Current Problems in Cancer 43 (2019) 222–227

Contents lists available at ScienceDirect

Current Problems in Cancer

journal homepage: www.elsevier.com/locate/cpcancer

Extramedullary relapse and discordant CD19 expression between bone marrow and extramedullary sites in relapsed acute lymphoblastic leukemia after blinatumomab treatment

Christos Demosthenous[a,*], Chrysavgi Lalayanni[a], Michalis Iskas[a], Vassiliki Douka[a], Nikoleta Pastelli[b], Achilles Anagnostopoulos[a]

[a] Department of Hematology and HCT Unit, General Hospital of Thessaloniki "George Papanicolaou", Thessaloniki, Greece
[b] Department of Pathology, General Hospital of Thessaloniki "George Papanicolaou", Thessaloniki, Greece

ARTICLE INFO

Keywords:
B acute lymphoblastic leukemia
Blinatumomab
Extramedullary relapse
CD19 expression

ABSTRACT

Blinatumomab, a bispecific T-cell engager antibody construct targeting CD19, has been shown to improve the outcome in patients with relapsed and/or refractory B-cell acute lymphoblastic leukemia. Treatment with blinatumomab demonstrated significant survival benefit over chemotherapy, supporting its use as a bridge therapy to allogeneic hematopoietic stem cell transplantation. Unfortunately, following initial response, approximately 50% of responding patients eventually relapse. At the time of failure, the majority of patients have CD19-positive blasts, yet a concerning number of CD19-negative relapses has been reported. In the data reported herein, we present an interesting case of a 42-year-old patient with primary refractory B-cell acute lymphoblastic leukemia who achieved complete morphologic remission after one cycle of blinatumomab as a single agent. Notably, and in the absence of extramedullary disease history, the re-

☆ **Funding:** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

* Correspondence to: Christos Demosthenous, Department of Hematology and HCT Unit, General Hospital of Thessaloniki "George Papanicolaou", 57010 Exohi, Thessaloniki, Greece.
E-mail address: christosde@msn.com (C. Demosthenous).

https://doi.org/10.1016/j.currproblcancer.2018.04.006

sponse in marrow coincided with the emergence of CD19-positive extramedullary relapse including sites of previous punctures for blood and bone marrow samples, as confirmed by biopsy, as well as parenchymal organs (eg breast and lung). During the second cycle of blinatumomab, a CD19-negative morphological relapse emerged. The loss of CD19 was a transient event, as leukemic cells partially regained it after chemotherapy. This study illustrates a challenging situation of relapsed and refractory acute lymphoblastic leukemia complicated with extramedullary disease after exposure to a bispecific T-cell engager antibody, such as blinatumomab. Physicians should maintain a high level of suspicion for the evolution of extramedullary leukemia. This pattern of resistance and/or relapse to blinatumomab resembles the graft-versus-leukemia effect after allogeneic transplantation (stronger in blood and marrow than in other tissues). Mechanisms of resistance to blinatumomab are not yet clear. Combination treatments for refractory patients and those at high risk for exramedullary disease may warrant future assessment.

Introduction

With current intensive chemotherapy, 90% of adult patients with acute lymphoblastic leukemia (ALL) achieve complete remission; however, more than 50% of the patients will eventually relapse.[1] Large retrospective studies demonstrated significant differences in terms of prognosis, with particularly poor response rates in early or refractory relapses.[2] Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative option for adult patients with refractory ALL and achievement of complete remission is an essential bridging step before allo-HSCT.[2,3] Precursor B-cell ALL (B-ALL) is the most common phenotype accounting for 80% of cases,[4] while the surface antigen CD19 is expressed on the surface of more than 90% of B-cell precursor ALL blasts.[5] Blinatumomab is a bispecific T-cell engager antibody construct which binds simultaneously to CD3-positive cytotoxic T cells and to CD19-positive B cells, allowing the patient's endogenous T cells to recognize and eliminate CD19-positive ALL blasts.[6] Treatment of patients with refractory or relapsed B-ALL with blinatumomab showed a significant survival benefit over chemotherapy,[7] thus supporting its use as a bridge to allogeneic stem cell transplant.

Case presentation

A 42-year-old Caucasian woman was diagnosed with precursor B-ALL on April 2017 ($CD10^+$, $CD19^+$, $HLA\text{-}DR^+$, cytoplasmic IgM^+) with a complex karyotype. She initially received treatment with cyclophosphamide, vincristine, doxorubicin and dexamethasone (DXM) achieving only a partial response. Accordingly, she later failed reinduction plus L-asparaginase as well as treatment with high dose of cytarabine (Ara-C) in combination with etoposide and DXM and methotrexate in combination with high dose Ara-C. Salvage chemotherapy with FLAG-Ida (fludarabine, high dose Ara-C, granulocyte colony-stimulating factor and idarubicin) was also ineffective (Fig 1a) and at that time, our decision was to test blinatumomab as a bridge to allo-HSCT.

Because of high blast count, she initially received a DXM pre-phase followed by continuous intravenous infusion of blinatumomab (9 μg/d for the first 7 days and 28 μg/d thereafter) for 4

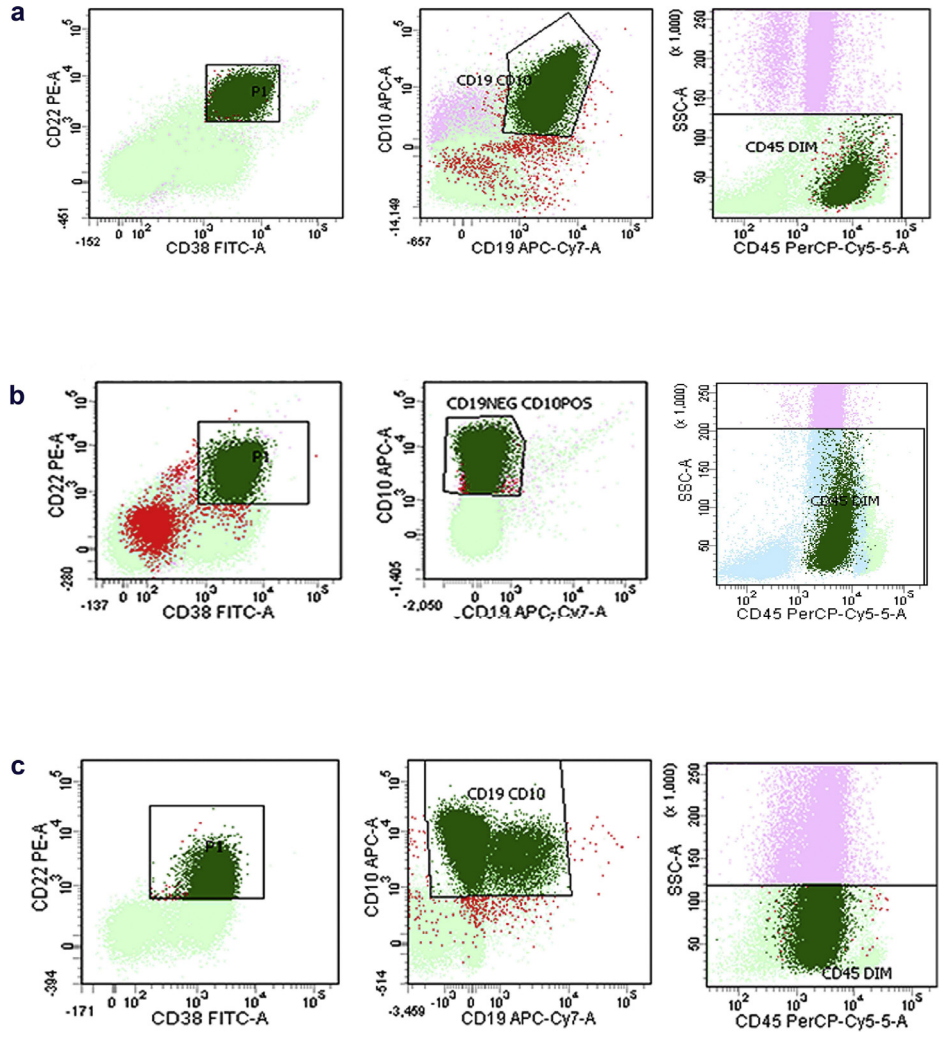

Fig. 1. Bone marrow flow cytometry revealing: (a) CD19 positive, (b) CD19 negative population, and (c) double population of CD19 negative and CD19 positive B-lymphoblasts.

weeks and a 2-week wash-out interval. Leukemic cells disappeared from peripheral blood by the second week of treatment and by the end of the first cycle the patient achieved a complete morphologic remission in the bone marrow for the first time. Minimal residual disease quantification by flow cytometry demonstrated a low proportion of CD19 expressing blasts (0.1%).

However, at the same time, subcutaneous nodules appeared at sites of previous punctures for venous blood and marrow samples (sternal manubrium, across the cephalic vein, venous catheter insertion site). Physical examination revealed a nodular mass on her left breast. Moreover, chest x-ray and chest computed tomography showed pulmonary nodules without any signs of infection. Biopsy of a subcutaneous nodule confirmed extramedullary relapse from CD19 positive ALL cells Fig 2 and a second blinatumomab cycle was initiated. Unfortunately, nodular enlargement progressed despite treatment, while bone marrow aspiration revealed morphological relapse, surprisingly this time, by CD19-negative ALL cells (Fig 1b). Cerebrospinal fluid evaluation after lumbar puncture ruled out central nervous system involvement and karyotype was

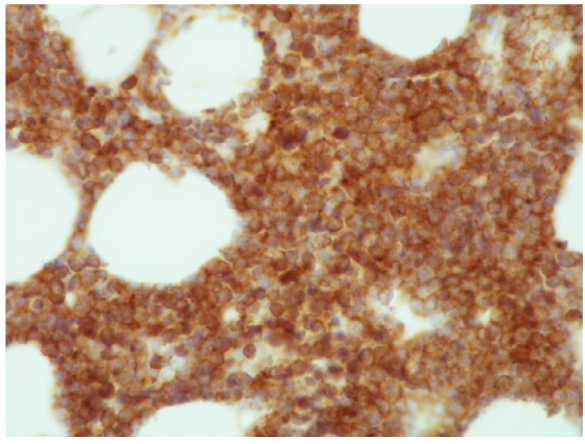

Fig. 2. CD19 positive stain in immunohistochemistry analysis of subcutaneous nodule biopsy.

unchanged. Rescue chemotherapy was administered to the patient, using cyclophosphamide, vincristine, DXM, mitoxantrone, Ara-C, methotrexate, and L-asparaginase. Despite a considerable size reduction of nodules at all sites, leukemia persisted in the bone marrow. This time, flow cytometric analysis showed reappearance of CD19 in a percentage of leukemic cells (Fig 1c). The patient received palliative treatment and succumbed to her disease a month later.

Discussion

Blinatumomab is a bispecific T-cell engaging monoclonal antibody that redirects cytotoxic T cells against leukemic B cells with its anti-CD3 and anti-CD19 arms. Treatment with blinatumomab contributed in significantly longer overall survival than conventional chemotherapy among adult patients with relapsed or refractory B-cell precursor ALL.[7] However, the CD19-negative ALL relapses ranging between 10% and 30% in retrospective and phase 2 studies,[8] currently represent a major treatment challenge. Leukemic cells slip out of the reach of the antibody by becoming CD19 negative. Assessment of the CD19 status at time of relapse is essential, as previous blinatumomab therapy may enhance drug resistance or even exclude a number of patients from the potential benefit of subsequent chimeric antigen receptor T-cell therapy.

Our case is the first report on discordant CD19 expression between marrow and extramedullary sites, providing valuable insights on the pathophysiology of blinatumomab-associated ALL resistance and relapse.

Minimal residual disease assessment in our patient after the first cycle showed a low frequency of CD19-expressing leukemic blasts. However, flow cytometry reevaluation during the second cycle detected a CD19-negative predominant blast population. Pre-existence of a minor CD19-negative population in the leukemia bulk has been proposed as a mechanism of resistance to blinatumomab and subsequent emergence of a CD19-negative relapse.[9,10]

It is of interest that our patient gradually lost CD19 expression on ALL blast cells and regained it after subsequent treatment, implying that the CD19 loss was a transient and rather isolated molecular event and not a relapse from a CD19 negative leukemic stem cell. The preserved ALL phenotypes (except for CD19 negativity) clearly exclude a myeloid shift as resistance mechanism.

Other tumor cell immune escape mechanisms have also been demonstrated as a disrupted CD19 membrane export in the postendoplasmic reticulum compartment.[10] Moreover, the emergence of extramedullary relapse post blinatumomab in rather unusual sites has been also described by others. In a retrospective study of relapsed and/or refractory B-ALL patients treated with blinatumomab, extramedullary relapse and/or resistance were quite common, affecting

one-third of treated patients. However most of them had a history of extramedullary leukemia, and this proved to be a significant risk factor for extramedullary relapse after blinatumomab. The expression of CD19 antigen in extramedullary sites was not reported.[8] Also, the fate of these CD19 negative relapses has not been reported, so it is unknown if any of them rendered CD19 positive again with time.

The pathogenesis of extramedullary disease postblinatumomab treatment remains poorly understood. Why extramedullary sites are more resistant to blinatumomab over bone marrow? Is it a matter of pharmacokinetics and lower drug concentrations or an immune-related phenomenon? In our case, it seemed that leukemic cell extravasation generated immunologic sanctuaries at sites of needle punctures in soft tissues. This pattern of relapse, including the presence of leukemic blasts in unusual sites such as the breast and possibly the lungs, resembles the T cell-mediated graft-versus-leukemia effect in the context of allo-HSCT, which is less prominent at extramedullary sites than bone marrow. Indeed, relapse at unusual sites was mostly described in patients with acute leukemia after allo-HSCT[11] and a lack of donor T cells has been reported at relapsed extramedullary sites.[12] In the allo-HSCT context, several immune escape mechanisms have been proposed, such as induction of T-cell anergy by ALL cells,[13] resistance to natural killer cells,[14] and reduced expression of costimulatory molecules.[15] A case of graft-versus-leukemia induction by blinatumomab for relapsed ALL after HSCT has been reported[16] supporting the key role of functioning T cells in the effective CD19 targeting by blinatumomab. The recruitment of T cells to nonhematopoietic tissues by blinatumomab may be deficient or delayed thus favoring the generation of immune-privileged sites. In the study reported herein, we show that extramedullary relapse, even in the absence of previous history, can occur during blinatumomab therapy and that CD19 loss may be a transient event. A high suspicion level for the emergence of extramedullary leukemia and biopsy of suspected lesions are strongly recommended.

Due to immune escape of ALL in extramedullary sites or by loss of CD19 expression, a considerable number of patients cannot be bridged to allo-HSCT. Mechanisms involved in blinatumomab resistance are not yet clear. Further assessment of blinatumomab earlier in the course of the disease or/and combination treatments for refractory patients and those at high risk for exramedullary disease may warrant extensive evaluation.

References

1. Linker C, Damon L, Ries C, et al. Intensified and shortened cyclical chemotherapy for adult acute lymphoblastic leukemia. *J Clin Oncol*. 2002;20:2464–2471.
2. Gokbuget N, Stanze D, Beck J, et al. Outcome of relapsed adult lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic factors, and performance of stem cell transplantation. *Blood*. 2012;120:2032–2041.
3. Fielding AK, Richards SM, Chopra R, et al. Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ECOG 2993 study. *Blood*. 2007;109:944–950.
4. Swerdlow SH. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon: International Agency for Research on Cancer; 2008.
5. Raponi S, De Propris MS, Intoppa S, et al. Flow cytometric study of potential target antigens (CD19, CD20, CD22, CD33) for antibody-based immunotherapy in acute lymphoblastic leukemia: analysis of 552 cases. *Leuk Lymphoma*. 2011;52:1098–1107.
6. Loffler A, Gruen M, Wuchter C, et al. Efficient elimination of chronic lymphocytic leukaemia B cells by autologous T cells with a bispecific anti-CD19/anti-CD3 single-chain antibody construct. *Leukemia*. 2003;17:900–909.
7. Kantarjian H, Stein A, Gokbuget N, et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. *N Engl J Med*. 2017;376:836–847.
8. Aldoss I, Song J, Stiller T, et al. Correlates of resistance and relapse during blinatumomab therapy for relapsed/refractory acute lymphoblastic leukemia. *Am J Hematol*. 2017;92:858–865.
9. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med*. 2013;368:1509–1518.
10. Braig F, Brandt A, Goebeler M, et al. Resistance to anti-CD19/CD3 BiTE in acute lymphoblastic leukemia may be mediated by disrupted CD19 membrane trafficking. *Blood*. 2017;129:100–104.
11. Lee KH, Lee JH, Choi SJ, et al. Bone marrow vs extramedullary relapse of acute leukemia after allogeneic hematopoietic cell transplantation: risk factors and clinical course. *Bone Marrow Transplantat*. 2003;32:835–842.
12. Goldberg SL, Mangan KF, Klumpp TR, et al. Lack of a graft-versus-leukemia effect in an immunologically privileged sanctuary site. *Bone Marrow Transplant*. 1994;14:180–181.
13. Cardoso AA, Schultze JL, Boussiotis VA, et al. Pre-B acute lymphoblastic leukemia cells may induce T-cell anergy to alloantigen. *Blood*. 1996;88:41–48.

14. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science*. 2002;295:2097–2100.
15. Han P, Story C, McDonald T, et al. Immune escape mechanisms of childhood ALL and a potential countering role for DC-like leukemia cells. *Cytotherapy*. 2002;4:165–175.
16. Khan MW, Gul Z. Blinatumomab may induce graft versus host leukemia in patients with pre-B ALL relapsing after hematopoietic stem cell transplant. *Clin Case Rep*. 2016;4:743–746.