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Brentuximab Vedotin Combined With Donor Lymphocyte Infusions for Early Relapse of Hodgkin Lymphoma After Allogeneic Stem-Cell Transplantation Induces Tumor-Specific Immunity and Sustained Clinical Remission

Introduction

Despite the high cure rate of Hodgkin lymphoma (HL) with conventional radiochemotherapy, HL is refractory in 15% to 30% of patients.[1] High-dose chemotherapy with autologous stem-cell transplantation is an option in this patient cohort and can induce enduring remissions in approximately 50% of patients, whereas the other 50% of patients relapse or have progressive disease and need additional treatment.[2] In such patients with chemotherapy-refractory disease who are otherwise clinically fit, allogeneic stem-cell transplantation (alloSCT) has been shown to have a curative potential as a result of the underlying graft-versus-lymphoma (GvL) effect.[3] Although reduced-intensity conditioning has lowered the treatment-related mortality, the reduced cytotoxicity of the conditioning regimen as well as the incomplete immune reconstitution in the early post-alloSCT phase leads to an increased risk of early relapse. Effective treatment options for relapse after alloSCT are limited because systemic therapy can be stem-cell toxic. Furthermore, thus far, it is not possible to specifically target allospecific immune responses to the tumor, and donor-lymphocyte infusions (DLIs), as another treatment option, can induce both antigen specific and nonspecific responses, which the latter resulting in potentially life-threatening graft-versus-host disease (GvHD). These complications emphasize the need for the control of tumor-cell proliferation and a strategy to specifically enhance GvL efficacy.

Brentuximab vedotin, which is a monoclonal anti-CD30 antibody coupled to the antitubulin agent monomethylauristatin E, recently demonstrated sustained responses in heavily pretreated patients with treatment refractory and progressive HL and also had a low adverse-effect profile.[4] It has been reported that treatment with brentuximab vedotin induces the release of cytokines such as interleukin-6, chemokine ligand 17, and tumor necrosis factor α.[4] This cytokine milieu and the liberation of tumor antigens induced by apoptosis could facilitate the induction of an antitumor immune response.

We hypothesized that treatment with brentuximab vedotin would be a promising approach for relapsed HL after alloSCT that selectively targets lymphoma cells and enhances the GvL response by the induction of immunogenic cell death. Furthermore, we assumed that the combination of this targeted therapy with DLI would have the potential to further increase the GvL effect.

Case Reports

In this article, we report on four heavily pretreated patients with relapsed HL after alloSCT who received brentuximab vedotin after inclusion into a compassionate-use program and the provision of written informed consent. Clinical characteristics of the patients are summarized in the Data Supplement. Blood sample collections before and after therapy and in vitro analyses of immune cells were approved by an institutional review board, and all patients gave their written informed consent.

Patients were treated consecutively from March 2011 to March 2012. Transplant settings and GvHD prophylaxes including antithymocyte globulins are summarized in the Data Supplement. In patient 2, a biopsy was performed because of clinical uncertainty, which then confirmed HL relapse.

We designed a treatment algorithm combining brentuximab infusions (1.8 mg/kg per day every 21 days) with DLI administration in an alternating regimen (Fig 1A). Three patients were free of GvHD initially (patients 1, 2, and 3) and, thus, received DLI in increasing doses that were continued as long as no signs of GvHD greater than I° occurred but to a maximum of five doses (Data Supplement). Patient 4 had limited chronic cutaneous GvHD before brentuximab treatment and did not receive additional DLI.

An evaluation of treatment response was performed clinically and every 2 to 3 months by using computed tomography scans according to established criteria.[5] Moreover, metabolic changes were analyzed by [^{18}F]fluorodeoxyglucose positron emission tomography/computed tomography scans by using the maximal standardized uptake value of the hottest respective lesion.

All four patients showed marked clinical and metabolic responses (Figs 1B and 1C; Data Supplement) with a median duration of disease control of at least 349 days (range, 259 to 366 days) after treatment initiation, which is still ongoing in three patients. Emergency local radiotherapy was given to patient 2 for lumbar spinal cord compression initially with the antibody therapy. Patient 4 received localized irradiation after five cycles of brentuximab for residual infiltration of thoracic vertebra 1/2.

Sensory polyneuropathy and mild thrombocytopenia were the most common adverse effects secondary to brentuximab therapy (Data Supplement), which were consistent with published data for nonallogeneic-transplanted patients.[4] Polyneuropathy occurred in a cumulative dose-dependent manner and resulted in treatment discontinuation in patient 4 after 10 cycles. Recently, Gopal et al[6] reported on the safety and feasibility of brentuximab without DLI after alloSCT, which provided additional support for the clinical potential of this substance.

Discussion

To assess the induction of a GvL effect, we developed an in vitro method by using cocultures of patient peripheral-blood mononuclear cells (PBMCs) (pre- and post-treatment) and well-characterized CD30$^+$ HL cell lines and controls as surrogate targets (HL: L1236 and L428; CD30$^+$ anaplastic large cell lymphoma: Karpas-299; B-cell acute lymphoblastic leukemia: Reh; and colon cancer: SW480). To rule out nonspecific allogeneic in vitro reactions, we cocultured patient PBMCs and respective cell lines for only 5 hours, and spontaneous T-cell activation was measured in parallel controls. As additional controls, we subjected PBMCs from a patient with AML after alloSCT and PBMCs from a healthy volunteer to the same conditions.

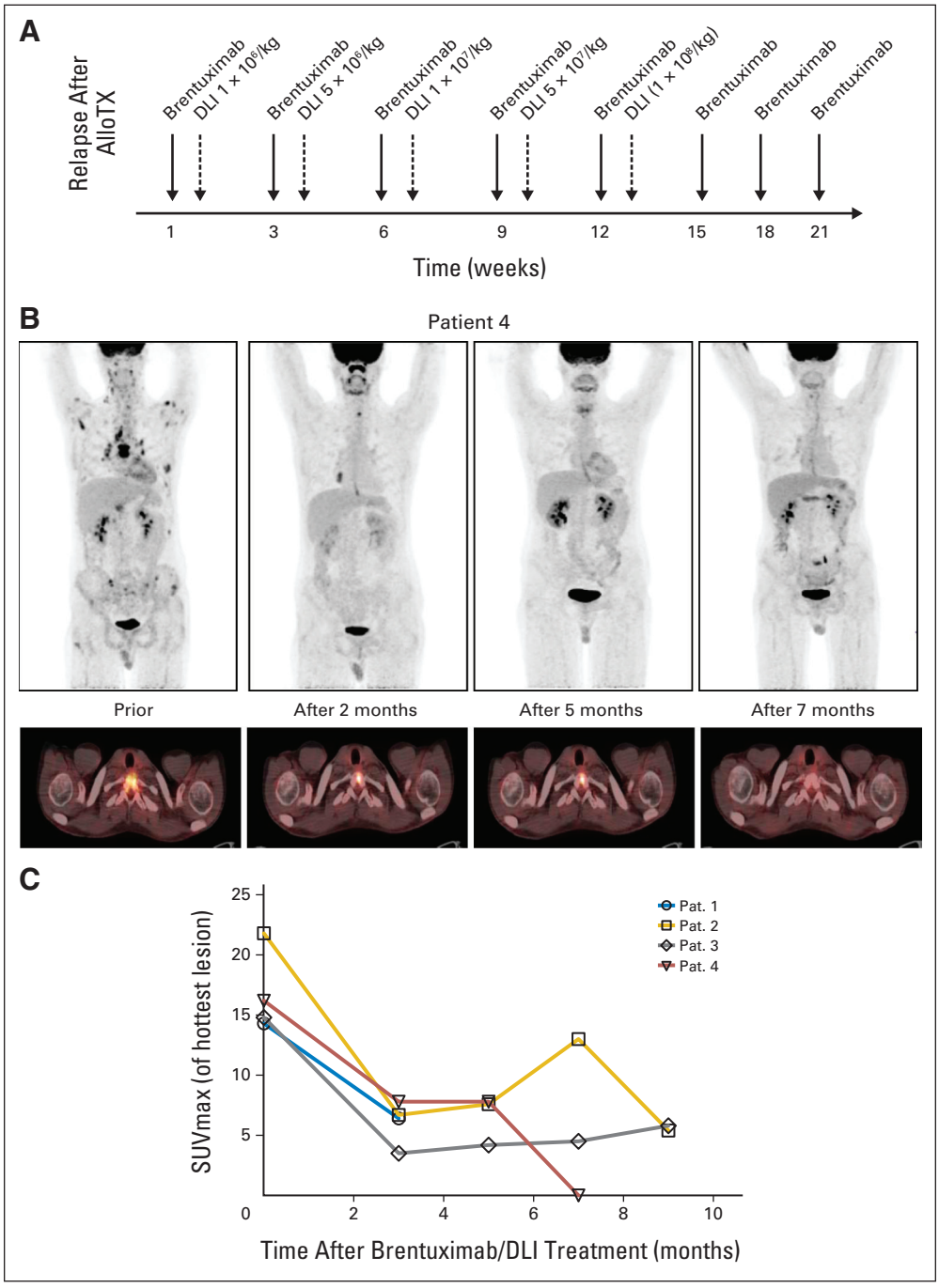

Fig 1.

First, we analyzed conventional cytotoxic T-cell (CTL) responses. We determined HLA-A2 expression, which is the most frequent major histocompatibility complex class I (MHC class I) allele in the white population (approximately 50%),[7] in the five cell lines. L1236, Reh, and SW480 expressed HLA-A2, whereas L428 and Karpas-299 were HLA-A2 negative. Only patient 1 was HLA-A2 positive (A*0201). In this patient, post-treatment CTLs specifically recognized the two HL cell lines (L1236 and L428) as demonstrated by the upregulation of CD69 (Fig 4A) and CD107a (data not shown). However, CTL reactivity with HLA-A2–negative L428 cells suggested the recognition in the context of additional MHC class I alleles. The allograft of this patient was positive for additional frequent MHC class I alleles (A*2402 [approximately 20%], B*3801 [approximately 10%], B*4001 [approximately 10%], C*0304 [approximately 10%], and C*1203 [approximately15%]), which explained the recognition of HLA-A2–negative L428 cells.

Next, we focused on T cells that expressed the natural-killer–cell c-type lectin receptor (CD161), which characterizes a new, heterogeneous population comprised of activated T cells, natural killer T-cell–like cells and T helper 17 (TH17) cells with immunomodulatory properties

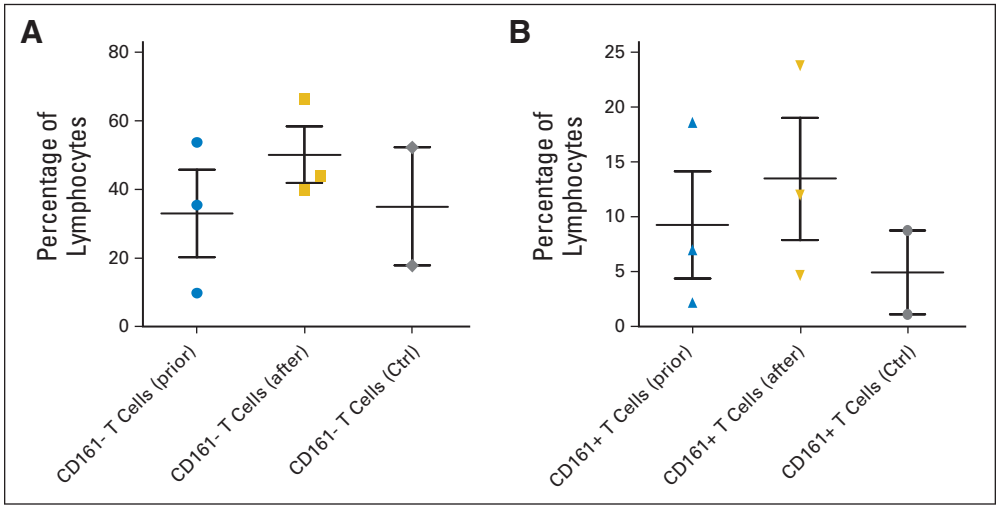

Fig 2.

depending on the surrounding microenvironment.[8,9] Although the distinct roles of these subpopulations are still subject to an intensive debate, emerging data underline their effect on tumor and transplant immunology.[10-12] For example, TH17 cells, which derive from $CD4^+$ T-cell precursors and strongly express CD161, have been described to be involved in the HL microenvironment and can suppress murine ocular lymphoma progression.[13-15]

In all patients, we found that circulating T cells, both $CD161^-$ and $CD161^+$, were increased after brentuximab and DLI treatment (Figs 2A and 2B; only data from three patients are shown because no pretreatment sample was available from patient 3).

In all patients, the fraction of $CD161^+$ T cells that coexpressed CD4 increased from 20% to 30% before treatment to greater than 50% after treatment, which suggested an induction of a predominant

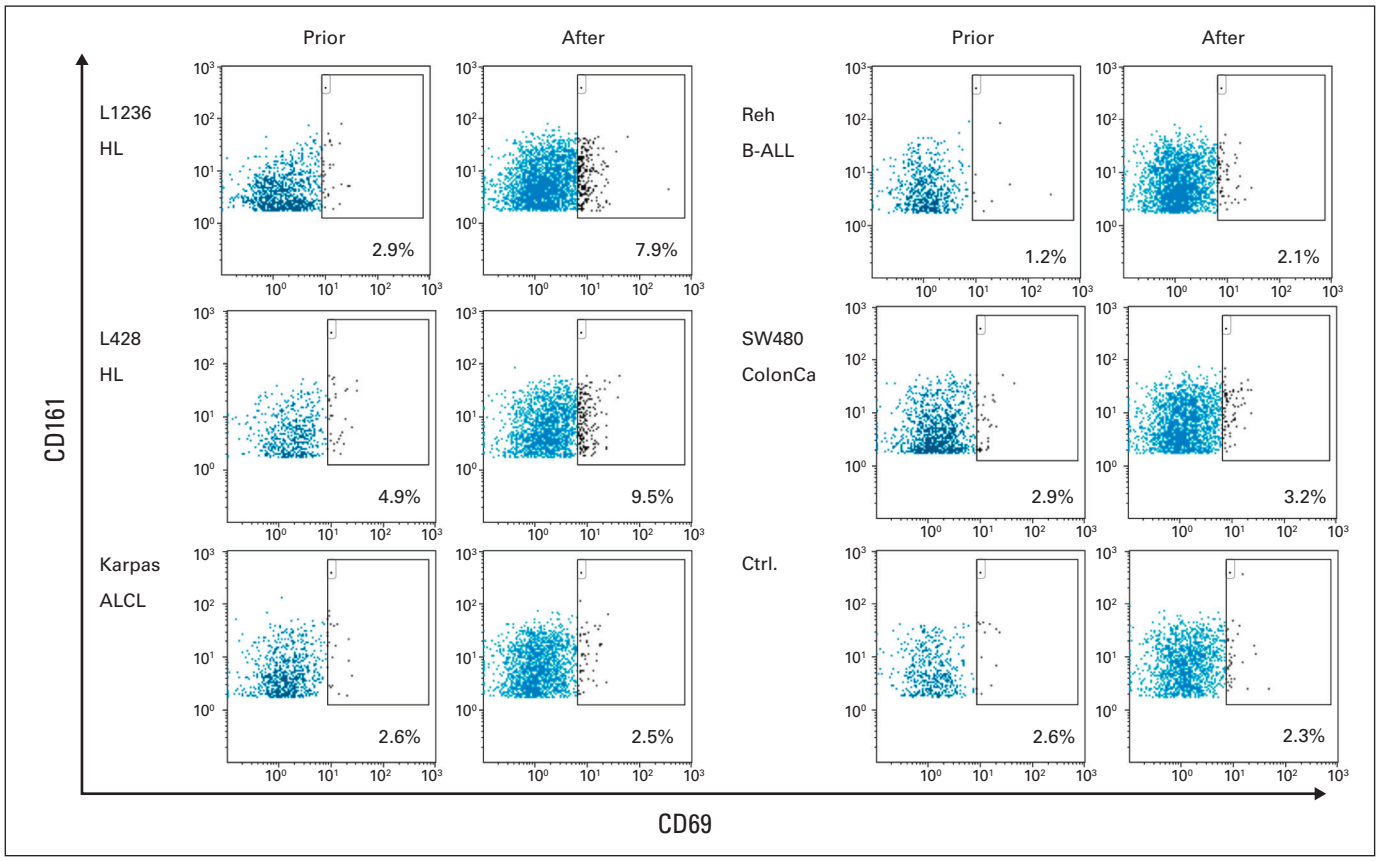

Fig 3.

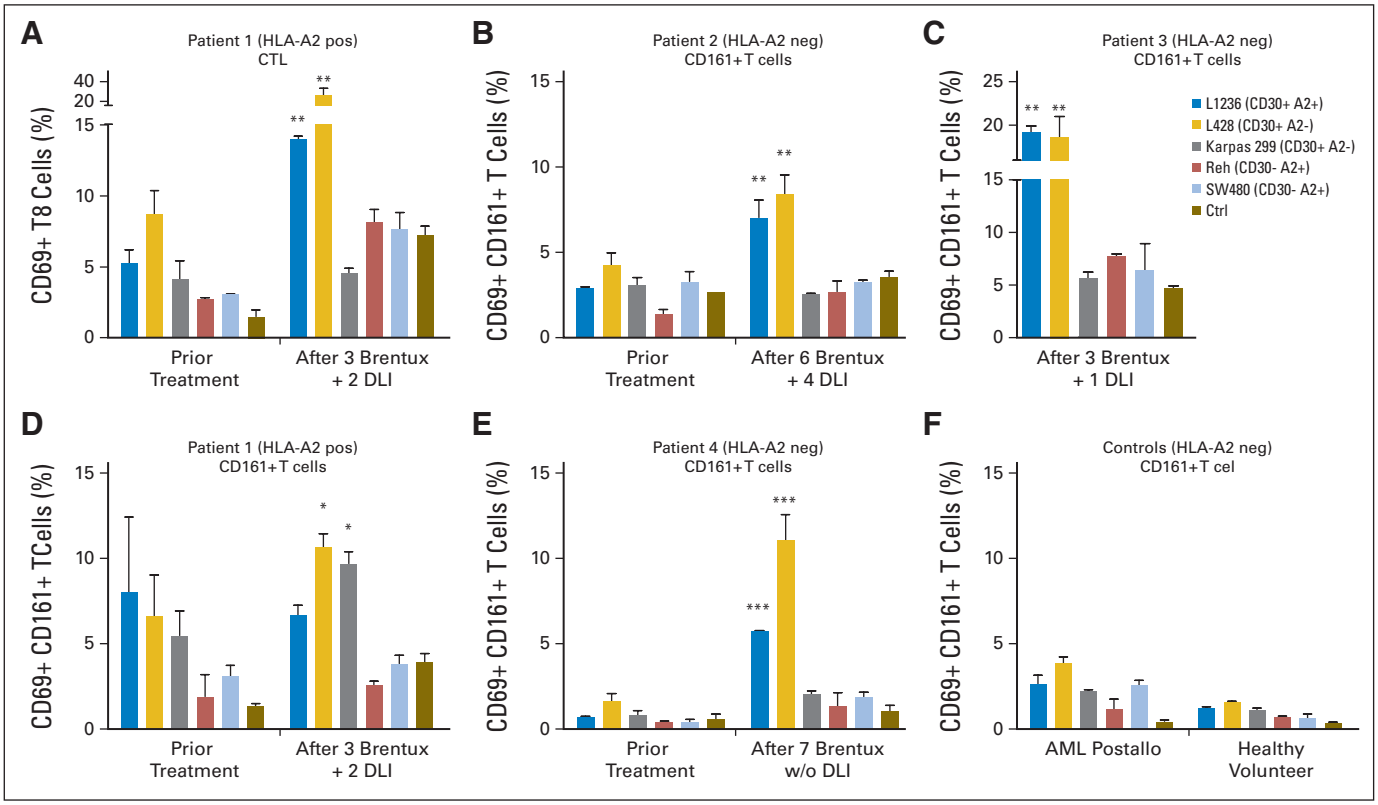

Fig 4.

TH17-like phenotype (data not shown). Interestingly, in patient 4, who only received brentuximab without DLI, we also could detect a 1.5-fold increase of circulating T cells, including TH17-like cells, after treatment, which suggested a DLI-independent effect.

Before treatment, the three patients who received the combination of brentuximab and DLI did not have clinical signs of GvHD, which implied only low immune activation, and we could not detect significant tumor-specific reactivity of $CD161^+$ T cells in vitro (Figs 3 and 4). In contrast, a significant increase of HL-specific $CD161^+$ T-cell activation, determined by the expression of CD69, was observed in all patients after treatment (Figs 4B to 4E; statistical analyis performed with Wilcoxon test with a *P* value being considered significant below .05). Reactive $CD161^+$ T cells mainly coexpressed CD4 (data not shown), which suggested a TH17-like phenotype of the HL-specific T cells. Of note, post-treatment $CD161^+$ T cells of patient 1 were also activated by $CD30^+$ Karpas-299 cells and showed a general higher reactivity (Fig 4D). The post-treatment blood sample of this patient was obtained at the clinical onset of acute GvHD, which could have explained the higher state of general immune reactivity (representative flow-cytometry results of patient 2 are shown in Fig 3; the induction of HL-specific $CD161^+$ T cells after treatment in each patient is shown in Fig 4; $CD161^+$ T cells from control individuals did not show any specific reactivity; Fig 4F) Because we detected significant and HL-specific activation of $CD161^+$ T cells only in PBMCs drawn after treatment, this suggests that the treatment led to a tumor-specific immune response that was absent before that therapy.

Clinically, except in patient 1, who developed severe acute GvHD that finally led to death subsequent to septic shock, all patients experienced manageable acute GvHD or, as in the case of patient 4, had stable limited chronic GvHD. The treatment of acute GvHD in patients 2 and 3 consisted of prednisolone, which could be tapered rapidly.

Importantly, patient 4, who did not receive DLI as a result of chronic skin GvHD, developed an equally high and significant HL-specific immune response after brentuximab vedotin treatment. Apparently, in this patient, chronic GvHD activity itself was initially not sufficient to induce sustained HL-specific GvL activity to maintain lymphoma remission. We hypothesize that the CD30-targeted therapy focused the HL-specific immune response against the tumor as a result of the release of tumor antigens and proinflammatory modulation of the tumor microenvironment.

In conclusion, we demonstrated that CD30-targeted lymphoma therapy with brentuximab vedotin induces sustained clinical responses and tumor-specific immunity in an allogeneic setting that warrants additional investigation.

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