

Graft versus Leukemia Could Participate of Efficacy of Blinatumomab in Patients with B-Lineage Acute Lymphoid Leukemia Relapsing after Stem Cell Transplantation

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Abstract

We report here the first clinical case of GvL post Blinatumomab treatment in acute lymphoblastic leukemia relapsing after allo stem cell transplantation. This effects accompanied a skin GvHD and has been documented by skin biopsy, immune-staining and FISH. We thus hypothesize that the GvHD may have contributed to disease eradication in this patient, the allogeneic setting reinforcing the immunological efficacy of blinatumomab through a GvL effect. Blinatumomab could actually represent a very interesting therapeutic approach in relapse and/or in prevention of relapse after alloSCT.

Keywords: ALL; Blinatumomab; Allogeneic stem cell transplantation; Relapse; GVHD

Introduction

Since the last three decades, outcome of adult patients with ALL has improved. Using pediatric-inspired treatment regimens, long-term overall survival (OS) is currently around 60%. However, 30 to 35% of the patients still relapsed. Allogeneic stem cell transplantation (alloSCT) is then recommended, at least in those who have not received alloSCT in first complete remission (CR). In patients relapsing after alloSCT, the outcome is very poor.

Blinatumomab is a bispecific T-cell engager (BiTE) antibody, with dual specificity for CD19 and CD3, which retargets cytotoxic T lymphocytes at the selected surface antigen on tumor cells. It engages T-cells with its anti-CD3 arm and redirects them via its anti-CD19 other arm to all B cells [1]. Blinatumomab seems to be associated with high response rates in patients with persistent minimal residual disease (MRD) after intensive chemotherapy [2,3], as well as in those with relapsing B-lineage ALL [4,5] and reported also in post-transplant relapse without signs of GVHD [6].

Acute GvHD is a major complication of alloSCT. The recipient tissues activate donor T lymphocytes. Activated T-cells then secrete cytokines, recruit additional inflammatory cells, induce the expression of histocompatibility antigens and focus the attack of donor immune effector cells on recipient targets. Cytolytic function of cytotoxic T-cells directly causes tissue damage and apoptosis [7,8]. T-cell depleted alloSCT is associated with a decrease of acute and chronic GvHD incidences, but an increase of relapse as cytotoxic T-cells also participate in reducing residual tumor cell level [9]. These data support an antileukemic effect associated with GvHD. After alloSCT, donor lymphocyte infusion (DLI) can thus be used for prevention or treatment of relapse [10,11]. Therefore, a beneficial graft-versus-leukemia (GvL) could be observed in parallel to GvHD, and this is implicating the same T-cells.

We report here the case of one patient with ALL relapsing after

alloSCT, who responded to blinatumomab therapy while presenting concomitant induced GvHD. We hypothesized that the effect of blinatumomab might be improved by triggering GvHD when used in the allogeneic setting.

Case Presentation

A 41-year old woman was diagnosed with B-lineage ALL in October 2011 using WHO classification. White blood cell count (WBC) was $10 \times 10^9/L$. She had no lymph node, liver or spleen enlargement, nor central nervous system (CNS) involvement. Karyotype analysis failed. IgVH rearrangement and *IKZF1* gene deletion were found in the leukemic cells, both used to monitor minimal residual disease (MRD). First-line treatment consisted in the pediatric-inspired GRAALL-2005 protocol [12]. Patient was resistant to the initial one-week corticosteroid prephase and presented persistent bone marrow leukemic blasts at day 14. She nonetheless reached CR after first induction cycle (defining as less than 5% marrow blasts, absence of blasts in PB, with recovery of hematopoiesis (neutrophils $>1000/mL$ and platelets $>100,000/mL$), without extra medullary disease), with a 3×10^{-1} MRD level. After the first consolidation phase, MRD level dropped to 6×10^{-3} . After the second consolidation phase, it was 4×10^{-3} . Given this suboptimal MRD early response, an alloSCT in first CR was performed in April 2012. The donor was her HLA identical sibling brother. The

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myeloablative conditioning regimen included total body irradiation (12 Grays) and cyclophosphamide (120 mg/kg). GvHD prophylaxis associated ciclosporine and methotrexate. One month after alloSCT, a grade III cutaneous GvHD occurred, without intestinal tract and liver involvement. Steroid therapy (2 mg/kg/day) was initiated with clinical efficacy after seven days of treatment. Steroid therapy was stopped on July 2012 without GvHD reoccurrence. Complete donor medullar and blood chimerism was checked 4 months after alloSCT. Ciclosporine was stopped after 6 months. Nine months after alloSCT, an ALL relapse using WHO classification was diagnosed with the same clinical, cytological and molecular characteristics than at baseline. WBC was 20G/L with 53% peripheral blood blasts and 94% bone marrow blasts. Blood chimerism was 53% donor. No CNS involvement was observed. The patient was then treated with blinatumomab, after enrollment in the MT103-211 trial [13], which evaluate this bispecific T-cell engager antibody in adult patients with relapsed ALL. Blinatumomab was administered at target dose of 28 µg/day (9 µg/day for the first seven day of the first cycle) for a total of 5 cycles. A cycle consists of a continuous intravenous at a constant flow rate over four weeks, followed by a treatment free interval of 2 weeks. Seven days after treatment initiation, a febrile maculopapular rash occurred (Figure 1). Rash was located in the extremities, the back, the trunk and the face, with more than 50% of the affected skin and was not associated with itching. No mucosal, ocular or toxic epidermal necrolysis signs were found. No new drugs and immunomodulating agents have been introduced the days before starting blinatumomab. A skin biopsy was performed before corticosteroid treatment initiation. Polymerase chain reaction (PCR) CMV, EBV, VZV, HSV1, HSV2, HHV6 were negative in blood and in biopsy. Skin biopsy was fixed in formaldehyde and alcohol (PFA) for 2 hours and further processed for paraffin embedding. Hematoxylin-eosin stain was performed on 3 µm-thick paraffin sections. Pathological skin examination revealed epidermal basal layer damages with apoptotic bodies and intra-epidermal lymphocytes, characteristic of acute GvHD, without pathological signs of toxic erythroderma or infection. Immuno-stainings showed a predominance of T-cells in the dermal infiltrate (Supplement Figure 1). Double immunofluorescent stainings were performed on 5 µm-thick paraffin sections using a monoclonal mouse-anti-human CD20 antibody (clone L26, Dako, Glostrup, Denmark) and a polyclonal rabbit-anti-human CD3 antibody (Dako, Glostrup, Denmark) as primary antibodies. FITC-conjugated donkey-anti-mouse and Texas Red-conjugated donkey-anti-rabbit antibodies (both from Abcam, Cambridge, UK) were used as secondary antibodies. Immunofluorescent staining using antibody against human CD20 does not show B-cells, excluding blast-cells infiltration (Figure 2). XY-Fluorescent *in situ* Hybridization (FISH) was performed on 5 µm thick sections as described in Janin et al. [14]. After proteinase K digestion, FISH was performed with the use of CEP X (Spectrum green)/Y (Spectrum orange) DNA probes (Vysis, Downers Grove, IL). XY-FISH showed numerous XX cells in the epidermis, and presence of XY cells in the perivascular areas may correspond to donor T-cells (Figure 2). No clinical sign of intestinal tract or liver GvHD was observed. Systemic corticosteroids were given (dexamethasone, 16 mg/day), and six days after beginning of this treatment, rash disappeared, allowing decreasing corticosteroids during one month, without symptom recurrence. At Day 15 of blinatumomab treatment, a bone marrow examination was performed and showed no persistent leukemic blasts in poor rich marrow. Blood chimerism, using real-time quantitative PCR, became 100% donor. At the end of first cycle of blinatumomab treatment (Day 29), patient was in CR with normal blood counts, no bone marrow residual blasts, and MRD undetectable at the 10⁻⁵ sensitivity level. Blood and medullar chimerism was 100%

donor. To date, the 5th cycle of blinatumomab has been completed and the patient is still in molecular CR and 100% donor medullar chimerism, without GVHD recurrence.

Discussion and Conclusion

We report here the first case of acute GvHD triggered by blinatumomab treatment in a patient with B-lineage ALL relapsing after alloSCT. Blinatumomab is a member of a novel class of antibodies that redirect T cells for selective lysis of tumor cells [1]. In the allogeneic setting, it could also potentially engage donor T-cells

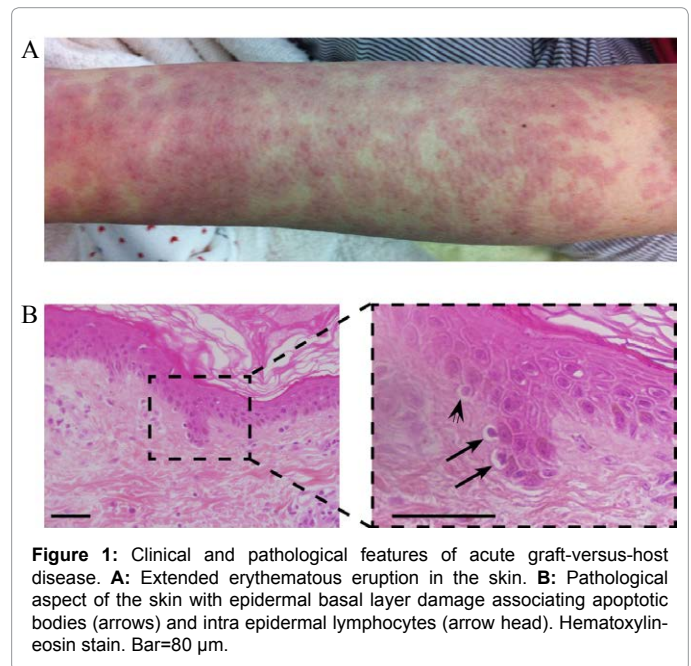


Figure 1: Clinical and pathological features of acute graft-versus-host disease. **A:** Extended erythematous eruption in the skin. **B:** Pathological aspect of the skin with epidermal basal layer damage associating apoptotic bodies (arrows) and intra epidermal lymphocytes (arrow head). Hematoxylin-eosin stain. Bar=80 µm.

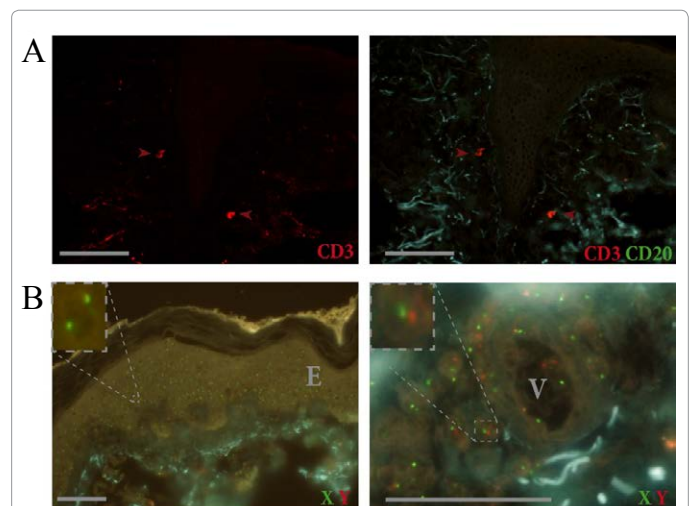


Figure 2: Immunofluorescent staining and FISH. **A:** Immunofluorescent staining using Texas Red labeled antibody directed against CD3 detects T-cells (arrow heads) in the superficial dermis. Double immunofluorescent staining using the same antibody directed against human CD3 and a FITC labeled antibody against human CD20 does not show B-cells associated to T-cells (red arrow heads) in the superficial dermis. Bar=80 µm. **B:** XY fluorescent *in situ* Hybridization shows on the left picture numerous XX cells in the epidermis (E) of the female patient, and on the right picture, presence of XY cells around a vessel (V) in the dermis. Bar=80 µm.

and reactivate donor memory T-cells implicated in GvHD and eventually in GvL. In this case, GvHD appeared one week after starting blinatumomab. GvHD prophylaxis was stopped since five months and no other immunomodulating agents were used in combination with blinatumomab, supporting a causal relationship with blinatumomab administration. Skin biopsy showed donor T-cells and no blasts infiltration. Concomitantly to GvHD, we observed peripheral blood and bone marrow blast clearance at day 15. At relapse, chimerism was 53% of donor cells and after the first blinatumomab cycle, blood chimerism was 100% of donor cell, reflecting a new expansion of the graft.

We thus hypothesize that the GvHD might have contributed to disease eradication in this patient, the allogeneic setting reinforcing the immunological efficacy of blinatumomab through a GvL effect. This observation supports further evaluation of blinatumomab in patients relapsing after alloSCT. Blinatumomab could actually represent a very interesting therapeutic approach in relapse and/or in prevention of relapse after alloSCT.

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A.M.R, N.D, E.L and T.C collected datas; A.J and C.L performed and analyzed tissue sections, CD3/CD20 and XY-FISH; D.B performed chimerism analysis; T.C and H.D designed the study; C.L, A.J, R.P.L, N.B, E.R, G.S, H.D, A.M.R and T.C edited the manuscript. A.M.R AND T.C wrote the manuscript.

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