Case Report

Extraordinarily elevated CD33 expression in $CD56^+CD3^-$ cells in the bone marrow of a patient with relapsed acute myeloid leukemia: a case report

Dongyao Wang[1,2], Liangquan Geng[1], Huilan Liu[1,2], Yongjun Fang[3], Zimin Sun[1,2]

[1]Department of Hematology, the First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, China; [2]Blood and Cell Therapy Institute, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, China; [3]Department of Hematology and Oncology, Children's Hospital of Nanjing Medical University, Nanjing, China

Correspondence to: Zimin Sun; Dongyao Wang. Department of Hematology, the First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, 443 Huangshan Road, Hefei 230027, China. Email: zmsun@ustc.edu.cn; dywsn@ustc.edu.cn.

Abstract: Based on genetic risk allogeneic stem cell transplantation (allo-SCT) is the only curative treatment for some forms of acute myeloid leukemia (AML). However, post-transplantation relapse remains a frequent cause of transplantation failure. Natural killer (NK) cells and CD8+ T cells are important effector lymphocytes with pivotal roles in tumor surveillance and anti-tumor immune response. In this study, a 14-year-old female patient with AML was treated with allogeneic peripheral blood stem cell transplantation (allo-PBSCT). Bone marrow relapse was found 6 months later. Thereafter, the patient was treated with DAE (daunorubicin, cytosine arabinoside, and etoposide), followed by IAE (idarubicin, cytosine arabinoside, and etoposide), and then MA (mitoxantrone and cytosine arabinoside) regimens. A series of experiments including Wright-Giemsa stain analyses, cytogenetic analysis and flow cytometry were conducted to investigate the characteristic of the patient. Although there was a short remission after the DAE regimen, the patient experienced another relapse after finishing the MA regimen. The $CD56^+CD3^-$ cells in the bone marrow showed severely impaired activation and anti-tumor function, while extraordinarily increased CD33 expression. Moreover, the proportion of multifunctional effector $CD8^+$ T cells remained stable, though they had high PD-1 expression. These findings revealed the dysfunction of abnormal $CD56^+CD3^-$ cells with high CD33 expression, which might be targetable and related to the relapsed/refractory AML after allo-SCT.

Keywords: Case report; natural killer (NK) cells; allogeneic stem cell transplantation (allo-SCT); acute myeloid leukemia (AML); CD33

Submitted Apr 27, 2021. Accepted for publication Aug 13, 2021.
doi: 10.21037/tcr-21-733
View this article at: https://dx.doi.org/10.21037/tcr-21-733

Introduction

Based on genetic risk allogeneic stem cell transplantation (allo-SCT) is the only curative treatment for some forms of acute myeloid leukemia (AML), most patients still ultimately experience relapse (1,2). Knowledge of the mechanisms underlying treatment resistance and relapse remains limited. There are several potential mechanisms underlying immune evasion post allo-HSCT. For example, AML cells cannot be well recognized if there is impaired expression or genomic loss of human leukocyte antigen (HLA) (2). Upregulation of immune-checkpoint molecules, such as PD-1 can contribute to the AML immune escape. The production of some anti-inflammatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor β (TGF-β) may also play a role in relapse. Moreover, anti-AML immune responses are lowered via reduced production of IL-15, which is important for NK cell activation (2,3).

Detection of minimal residual disease (MRD) by flow

cytometry in patients with AML has been widely used to guide clinical management. The expression of CD33, a member of the sialic acid-binding immunoglobulin-like lectin family and assumed to be restricted to the myeloid lineage of immune cells, is generally used in MRD detection (4,5). Natural killer (NK) cells originate from hematopoietic stem cells (HSCs), primarily in the bone marrow, and play crucial roles in tumor surveillance and controlling tumor invasion (6,7). The NK cell function is finely tuned by activating and inhibitory receptors, allowing NK cells to discriminate between normal and aberrant cells (8). NK cells can kill AML directly via cytotoxicity and cytokines, such as IFN-γ, TNF-α, and CD107a. Therefore, it is important to maintain function of NK cells. A recent study reported that CD33 expression on NK cells could be a potential confounder for MRD detection, although the percentage of $CD33^+$ NK cells was low (4). However, little is known about the interrelationships between extraordinarily high CD33 expression on $CD56^+CD3^-$ cells and the impaired anti-tumor function of $CD56^+CD3^-$ cells, as well as other immune characteristics in the bone marrow of patients with relapsed/refractory AML.

Here, we examined the characteristics and the impaired function of $CD56^+CD3^-$ cells and demonstrated the abnormal proportion of $CD33^+$ $CD56^+CD3^-$ cells in the bone marrow of a patient with relapsed AML after allo-HSCT and remission induction, to ascertain whether abnormal $CD56^+CD3^-$ cells are related to relapse.

We present the following article in accordance with the CARE reporting checklist (available at https://dx.doi.org/10.21037/tcr-21-733).

Case presentation

The case report was approved by the institutional review board of the First Affiliated Hospital of University of Science and Technology of China (2021-N(H)-120). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal. Patient data were collected via retrospective chart review, which included clinical characteristics; comprehensive hematopathologic examination, such as Wright-Giemsa stain analyses, cytogenetic analysis and flow cytometry (9) (Appendix 1); and clinical outcome.

The characteristics of the 14-year-old girl were shown in (Table S1). The girl was diagnosed with AML-M1 in the First Affiliated Hospital of Anhui Medical University, in May 2019. Her karyotype was 46 XX, t(2; 17)(q31; q25), +8, t(16; 21)(p11; q22). PML/RARA and AML1/ETO fusion genes were not present. Then she received an allogeneic peripheral blood stem cell transplant (allo-PBSCT) from the matched sibling donor (MSD) in Children's Hospital of Nanjing Medical University (15/November/2019). Relapse was confirmed in the bone marrow 6 months later. Thereafter, remission was induced using the DAE (daunorubicin, cytosine arabinoside, and etoposide) regimen once a month. This patient received daunorubicin (20 mg/m^2/day) for 3 days (day 1–3), etoposide (VP-16, 120 mg/m^2/day) for 5 days (day 1–5), and cytarabine (100 mg/m^2/d) twice a day for 7 days (day 1–7). On 21/June/2020, MRD was found to be negative and the patient achieved complete remission (CR). The patient was then given the IAE (idarubicin, etoposide, and cytosine arabinoside) regimen: idarubicin (40 mg/m^2/day) for 3 consecutive days, then etoposide (120 mg/m^2/day) for 5 consecutive days, followed by cytarabine (100 mg/m^2/d) twice a day for 7 consecutive days. On 29/July/2020, a secondary breast tumor was found. Thereafter, the patient was treated with the MA (mitoxantrone and cytosine arabinoside) regimen once a month for 2 months: mitoxantrone (10 mg/m^2/day) for 3 consecutive days and cytarabine (100 mg/m^2/d) twice a day for 7 consecutive days. During the MA regimen, the patient also received radiotherapy 12 times. Relapse was confirmed again on 14/October/2020 in bone marrow after the last radiotherapy with the treatment of anti-CD38 antibody (*Figure 1*).

Through morphologic analysis of the leukemia cells in the bone marrow, we found that primitive cells accounted for 74% of the nucleated cells (*Figure 2A*). Thereafter, to investigate the immunological characteristics and the possible mechanism underlying the AML relapse, we isolated mononuclear cells from the bone marrow and systematically analyzed molecular expression related to NK cell function. The percentage of $CD56^+CD3^-$ cells was nearly 90% when gated from $CD45^+$ cells. Furthermore, CD33, generally assumed to be restricted to the myeloid lineage of immune cells [and not to erythrocytes, platelets, T cells, B cells, or NK cells (4)] was extraordinarily highly expressed on the $CD56^+CD3^-$ cells (*Figure 2B*). The antibody used in this study were shown in (Table S2). As

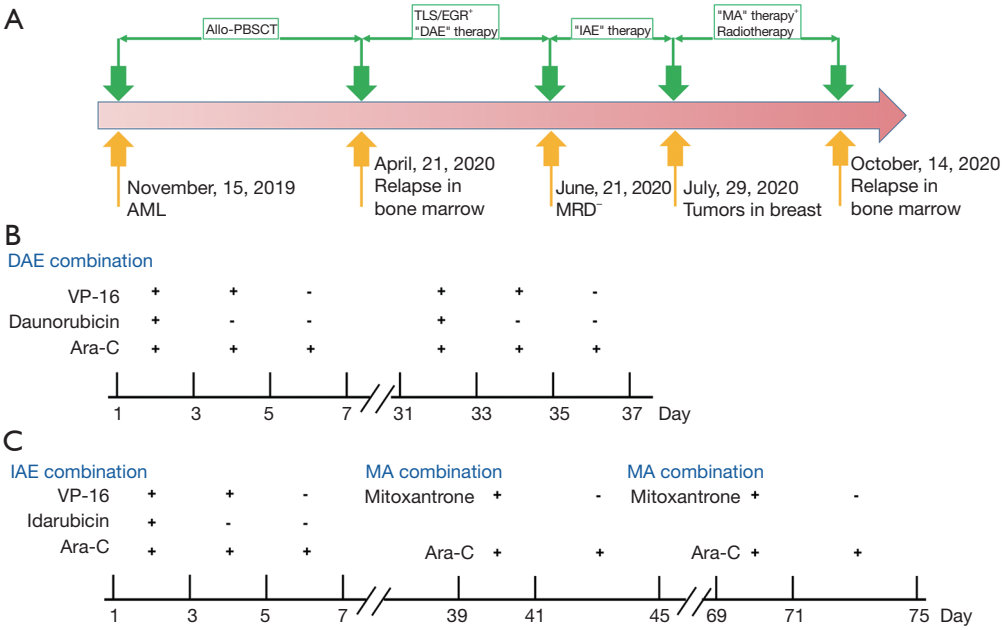

Figure 1 Disease progression and treatment course. A 14-year-old girl with acute myeloid leukemia (AML) received allogeneic peripheral blood stem cell transplantation (allo-PBSCT) from the matched sibling donor (MSD) on November 15, 2019. Relapse was confirmed in the bone marrow 6 months later (A). Thereafter, remission was induced using the DAE (daunorubicin, cytosine arabinoside, and etoposide) regimen (B). MRD was found to be negative 2 months later, which was followed by the IAE (idarubicin, cytosine arabinoside, etoposide) regimen (C). On July 29, 2020, a secondary breast tumor was found. Next, the patient was treated with the MA (mitoxantrone and cytosine arabinoside) regimen, anti-CD38 antibody therapy, and radiotherapy. Relapse was confirmed again in the bone marrow on October 14, 2020 (A-C). The characters "+" and "–" represented the therapy "with" or "without" the indicated medicine, respectively.

the activation of the $CD56^+CD3^-$ cells was limited, we then analyzed CD69, CD38, NKG2D and NKp30 expression and found that their expressions were low (*Figure 2C,2D*). We characterized four populations of $CD56^+CD3^-$ cells defined by CD11b and CD27, which represent the distinct stages of human NK cells from different tissues (10). The majority of $CD56^+CD3^-$ cells had the $CD11b^-CD27^-$ phenotype, which is reported to be an immature phenotype (11). Additionally, the $CD56^+CD3^-$ cells had low expression of NKG2A, KLRG1, and PD-1 (*Figure 2E*). Moreover, human NK cells can be classified into two main classic subsets dependent on CD56 and CD16: $CD56^{bright}CD16^-$ and $CD56^{dim}CD16^+$ NK cells (12). The former is considered efficient cytokine producers. The proportion of these cells was nearly 99% (*Figure 2E*). Then, to demonstrate the effector functions of the NK cells, we stimulated the mononuclear cells with PMA and ionomycin in the presence of monensin for 4 h. We found that the $CD56^+CD3^-$ cells from the bone marrow exhibited an extremely low percentage (<0.1%) of polyfunctional effector IFN-γ^+TNF-α^+, IFN-γ^+CD107a$^+$, and IFN-γ^+GranzymeB$^+$ cells (*Figure 2F*). $CD8^+$ T cells also play an important anti-tumor role, here we demonstrated that the proportion of polyfunctional effector IFN-γ^+TNF-α^+, IFN-γ^+CD107a$^+$ and IFN-γ^+GranzymeB$^+$ $CD8^+$ T cells was normal (*Figure 2G*). This implied that the anti-tumor effect was heavily impaired in $CD56^+CD3^-$ cells but not in $CD8^+$ T cells. Furthermore, we demonstrated that Ki67 was highly expressed on these abnormal $CD56^+CD3^-$ cells, but not on T cells (*Figure 2H*). Moreover, we verified the low expression of the early-activation marker CD69 on T cells (*Figure 2I*) and the high expression of PD-1 on $CD8^+$ T cells (*Figure 2J*).

Discussion

Recently, Eckel *et al.* reported that CD33 expression on NK cells in AML was a potential confounder for MRD detection, and nearly 18% of MRD^+ patients were CD33 positive. Furthermore, the $CD33^+$ NK population accounted for a mean of 11.4% of NK cells (median 11.9%, range,

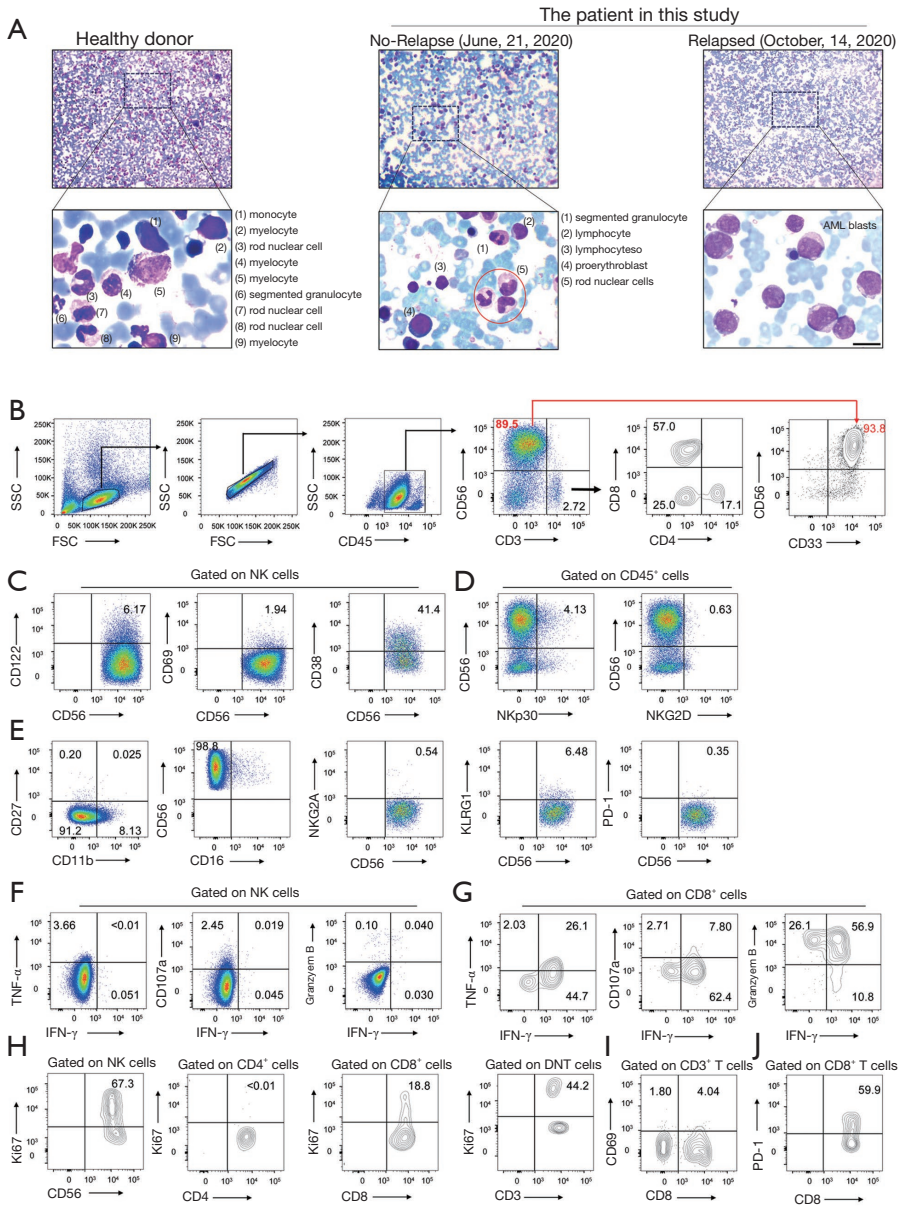

Figure 2 Extraordinarily increased CD33$^+$CD56$^+$CD3$^-$ cells with poor function in bone marrow at relapse. Mononuclear cells were derived from the bone marrow of the patient with relapsed acute myeloid leukemia (AML). (A) Wright-Giemsa stain analyses of the leukemia cells in bone marrow smears before (left) and at (right) AML relapse. Scale bar = 20 μm. (B) Flow cytometry gating strategy for CD56$^+$CD3$^-$, CD8$^+$ T, and CD4$^+$ T cells, derived from bone marrow samples. (C) Density plots of CD122, CD69, and CD38 expression in gated CD56$^+$CD3$^-$ cells. (D) Density plots of NKp30 and NKG2D expression in gated CD45$^+$ cells. (E) Density plots of CD11b, CD16, NKG2A, KLRG1, and PD-1 expression in gated CD56$^+$CD3$^-$ cells. (F) Intracellular cytokine staining of IFN-γ$^+$TNF-α$^+$, IFN-γ$^+$CD107a$^+$, and IFN-γ$^+$GranzymeB$^+$ CD56$^+$CD3$^-$ cells in gated NK cells. Mononuclear cells from the bone marrow were stimulated with phorbol 12-myristate 13-acetate (PMA) (50 ng/mL; Sigma) and ionomycin (1 μg/mL; Calbiochem) in the presence of monensin (10 μg/mL; Sigma) for 4 h. (G) Intracellular cytokine staining of IFN-γ$^+$TNF-α$^+$, IFN-γ$^+$CD107a$^+$, and IFN-γ$^+$GranzymeB$^+$ CD8$^+$ T cells in gated CD8$^+$ T cells. Mononuclear cells from bone marrow were stimulated with PMA (50 ng/mL) and ionomycin (1 μg/mL) in the presence of monensin (10 μg/mL) for 4 h. (H) Density plots of Ki67 expression in gated NK cells and CD4$^+$ T cells, CD8$^+$ T cells, and double negative T (DNT) cells (CD4$^-$CD8$^-$). (I) Density plot of CD69 expression in gated CD3$^+$ T cells. (J) Density plot of PD-1 expression in gated CD8$^+$ T cells.

8.0–15.3%) (4). Here, we demonstrated that CD33 might not only be a potential confounder for MRD detection, but also a targetable molecule in our relapsed/refractory AML patient. We verified that the CD33$^+$CD56$^+$CD3$^-$ population accounted for 93.8% of the CD56$^+$CD3$^-$ cells. Additionally, we found low expression of the activating receptors NKp30 and NKG2D. Importantly, we observed that CD56$^+$CD3$^-$ cells had downregulated granzyme B, TNF-α, CD107a, and IFN-γ, suggesting impaired functionality. In addition, we speculated that these markers might be useful for predicting immune states in the tumor microenvironment. Given that several CD33-targeted therapies have been used in AML, and that they can mediate killing of AML blasts by CD56$^+$CD3$^-$ cells (13-15), more attention should be paid to the influence of CD56$^+$CD3$^-$ cells expressing CD33.

Taken together, these results showed that CD56$^+$CD3$^-$ cells in the bone marrow of this patient with relapsed AML had abnormally high CD33 expression and severely impaired anti-tumor function, however, the immune function of CD8$^+$ T cells in the bone marrow seemed to be normal. This study provides new perspectives on the mechanism underlying relapse after allo-SCT, and treatment with anti-CD33 monoclonal antibody may be worthy of attention.

Acknowledgments

The authors thank their colleagues at the First Affiliated Hospital of the University of Science and Technology of China for collaboration on the care of the patient. In addition, we appreciate the help from The Charlesworth Group, a high-quality publishing services company, for English language editing.

Funding: This work was supported by China postdoctoral science foundation (2020M671910), and the Fundamental Research Funds for the Central Universities (WK9110000168, and WK9110000001).

Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at https://dx.doi.org/10.21037/tcr-21-733

Peer Review File: Available at https://dx.doi.org/10.21037/tcr-21-733

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/tcr-21-733). All authors report that this work was supported by China postdoctoral science foundation (2020M671910), and the Fundamental Research Funds for the Central Universities (WK9110000168, and WK9110000001). The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The case report was approved by the institutional review board of the First Affiliated Hospital of University of Science and Technology of China (2021-N(H)-120). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

References

1. Bullinger L, Ehrich M, Döhner K, et al. Quantitative DNA methylation predicts survival in adult acute myeloid leukemia. Blood 2010;115:636-42.
2. Christopher MJ, Petti AA, Rettig MP, et al. Immune Escape of Relapsed AML Cells after Allogeneic Transplantation. N Engl J Med 2018;379:2330-41.
3. Carlsten M, Järås M. Natural Killer Cells in Myeloid Malignancies: Immune Surveillance, NK Cell Dysfunction, and Pharmacological Opportunities to Bolster the Endogenous NK Cells. Front Immunol 2019;10:2357.
4. Eckel AM, Cherian S, Miller V, et al. CD33 expression on natural killer cells is a potential confounder for residual

disease detection in acute myeloid leukemia by flow cytometry. Cytometry B Clin Cytom 2020;98:174-8.
5. Freeman SD, Kelm S, Barber EK, et al. Characterization of CD33 as a new member of the sialoadhesin family of cellular interaction molecules. Blood 1995;85:2005-12.
6. Sinha C, Cunningham LC. An overview of the potential strategies for NK cell-based immunotherapy for acute myeloid leukemia. Pediatr Blood Cancer 2016;63:2078-85.
7. Locatelli F, Pende D, Falco M, et al. NK Cells Mediate a Crucial Graft-versus-Leukemia Effect in Haploidentical-HSCT to Cure High-Risk Acute Leukemia. Trends Immunol 2018;39:577-90.
8. Li H, Er Saw P, Song E. Challenges and strategies for next-generation bispecific antibody-based antitumor therapeutics. Cell Mol Immunol 2020;17:451-61.
9. Zheng X, Qian Y, Fu B, et al. Mitochondrial fragmentation limits NK cell-based tumor immunosurveillance. Nat Immunol 2019;20:1656-67.
10. Fu B, Zhou Y, Ni X, et al. Natural Killer Cells Promote Fetal Development through the Secretion of Growth-Promoting Factors. Immunity 2017;47:1100-1113.e6.
11. Fu B, Li X, Sun R, et al. Natural killer cells promote immune tolerance by regulating inflammatory TH17 cells at the human maternal-fetal interface. Proc Natl Acad Sci U S A 2013;110:E231-40.
12. Forconi CS, Cosgrove CP, Saikumar-Lakshmi P, et al. Poorly cytotoxic terminally differentiated CD56negCD16pos NK cells accumulate in Kenyan children with Burkitt lymphomas. Blood Adv 2018;2:1101-14.
13. Sweeney C, Vyas P. The Graft-Versus-Leukemia Effect in AML. Front Oncol 2019;9:1217.
14. Walter RB. Investigational CD33-targeted therapeutics for acute myeloid leukemia. Expert Opin Investig Drugs 2018;27:339-48.
15. Jitschin R, Saul D, Braun M, et al. CD33/CD3-bispecific T-cell engaging (BiTE®) antibody construct targets monocytic AML myeloid-derived suppressor cells. J Immunother Cancer 2018;6:116.

Cite this article as: Wang D, Geng L, Liu H, Fang Y, Sun Z. Extraordinarily elevated CD33 expression in $CD56^{+}CD3^{-}$ cells in the bone marrow of a patient with relapsed acute myeloid leukemia: a case report. Transl Cancer Res 2021;10(11):5022-5027. doi: 10.21037/tcr-21-733

Appendix 1 Wright-Giemsa stain analyses and flow cytometry

Bone marrow aspirates from the patient were treated with Wright-Giemsa stain (cat. no. G1020; Solarbio) according to the manufacturer's protocol and the morphological changes were observed under an optical microscope. Mononuclear cells were firstly derived from the bone marrow and the mononuclear cell suspensions were then treated with mouse serum to block the binding to non-specific Fc receptors. Next, to assess the surface and intracellular markers, the cells were stained with anti-human monoclonal antibodies, from BD Biosciences and BioLegend, in accordance with the manufacturer's instructions. Intracellular cytokine staining involved 4 h of stimulation of the cells with phorbol 12-myristate 13-acetate (PMA) (50 ng/mL; Sigma) and ionomycin (1 μg/mL; Calbiochem) in the presence of monensin (10 μg/mL; Sigma). Data were collected using an FCM LSR II flow cytometer (BD Biosciences, USA) and analyzed using FlowJo software (Tree Star, USA).

Table S1 Baseline characteristics of AML patient

Characteristic	Value
Gender (M/F)	F
Age, years	14.0
White blood cell count, $\times10^9$/L	26.8
Red blood cell count, $\times10^{12}$/L	2.95
Nucleated red blood cell count, $\times10^9$/L	0.02
Nucleated red blood cell (%)	0.10
Immature reticulocytes (%)	29.4
Platelet count, $\times10^9$/L	55.0
Lymphocyte count, $\times10^9$/L	3.19
Lymphocytes (%)	11.9
Neutrophil count, $\times10^9$/L	3.54
Neutrophils (%)	13.2
Monocyte count, $\times10^9$/L	3.54
Monocytes (%)	74.7
Eosinophil count, $\times10^9$/L	0.02
Eosinophils (%)	0.10
Basophil count, $\times10^9$/L	0.03
Basophils (%)	0.10
Hemoglobin (g/L)	87.0

https://dx.doi.org/10.21037/tcr-21-733

Table S2 Antibodies used in flow cytometric analysis

Antibody	Brand	Cat. No.
APC-CY7 Mouse Anti-Human CD3	BD Bioscience	Cat# 557832, RRID:AB_396890
FITC Mouse Anti-Human CD16	BD Bioscience	Cat# 555406, RRID:AB_395806
FITC Mouse Anti-Human CD27	BD Bioscience	Cat# 555440, RRID:AB_395833
APC-Cy7 Mouse Anti-Human CD14	BD Bioscience	Cat# 557831
APC Mouse Anti-Human CD107a	BD Bioscience	Cat# 560664, RRID:AB_396135
PerCP-CY5.5 Mouse Anti-Human CD38	BD Bioscience	Cat# 551400, RRID:AB_394184
FITC Mouse Anti-Human CD4	BD Pharmingen	Cat# 555346, RRID:AB_395751
PE-Cy7 Mouse Anti-Human CD8	BD Pharmingen	Cat# 557746, RRID:AB_396852
FITC Mouse Anti-Human IFN-γ	BD Bioscience	Cat# 554700, RRID:AB_395517
PE Mouse Anti-Human CD69	BD Bioscience	Cat# 555531, RRID:AB_395916
PE Mouse Anti-Human PD-1	BD Bioscience	Cat#560795
7-AAD	BD Bioscience	Cat# 559925
APC Mouse Anti-Human HLA-DR	BD Bioscience	Cat# 559866, RRID:AB_398674
PerCP-CY5.5 Mouse Anti-Human CD123	BD Bioscience	Cat# 558714, RRID:AB_891359
FITC Mouse anti-human Lineage Cocktail	BD Bioscience	Cat# 348801, RRID:AB_10612570
APC-CY7 Mouse Anti-Human CD11c	Biolegend	Cat# 337218
PE Mouse Anti-Human NKG2D	BD Bioscience	Cat# 557940
PE Mouse Anti-Human TNF-α	BD Bioscience	Cat# 559321, RRID:AB_397219
Alexa Fluor® 647 Mouse Anti-Human NKP30	BD Bioscience	Cat# 558408, RRID:AB_398454
FITC Mouse IgG1, κ	BD	Cat# 555748, RRID:AB_396090
PE Mouse IgG1, κ	BD	Cat# 55749, RRID:AB_396091
PerCP-Cy5.5 Mouse IgG1, κ	BD	Cat# 552834, RRID:AB_394484
PE-Cy7 Mouse IgG1, κ	BD	Cat# 557872, RRID:AB_396914
Alexa Fluor 647 Mouse IgG1, κ	BD	Cat# 557714, RRID:AB_396823
APC-Cy7 Mouse IgG1, κ	BD	Cat# 557873, RRID:AB_396915
APC-Cy7 Mouse IgG1, κx	Biolegend	Cat# 400161, RRID:AB_11125373
PE Mouse IgG2a, κ	BD	Cat# 555574, RRID:AB_395953
PerCP-Cy5.5 Mouse IgG2a, κ	BD	Cat# 558020, RRID:AB_396989
PE-Cy7 Mouse IgG2b, κ	Biolegend	Cat# 400325

No., number; Cat., catalog; RRID, Research Resource Identifier.

https://dx.doi.org/10.21037/tcr-21-733