Brief report

Costimulated tumor-infiltrating lymphocytes are a feasible and safe alternative donor cell therapy for relapse after allogeneic stem cell transplantation

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Donor lymphocyte infusion (DLI), a standard relapse treatment after allogeneic stem cell transplantation (AlloSCT), has limited efficacy and often triggers GVHD. We hypothesized that after AlloSCT tumor-infiltrating donor lymphocytes could be costimulated ex vivo to preferentially activate/expand antitumor effectors. We tested the feasibility and safety of costimulated, tumor-derived donor lymphocyte (TDL) infusion in a phase 1 trial. Tumor was resected from 8 patients with B-cell malignancy progression post-AlloSCT; tumor cell suspensions were costimulated with anti-CD3/anti-CD28 Ab-coated magnetic beads and cultured to generate TDL products for each patient. Costimulation yielded increased proportions of T-bet8FoxP3– type 1 effector donor T cells. A median of 2.04 × 107 TDL/kg was infused; TDLs were well tolerated, notably without GVHD. Two transient positron emission tomography (PET) responses and 2 mixed responses were observed in these refractory tumors. TDL are a feasible, tolerable, and novel donor cell therapy alternative for relapse after AlloSCT. This trial is registered at clinicaltrials.gov as no. NCT00445666. (Blood. 2012;119(12):2956-2959)

Introduction

Donor lymphocyte infusion (DLI) is standard relapse treatment after allogeneic hematopoietic stem cell transplantation (AlloSCT), with durable responses reported in relapse of chronic hematologic malignancies, but is not available for cord blood or many unrelated donor recipients. DLI response rates vary but, except for early relapse of chronic myelogenous leukemia, are generally < 50%.1-3 Responses are strongly associated with GVHD, affecting up to 60%, with up to 11% treatment-related mortality.4 We hypothesized that relapsed tumor post-AlloSCT would be infiltrated with tumor-specific donor lymphocytes that might be primed but inadequately costimulated for tumor cell killing. Their costimulation and expansion ex vivo might yield a donor lymphocyte product with enhanced tumor specificity and graft-versus-tumor (GVT) potency. Safety of ex vivo CD3/CD28-costimulated DLI has been demonstrated.5-8 as has feasibility of CD3/CD28-expanded marrow lymphocytes from myeloma patients.9 Preclinical feasibility studies with tumor-infiltrating donor lymphocytes10 provided the basis for a phase 1 clinical trial of tumor-derived donor lymphocyte (TDL; BB-IND13226, MRB) infusion for relapsed B-lymphoid malignancy.

Methods

Eligible adults had: progression of B-cell malignancies despite full-donor T-cell engraftment after AlloSCT; trial of immune suppression withdrawal; DLI (if available); minimal active GVHD; and resectable tumor. The National Cancer Institute Institutional Review Board approved this study, and all subjects provided informed, written consent in accordance with the Declaration of Helsinki. Primary endpoints were feasibility (at least 80% success in generating the TDL product) and safety (< 60% grade 2-4 acute/late-acute GVHD within 28 days). Tumor response was a secondary end point. Accrual stopped subsequent to a planned interim analysis of the first 8 of 15 subjects, when the investigator-held investigational new drug application changed institutions.

After surgical tumor harvest, single-cell suspensions were prepared and costimulated by incubating with anti-CD3/anti-CD28 Ab-coated magnetic beads (BB-IND6675, CHJ) at a 3:1 bead/T-cell ratio and expanded in TDL media (supplemental Figure 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article). Cultures were harvested when yields reached infusion targets, with ≥ 50% CD3+ T cells and ≤ 5% CD19+ tumor cells by flow cytometry. Expanded TDL products were cryopreserved and tested for release criteria (sterility, donor chimerism ≥ 90%, and purity: residual tumor < 5% by clinical pathology and microscopic bead count < 100 per 3 × 106 cells). TDL dose was determined by culture yield, with 1-100 × 106 TDL/kg infused without additional immunophylaxis or exogenous cytokines. Subjects were monitored for toxicity11 for 24 hours in-hospital and at 1, 2, 4, 8, and 12 weeks; response was assessed at 4, 8, and 12 weeks (supplemental Figure 1).12,13

Fresh tumor-infiltrating lymphocytes and the expanded TDL products were phenotyped with multicolor flow cytometry for T-cell and effector subsets; TDL clonality was assessed with PCR for TCR-γ-chain (TRG locus) gene rearrangements and Vβ spectratyping; and tumor-associated
<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Primary diagnosis</th>
<th>Donor</th>
<th>Conditioning (TCD)</th>
<th>TTP post-AlloSCT, mo</th>
<th>Relapse duration, mo</th>
<th>Maximum prior GVHD</th>
<th>Acute skin/ liver/GI</th>
<th>Chronic grade</th>
<th>Post-Allo ChemoRx</th>
<th>Post-Allo donor cells</th>
<th>Tumor TDL</th>
<th>Cell dose, 10^6/kg</th>
<th>Toxicity</th>
<th>Response (duration)</th>
<th>Survival, mo*</th>
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<td>3</td>
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<td>98</td>
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<td>100</td>
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<td>Axila</td>
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</table>

Acute indicates acute GVHD stage per Keystone Consensus Criteria; chronic, chronic GVHD grade per IBMTR Criteria (then in we); CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; GI, gastrointestinal; HL, Hodgkin lymphoma (1° Ref, primary refractory; Rel, relapsed); MA, myeloablative; MRD, matched, related (sibling) donor; MUD, matched unrelated donor; R-ATG, rituximab; RIC, reduced-intensity conditioning; SD, stable disease; TCD, in vivo T-cell depletion for GVHD prophylaxis.

Acute GVHD staged per Keystone Consensus Criteria; Chronic GVHD graded according in IBMTR Criteria, then in use.

*Survival censored July 21, 2011. Bold values indicate deceased; italicized values last contact.

†PN12 received one cycle of cyclophosphamide, vincristine, doxorubicin and dexamethasone (Hyper-CVAD) after harvest, TDL infusion on day 19.

†PN2 TDL product required extension of culture beyond the expansion target in order to meet criteria for residual tumor contamination.

†PN6 required a second tumor harvest; the initial excision yielded a wholly sclerotic specimen.

§PNS presented to his local emergency room 22 days after TDL infusion with fever and shortness of breath. Computed tomography (CT, pulmonary embolism-proto) demonstrated stable tumor with new perinodular infiltrates. He refused bronchoscopy and was treated with broad-spectrum antimicrobials without improvement. He consented and underwent bronchoscopy with bronchoalveolar lavage 1 week later, and required intubation for the procedure. Microbiologic stains and cultures and cytopathologic examination were nondiagnostic. He was treated with steroids with prompt resolution of hypoxia and infiltrates, was extubated on the third day after the procedure, and was discharged 4 days later. Hypoxia and infiltrates recurred during rapid steroid taper and again resolved with increased oral steroid dose. Symptoms flared again with taper; he was treated with gemcitabine, vinorelbine, and liposomal doxorubicin with resolution of infiltrates and complete remission of HL.

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Ag-specific CD8+ donor T cells were sought with an MHC/peptide tetramer library.14 Proliferative alloresponses and antitumor cytotoxicity were assessed in mixed lymphocyte culture, with CFSE and caspase-8 cleavage, respectively (supplemental Methods).

Results and discussion

Primary feasibility and safety aims were met with the initial 8 patients (Table 1). All surgically harvested tumors demonstrated donor lymphocyte infiltration; T cells comprised 0.5% to 71% of tumor cell suspensions, with donor origin confirmed by short tandem repeat chimerism analysis. CD3/CD28 costimulation and culture yielded clinical TDL products for 8 of 8 patients (95% CI for feasibility: 63.1%-100%), generating a median of 2.04 × 10^6 TDL/kg. A median 95% of TDL were CD3+ and CD8+ (supplemental Methods).

Figure 1. [18FDG-PET responses.](A) [18FDG-PET before and at 4 and 8 weeks after TDL. PN6, a 36-year-old with primary-refractory HL with unabated disease progression following myeloablative conditioning and a 6 of 6 HLA-matched sibling-donor AlloSCT with posttransplantation in vivo T-cell depletion with cyclophosphamide. Transient responses followed radiation and gemcitabine, vinorelbine, and liposomal doxorubicin therapies. Twenty-eight months post-AlloSCT, TDL were generated from a right axillary tumor harvest and 26.3 × 10^6 TDL/kg were infused. Moderate pain and tenderness at sites of chest wall tumor and worsening of pleural effusions developed 1 week after infusion. At restaging, [18FDG-PET-CT] demonstrated decreasing standardize uptake values (SUV) in all lesions over 3 consecutive months. By 4 months, however, PET showed increasing SUV in prior sites of disease, which correlated with clinical progression. Shown are coronal maximum intensity projection (MIP) images with a cutoff intensity of 2.5 SUV. (B) Transverse [18FDG-PET-CT fusion images demonstrate decreased SUV in PN8, a 35-year-old with relapsed HL, whose disease progressed from best partial response 6 months after reduced-intensity conditioning and a T-cell replete, 6 of 6 HLA-matched sibling-donor AlloSCT. The patient was treated with vinblastine and subsequently with 3 cycles of dose-adjusted EPOCH (etoposide, vincristine, adriamycin, prednisone, and cyclophosphamide) plus DLI. Seventy months from AlloSCT, TDL were generated from a right axillary tumor harvest, and 91.2 × 10^6 TDL/kg were infused. Grade 2 cytokine release syndrome (culture-negative fevers to 39.5°C, mild hypotension, and tachycardia) was observed from 4 to 24 hours after infusion.[18FDG-PET-CT was stable at 4 weeks, then demonstrated decrease in SUV in multiple sites of disease at 2 months; tumor size was stable by CT. At 4 months, although PET-CT did not show clear progression, the patient developed severe radicular pain associated with tumor-involved cervical lymph nodes requiring additional therapy.

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All patients received TDL infusion. Acute toxicity was minimal, with grade 2 cytokine release syndrome in one patient (PN8). None developed GVHD (95% CI for safety: 0%-36.9%). One patient (PN5) developed grade 4 pneumonia 22 days after infusion, with acute onset of fever, hypoxia, and pulmonary infiltrates surrounding parenchymal tumor (95% CI for incidence of severe adverse events: 0.3%-52.7%). Extensive evaluation was nondiagnostic and rapid resolution followed steroid treatment (Table 1).

Five patients were evaluable for response. PN1, PN2, and PN3 experienced unabated, rapid progression, and clinical deterioration requiring additional treatment and/or initiation of hospice before 4-week restaging. Four of the 5 subsequent patients maintained stable disease through at least 2 evaluations and each manifested some evidence of antitumor activity. PN6 and PN8 demonstrated transient responses by positron emission tomography (PET), with decreased ^18FDFG uptake in all measurable lesions (Figure 1), and PN4 and PN7 demonstrated mixed responses. PN5 may also have had an antitumor effect from TDL, if pneumonitis reflected a tumor-directed immune response. Interestingly, both PET responses were in patients with Hodgkin lymphoma (HL) whose tumors were enriched with donor-derived reactive cells. Plausibly, in HL, gain of type 1 effector function and retained tumor homing permitted infused TDL to transiently disrupt the HL microenvironment.17,18

Ex vivo expansion of TDL by CD3/CD28 costimulation is feasible, generating large numbers of type 1 effector donor T cells. TDL appear relatively safe; remarkably, no GVHD was observed after infusing these type 1 polarized TDL. Although significant GVHD did exclude enrollment, 4 of 8 subjects had received in vivo T cell–depleted allologs so likely were at significant risk for DLI-associated GVHD. TDL may be a therapeutic option for cord blood recipients and others without access to DLI. Suggestive tumor-directed biologic effects and potential GVT activity in these few, heavily treated patients warrants further investigation of this therapeutic approach. Planned evaluation of pre-TDL immune depletion to support TDL expansion and effector function in vivo may improve magnitude and durability of responses.

Acknowledgments

The authors acknowledge the critical contributions of the following current and former coworkers: Charles “Charley” Carter (deceased), E. J. Read, Mark Dudley, and Steven A. Rosenberg provided assistance in development of cell processing methods; Richard Sherry performed surgical tumor harvests; Ellen Mann coordinated tumor specimen acquisition; Mark Raffeld performed PCR on TDL for TCR-γ-chain gene rearrangements, and Armando Filie provided cytopathologic assessment of TDL products from HL tumors. They thank innumerable clinical staff of the National Institutes of Health (NIH) Clinical Center for their outstanding patient care. Finally, they express sincere gratitude to the patients and their families for their generous, essential, and invaluable contributions to this research.

This work was supported by the Intramural National Cancer Institute, Center for Cancer Research (Bethesda, MD).

Authorship


Conflict-of-interest disclosure: C.H.J. has received royalties from United States government–owned patents and patent applications in the field of adoptive immunotherapy; this arrangement is under compliance with the policies of the University of Pennsylvania. The remaining authors declare no competing financial interests.

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References

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