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LETTER TO THE EDITOR

αβ-T-cell depleted donor lymphocyte infusion for leukemia relapse after allogeneic stem cell transplantation

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Relapse of hematological disease remains one of the major challenges after allogeneic stem cell transplantation (allo-SCT). Adoptive immunotherapy with donor lymphocyte infusions (DLIs) has been established as a therapy option to treat relapse after allo-SCT since the early 1990s.[1,2] The second major challenge after allo-SCT is GvHD. As DLIs contain considerable numbers of alloreactive CD3+ αβ-TCR+ T cells, GvHD is a potential side effect of DLI that may limit its application. After DLI administration, acute GvHD is reported to occur in 30–40% and chronic GvHD in 40–60%.[3,4] Nevertheless, DLI has become a well-established procedure to treat relapse after allo-SCT. This is because of the GvL effect mediated by the donor immune cells contained in the DLI that include not only alloreactive CD3+ αβ-TCR+ T cells but also CD3+ γδ-TCR+ T cells as well as CD3− CD56+ natural killer (NK) cells. The latter two subsets are of particular interest because, in contrast to alloreactive CD3+ αβ-TCR+ T cells that recognize mismatched minor or MHC antigens of the patient and therefore can induce GvHD, they recognize their target cells in a non-MHC restricted manner. In several SCID mouse tumor models it has been shown that γδ-T cells exert antitumor activity and tumor surveillance *in vivo*.[5,6] Based on this, γδ-T cells are potentially able to mediate GvL without causing GvHD. Protocols for selective depletion of CD3+ αβ-T cells as well as CD19+ B cells have been developed for haploidentical allo-SCT especially in pediatric patients.[7–9] In contrast, DLI depleted of CD3+ αβ-T cells for the treatment of relapse post allo-SCT has—to the best of our knowledge—not been reported so far.

A female patient with nucleophosmin (NPM1)-positive AML and complex-aberrant karyotype underwent allo-SCT in hematological remission but in molecular persistence with HLA-identical (10/10 HLA-A, -B, -C, -DRB1, -DQB1) stem cells from a male unrelated donor after conditioning with TBI (12 Gy) and fludarabine 30 mg/m^2 over 5 days. GvHD prophylaxis consisted of anti-thymocyte globulin (ATG Fresenius 10 mg/kg body weight per day) on day −4 to day −2, cyclosporine A from day −1 and short courses of methotrexate on days +1, +3, +6 and +11. Bone marrow (BM) examination 4 weeks after allo-SCT documented hematological remission with 100% donor chimerism by

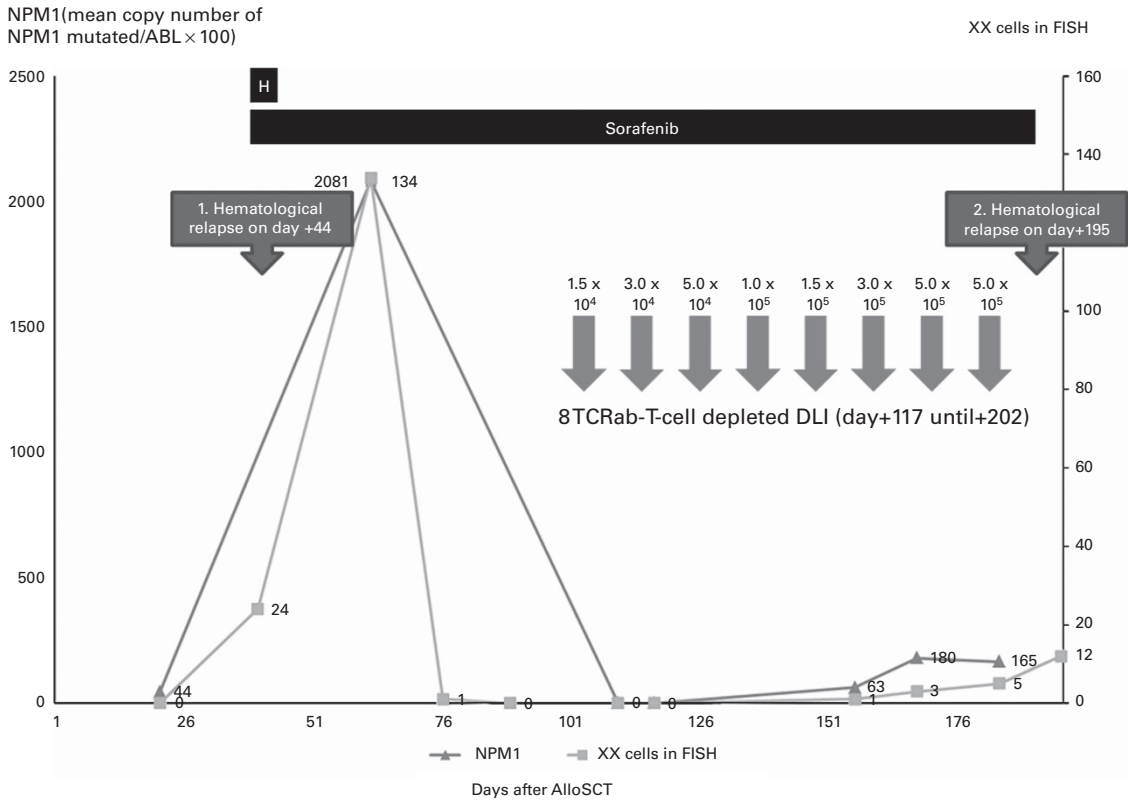

Figure 1. Minimal residual disease (MRD) and chimerism kinetics post transplantation. The *x* axis shows the days post allo-SCT, the left *y* axis shows the ratio given as mean copy number of NPM1 mutated/ABL1 × 100 in qPCR (triangles) and the right *y* axis the number of cells bearing the patient-specific X chromosome in FISH. All measurements were performed on PB except for those from days +21 and +62 that were performed on BM. Dates of first and second hematological relapse, hydroxyurea (H) and sorafenib treatment as well as DLI administrations are indicated. A full colour version of this figure is available at the *Bone Marrow Transplantation* journal online.

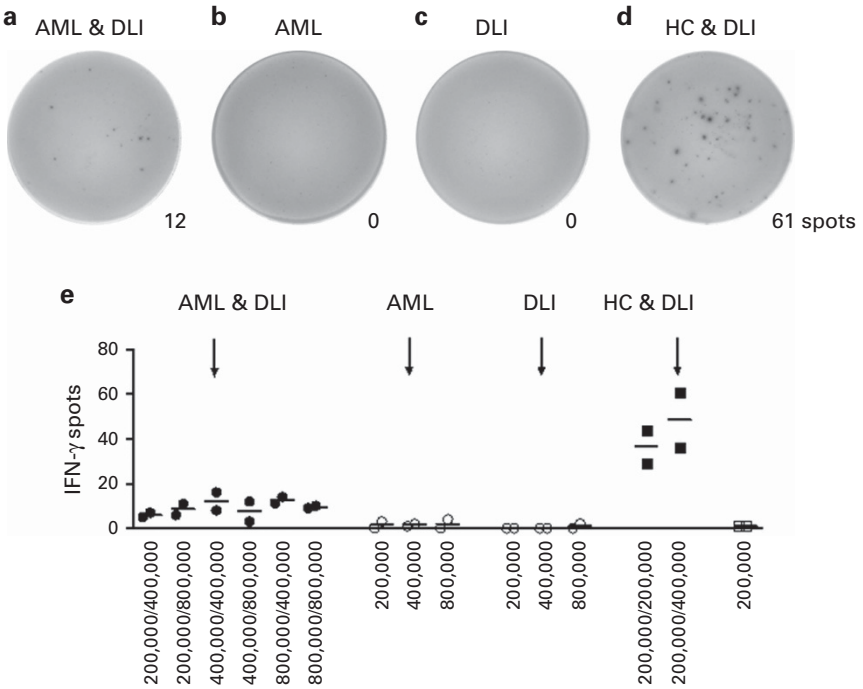

Figure 2. Recognition of patient AML blasts by αβ-T-cell depleted DLIs. αβ-T-cell depleted DLIs were incubated with AML blasts and the secretion of IFN-γ was determined by ELISpot assay (**a**). These AML blasts were collected from the patient at day +208 post allo-SCT containing 42% AML blasts in PB (AML). AML blasts (**b**) or DLIs (**c**) grown alone served as negative controls, and DLIs stimulated by third-party PBMCs from 10 healthy controls (HC) as positive controls (**d**). Apart from the cell numbers as described in the Materials and methods section, other numbers and ratios of cells were used as shown in detail in (**e**). ELISpot assays were performed as duplicate cultures, and the horizontal lines indicate mean values. Conditions displayed as images in (**a**–**d**) are marked by an arrow in (**e**).

quantitative PCR (qPCR), but NPM1 was still positive. Despite rapid reduction of immunosuppression the patient suffered a fulminant AML relapse on day +44 after allo-SCT. Immunosuppression was completely tapered which led to a histologically proven cutaneous GvHD grade II. Treatment with hydroxyurea and subsequently sorafenib was initiated on day +47. This led to normalization of leukocyte and blast counts. Hydroxyurea was terminated after 4 days and sorafenib was decreased because of side effects from 800 to 200–400 mg/day.

Given the dismal prognosis with high-risk AML and very early relapse after allo-SCT, αβ-T-cell depleted DLIs were applied to the patient after written informed consent, in accordance with the declaration of Helsinki. The administration of αβ-T-cell depleted DLI was approved by the Legal Department of the University Hospital Essen. Unstimulated PB mononuclear cells (PBMCs) were collected from the original unrelated stem cell donor by leukapheresis and subjected to depletion of αβ-TCR+ T cells using the CliniMACS device (Miltenyi Biotec, Wiesbaden, Germany) under good manufactory practice conditions, according to the manufacturer's recommendations. CD19+ B cells were not depleted from the leukapheresis product. The cellular composition of the modified DLI was determined by immune phenotyping using the following monoclonal antibodies: anti-TCRαβ phycoerythrin (PE) and anti-TCRγδ FITC (both from Miltenyi Biotec, Bergisch Gladbach, Germany), anti-CD3 PC7 and ECD, anti-CD19 PE, anti-CD16 PC7, anti-CD56 PE, anti-CD14 PC7 and anti-CD45 ECD and FITC (all the aforementioned antibodies by Beckman Coulter, Krefeld, Germany). After manipulation, only trace amounts of αβ-TCR+ T cells (0.0045%) remained in the product. The γδ-TCR+ T cells represented 82% of the remaining CD3+ T cells, amounting to 4.68% of cells in the product. Concomitantly, the percentages of CD56+ NK cells and of CD19+ B cells were increased that represented 8.4% and 26.7% of the final DLI product, respectively. Compared with the unmanipulated leukapheresis, there was a 2.6-fold increase in γδ-TCR+ T cells and in CD56+ NK cells, and a 2.2-fold increase in B cells.

Eight consecutive increasing doses of αβ-T-cell depleted DLIs were administered between days +117 and +202. Despite the rapid increase in dosage and short intervals between the applications, the DLIs were well tolerated. The histologically confirmed cutaneous GvHD that had appeared after withdrawal of immunosuppression resolved completely during the course of DLI administration. Complete hematologic and molecular remission was maintained under DLI treatment for 39 days until day +156, when NPM1 became positive again, paralleled by increasing chimerism by both FISH and qPCR. A second hematologic relapse with 6% blasts in the PB was documented on day +196. The patient died on day +209 after allo-SCT because of the uncontrolled leukemia relapse and with multiorgan failure (Figure 1).

The ability of the αβ-T-cell depleted DLIs to recognize the patient's AML cells was tested by ELISpot assays for interferon-γ (IFN-γ) production, as described previously.[10,11] Briefly, 400 000 αβ-T-cell depleted DLIs were stimulated for a total of 3 days with irradiated PBMCs from the patient at second relapse on day +208 after allo-SCT, containing 42% AML blasts as evidenced by morphologic counts in a 1:1 ratio. The same numbers of αβ-T-cell depleted DLIs cultured in medium alone or stimulated with irradiated PBMCs from 10 HLA disparate healthy volunteers in a 2:1 ratio were used as controls. Overnight cultures were transferred to ELISpot plates (Ready-MTP basic Human IFN-γ, Lophius Biosciences, Regensburg, Germany) for further 2 days before determination of IFN-γ spot numbers with an ELISpot plate reader (AID iSpot FluoroSpot, Autoimmun Diagnostika GmbH, Strassberg, Germany) as described previously.[12] αβ-T-cell depleted DLIs displayed allo-responses against AML blasts (12 spots, mean number); the frequency of cells producing IFN-γ was 12 per 400 000 DLIs (0.003%, Figure 2a). The secretion of IFN-γ by DLIs after stimulation with AML blasts can be considered as a surrogate

marker of antileukemic immune responses, as IFN-γ is a key cytokine of cytotoxic T cells. Negative controls, AML blasts or DLIs grown alone did not show IFN-γ spots (Figures 2b and c). DLIs also responded to third-party PBMCs, a pool of 10 HLA disparate healthy controls who were used as positive control (61 spots, Figure 2d).

As DLIs imply an unpredictable GvHD risk, particularly the pre-emptive use of DLI has to be meticulously weighed in each patient taking into account the beneficial antileukemic effect and the potential risk of GvHD. The method of αβ-T-cell depletion has been developed to manipulate the graft in haploidentical allo-SCT. Recently, a study with αβ-T-cell depleted haploidentical allo-SCT in 34 adult patients with acute leukemias reported a very low incidence (5.9%) of acute GvHD III–IV.[13] αβ-T-cell depletion has also been successfully implemented in stem cell boosts to treat graft failure after allo-SCT.[14] The present case, however, represents—to the best of our knowledge—the first application of αβ-T-cell depleted DLIs for the treatment of AML relapse post transplant. Despite repeated and increasing doses of DLIs, no new clinical signs of GvHD appeared in our patient. It is tempting to speculate that NK- and/or γδ-TCR+ T cells administered in the αβ-T-cell depleted DLIs might have actively contributed to the resolution of GvHD, for instance by depleting residual patient antigen presenting cells.[15] The αβ-T-cell depleted DLIs displayed limited but detectable activity against the patient's AML blasts *in vitro*. It should be noted that the AML blasts available for the *in vitro* ELISpot assays were obtained during the second relapse at day +208, that is, after several rounds of DLI administration. The limited *in vitro* activity of DLI against the blasts is therefore in line with the inability of the DLIs at that time point to control the leukemia *in vivo*. A possible reason might be the onset of immune escape mechanisms favoring the outgrowth of DLI-resistant AML under treatment. Hematological remission had indeed been already induced pre-DLI by the combined effects of withdrawn immunosuppression, hydroxyurea and sorafenib, the latter being administered during the entire course of DLI. Therefore, it is impossible to dissect the degree of contribution by DLI to the patient's transient remission of in total 80 days. Clearly, our αβ-T-cell depleted DLIs were unable to eventually prevent the hematological fatal relapse. This could be because of the particularly aggressive, highly proliferative AML of this patient. Conventional DLIs for relapsed AML have response rates of ~30% and in many cases are not durable, possibly because of their rapid growth kinetics.[3,4] Given their low GvHD risk, αβ-T-cell depleted DLIs might have the potential to improve these results. However, probably higher cumulative cell dosage and shorter intervals are needed to counteract the dynamics of highly proliferative leukemias. Moreover, pre-emptive αβ-T-cell depleted DLIs could be considered in high-risk patients with potentially improved efficacy at lower tumor burden. Taken together, we believe that despite the ultimately negative outcome, our case demonstrates the safety and potential efficacy of αβ-T-cell depleted DLIs as innovative treatment option for patients at high risk of relapse after allo-SCT.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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L Kordelas[1], U Buttkereit[1], M Lindemann[2], M Koldehoff[1], V Klisanin[1], PA Horn[2], K Fleischhauer[3] and DW Beelen[1]

[1]*Department of Bone Marrow Transplantation, University Hospital Essen, Essen, Germany;*

[2]*Institute for Transfusion Medicine, University Hospital Essen, Essen, Germany and*

[3]*Institute for Experimental Cellular Therapy, University Hospital Essen, Essen, Germany*

E-mail: lambros.kordelas@uk-essen.de

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