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Molecular remission induced by gemtuzumab ozogamicin associated with donor lymphocyte infusions in *t*(4;11) acute lymphoblastic leukemia relapsed after transplantation

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TO THE EDITOR

The prognosis of acute lymphoblastic leukemia (ALL) relapsing after hematopoietic cell transplantation is poor, particularly when relapse occurs early.[1] The outcome of a second transplantation is usually dismal, due to either transplant-related mortality or disease progression, and the efficacy of donor lymphocyte infusions (DLI), alone or combined with cytoreductive therapy, is still controversial in ALL, where the burden of disease is usually high and rapidly increasing.[1,2] Therefore, innovative protocols, including targeted therapy, can be additional tools, available to some patients with appropriate antigen expression. We report the case of a 3-month-old girl, diagnosed with ALL in May 2000 and treated according to the Interfant 99 Protocol, combining therapeutic patterns from both ALL and AML protocols.[3] White blood cell count at the onset was 58×10^9/l, initial response to steroid was good and remission was obtained on day $+15$. The disease course was monitored by means of morphological criteria and immunophenotyping, which showed TdT^+, $CD19^+$, $CD34^+$, $CD10^-$, $CD33^-$ blasts at diagnosis. Minimal residual disease (MRD) was assessed by means of real-time quantitative reverse transcriptase-polymerase chain reaction (RQ-RT-PCR) of the fusion gene MLL/AF4 transcript, generated by the *t*(4;11) type e11-e4, and by RQ-PCR of the heavy-chain immunoglobulin gene (IgH) rearrangement DH_2-JH_6.[4,5] During the front-line treatment, the first remission was confirmed by molecular criteria, and was maintained thereafter up to overt relapse, which occurred 18 months later, during maintenance therapy. At relapse all blasts were found: TdT^+, $CD19^+$, $CD10^-$, $CD33^+$. MLL/AF4 and IgH RQ-PCR results after relapse, normalized for the Abelson gene transcript and the albumin gene, respectively, as previously described, are shown in Figure 1.[4,5] The second remission, reinduced by the 'FLAG-DaunoXome' block, was only morphological, since MRD was persistently positive, at the level of 2×10^{-4}.[6] In February 2002, at 24 months of age, the child underwent bone marrow transplantation from an unrelated donor, fully matched at the A,B,C,DRB1,DRB3,DQB1,DPB1 HLA loci, defined by high-resolution genomic typing. The conditioning regimen consisted of busulfan, etoposide, and cyclophosphamide; cyclosporine, methotrexate, and antithymocyte globulins (ATG) were given as graft-versus-host disease (GVHD) prophylaxis. After receiving unmanipulated bone marrow, containing $7 \times 10^6 CD34^+$ cells/kg and $1 \times 10^8 CD3^+$ cells/kg, myeloid engraftment occurred at day $+12$, while platelet engraftment was delayed due to severe and persistent hemorrhagic cystitis. The child developed skin and gut acute GVHD, grade II overall, which was initially treated with steroid, then combined with ATG 2.5 mg/kg for three alternate days. Neither toxicity nor GVHD involved the liver. Morphological and molecular remission and full donor chimerism were documented up to 6 months after transplantation, when MRD analysis became positive and low-dose steroid, still ongoing for mild chronic GVHD, was discontinued. After 3 weeks, 7% of blasts were detectable in the bone marrow and a subtle recipient band appeared at chimerism analysis. The donor was unavailable for second donation, but willing to donate unstimulated blood, yielding enough cells for DLI treatment, given the low recipient weight. Immunophenotyping confirmed a high level of CD33 antigen expression in virtually all blasts, and treatment with gemtuzumab ozogamicin (GO) followed by DLI was planned, after extensive discussion with the parents.[7] Two infusions of GO were given at a 2-week interval at doses of 5 and 2 mg/sm, respectively; no toxicity other than severe pancytopenia was reported, in particular AST and ALT were consistently <35 UI/l and bilirubin was consistently <0.3 mg/dl. Morphological remission and MRD reduction were achieved after the second dose. A first DLI, at the dose of $5 \times 10^6 CD3^+$ cells/kg, was delivered 2 weeks after the second GO infusion, and caused skin, gut, and liver GVHD, requiring steroid treatment. After 2 months, molecular remission and full donor chimerism were documented and a second DLI, at the same dose, was given; once again, GVHD flared and responded only partially to treatment with ATG. In the third month after second DLI, morphological remission with full donor chimerism was documented, but MRD became slightly positive, consistently for the two markers. To date, 18 months after transplantation and 8 months after last DLI, the child is active and playful, despite immunosuppression for liver GVHD, and MRD positivity is decreasing, reaching a level lower than in any previous positive sample after the post-transplant relapse, close to the sensitivity level of the technique (10^{-4}).

ALL relapsing after transplantation has dismal prognosis; in this case, the prognosis was worsened by youngest age, cytogenetic abnormality, and previous high-intensity front-line and reinduction treatments.[3] Different groups failed to show the prognostic value of CD33 positivity, which was reported in 15% of childhood ALL overall, and in a smaller proportion of infant ALL, despite a more immature immunophenotype and possible oligoclonality at the onset, which could account for clonal variations at relapse.[3] Among 16 consecutive infant ALL at our institution, the CD33 antigen was expressed in two at disease

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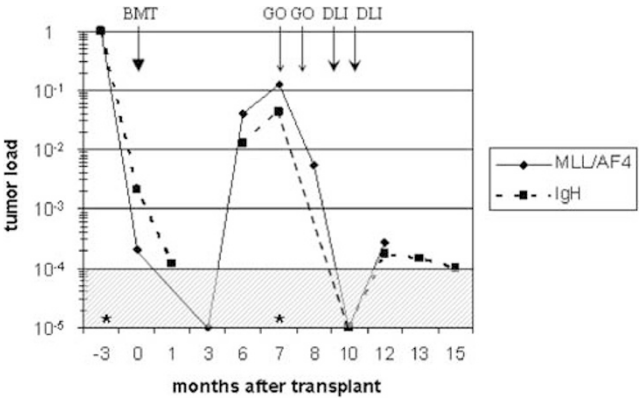

Figure 1 Monitoring of leukemic cell burden before and after transplantation. Two molecular markers were used to monitor the kinetic of tumor load during the course of the disease; the 'AML/AF4' fusion transript for the *t*(4;11) was tested by real-time quantitative (RQ) reverse transcriptase (RT)-PCR, while the immunoglobulin heavy-chain 'IgH' gene rearrangement was analyzed by RQ-PCR on DNA. Normalized tumor load levels reported on the *Y*-axis are referred to relapse before transplantation. The sensitivity of both the methods was 10^{-4}; all tests below the detection limit, indicated by the shaded area, are to be considered negative. The two marker analyses yielded consistent results. Relapses before and after transplant are marked as '*' on the *X*-axis. Arrows on the top of the figure point out clinically relevant time points: transplantation (BMT), GO and DLI. At this time, the child is in third remission, 18 months after transplantation and 8 months after second DLI with very low level of residual disease.

onset and in additional two at relapse; despite small numbers, an intense pattern of CD33 expression was associated with resistant disease (data not shown). In this child, an exhaustive molecular monitoring made way for early intervention before overt relapse, but discontinuation of immunosuppression was not effective in preventing relapse, despite apparent full donor chimera. The role of DLI is well established in chronic myelogenous leukemia, but only single cases or short retrospective series document the graft versus leukemia (GVL) effect in ALL, closely associated with GVHD; the overall probability of durable remission after DLI, possibly even overestimated, due to reporting bias on single cases, is quoted between 0 and 10%, with best results if delivered after cytoreductive chemotherapy.[1,2] Instead of conventional approaches, due to the high level of CD33 antigen expression in virtually all blasts, targeted therapy was considered, in an attempt to spare toxicity without jeopardizing efficacy.[7] The same criterion allows the use of GO in the elderly, a setting in which toxicity is unacceptably severe. GO results are encouraging in resistant acute myeloid leukemia, and a recent study reported its efficacy for CD33-positive ALL.[7] Although liver toxicity attributable to GO, extensively described, particularly after transplantation, was not observed in our patient, the second dose was lowered, as suggested by previous experiences in transplanted patients.[8] As previously extensively reported, unfortunately GVL was never separated from GVHD, which required immunosuppression, potentially jeopardizing GVL; whether ongoing immunotherapy will ultimately clear MRD is still to be assessed. As expected, the targeted therapy did not eradicate the disease, but we can speculate that GO, by reducing the tumor load to the molecular level, allowed DLI, often failing in ALL, the chance to be effective.

In conclusion, this case suggests that GO can induce remission of CD33-positive ALL and can be associated with DLI to treat relapse after transplantation. Whether the combination of GO and DLI could be successfully adopted in the earlier relapse phase of MRD positivity is appealing, but still to be assessed.

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