1 immune reconstitution posttransplant with restricted donor CD8+ T-cell expansion posttransplant, low-level persistent mixed T-cell chimerism,44 low-intensity conditioning,35 and Sirolimus prophylaxis.46 With respect to this latter point, in a recent study performed by our collaborators,47 the rate of acute grade 2 to 4 GVHD in recipients of a matched related donor transplant after reduced-intensity conditioning and GVHD prophylaxis consisting of cyclosporine plus a short course of peritransplant Sirolimus without T-Rapa cell DLI was 27% (6 of 22 cases). Classical chronic GVHD was primarily of mild-to-moderate global severity. A substantial proportion of patients did not develop acute or chronic GVHD. As such, the overall GVHD profile of the current platform was not excessive, particularly considering that ex vivo activated donor T cells were administered in the context of a T-cell–replete peripheral blood allograft.

This study provided a rigorous clinical test for immune GVT effects because of the low intensity of host conditioning and because of the patient population, which had relatively low percentages of patients having a low-risk malignancy diagnosis (22.5%), receiving transplantation in complete remission (18%), and having chemotherapy-sensitive disease (50%). By comparison, previous studies using other low-intensity conditioning methods had higher frequencies of patients transplanted in remission and having low-risk diagnoses.4,48 In our trial, sustained complete remissions were achieved in some patients with primary refractory disease and diagnoses such as refractory chronic myelogenous leukemia (CML) that are difficult to eradicate with reduced-intensity transplantation.49 As such, the current transplant platform appears to be suitable in terms of antitumor potency for the majority of patients typically considered for reduced-intensity transplantation. However, T-Rapa cell infusion did not promote sufficient GVT effects in patients with refractory diffuse large cell NHL or multiply relapsed AML; ongoing efforts seek to overcome this GVT limitation through infusion of T-Rapa cells with an increased Th1 phenotype21 or enhanced tumor specificity through incorporation of chimeric antigen receptors.50

In summary, in this first human clinical trial of ex vivo manufactured allogeneic T-Rapa cells, we have demonstrated that the combined use of ex vivo and in vivo Sirolimus can be combined to provide a new platform for the safe implementation of low-intensity allogeneic HCT. These phase 2 clinical trial results suggest that the CD4-purified T-Rapa cells mediated distinct effects in vivo when administered as a preemptive DLI, in particular the rapid conversion of mixed chimerism toward full donor chimerism that was predominated by CD4+ T cells of a balanced Th2/Th1 cytokine phenotype. Preemptive T-Rapa cell infusion after low-intensity allogeneic HCT therefore represents a suitable platform for further studies. Our demonstration that the T-Rapa cell product can be safely administered in a multicenter manner indicates that it will be feasible to perform subsequent randomized studies comparing T-Rapa cell–based therapy to other types of DLI or other transplant regimens.

Acknowledgments

The authors thank numerous individuals who made outstanding contributions to this work, including Bazetta Blacklock-Schuver and Sheila Phang for their creative and efficient efforts in patient
recruitment and care coordination; Roger Kurlander, NIH Department of Laboratory Medicine, for his collaboration and professionalism in chimerism analysis; Maryalice Stetler-Stevenson and Constance Yuan for their care in providing clinical flow cytometry analyses; Jeanne Odom, Paula Layton, and Brenna Hansen for excellence in research nursing; Vicki Fellowes, for her irreplaceable efforts toward the development and implementation of T-Rapa cell manufacturing; Xiao-Yi Yan, Sarfraz Memon, Shoba Amarnath, Tania Felizardo, Jason Foley, and Yelena Kogan for their efforts with respect to laboratory studies and immunology end points; Elizabeth J. Read, for her involvement in cell manufacturing at the NIH during initial aspects of study implementation; Daniele Avila, Amanda Urban, Jennifer Mann, and Tiffani Taylor for their excellence and dedication in the care of protocol patients; Suzanne Murphy for expert assistance with the protocol and Investigational New Drug Application; the NIH Clinical Center and Department of Nursing; the Medical Oncology Fellows at the NCI; and numerous individuals at HUMC, including Tatyana Feldman, Anthony Mato, Carol Carini, and Andrea Ortega.

This work was supported by the Intramural Research Program, NCI Center for Cancer Research; and was also supported by the NCI Center for Treatment and Evaluation Program, in particular Dr Howard Streicher, for provision of IL-4.

Authorship

Contribution: D.H.F. designed the trial, provided patient care, performed data interpretation, and wrote the manuscript; M.E. M. performed laboratory end point experiments and data interpretation and assisted in writing manuscript; S.M.S. performed data analysis and interpretation; D.C.H. provided patient care and performed data interpretation; D.S. and H.M.K. provided clinical product manufacturing and data interpretation; F.T.H. performed laboratory end point experiments and data analysis and assisted in writing the manuscript; L.C. and M.S. performed laboratory end point experiments; S.F.L. participated in protocol design and transfusion medicine support; J.M. provided patient care and participated in protocol design; J.C. G.-B. and C.S. provided patient care and performed data analysis; N.M.H. and D.D.H. provided patient care; S.Z.P. provided patient care and performed data analysis; S.R., A.G., and M.D. participated in protocol design and provided patient care; R.K. participated in protocol design; A.P. provided patient care and research support; B.L.L. and C.H.J. participated in protocol design and manufacturing of clinical cellular product; R.E.G. participated in protocol design, provided patient care, and assisted in writing the manuscript; and M.R.B. participated in protocol design, provided patient care, performed data analysis, and assisted in writing the manuscript.

Conflict-of-interest disclosure: D.H.F., R.E.G., B.L.L., and C.H.J. are listed as coinventors on a related patent, Rapamycin-Resistant T Cells and Therapeutic Uses Thereof (US Patent 7,718,196; May 18, 2010). The remaining authors declare no competing financial interests.

Correspondence: Daniel H. Fowler, 10 Center Dr, Building 10, CRC, 3-3330, Bethesda, MD 20892; e-mail: dhfowler@helix.nih.gov.

References

17. Mariotti J, Fowler DH. Th2 cell therapy of established acute graft-versus-host disease requires IL-4 and IL-10 and is abrogated by IL-2 or host-type antigen-presenting cells. Biol Blood Marrow Transplant. 2008;14(9):959-972.


Phase 2 clinical trial of rapamycin-resistant donor CD4⁺ Th2/Th1 (T-Rapa) cells after low-intensity allogeneic hematopoietic cell transplantation