haematologica
the hematology journal

Early Release Paper

Human acute myeloid leukemia CD34$^+$CD38$^-$ stem cells are susceptible to allorecognition and lysis by single KIR-expressing natural killer cells

by Ulrich Langenkamp, Uwe Siegler, Simon Jörger, Stefan Diermayr, Alois Gratwohl, Christian P. Kalberer, and Aleksandra Wodnar-Filipowicz

Haematologica 2009 [Epub ahead of print]

doi:10.3324/haematol.2009.005967

Haematologica (pISSN: 0390-6078, eISSN: 1592-8721, NLM ID: 0417435, www.haematologica.org) publishes peer-reviewed papers across all areas of experimental and clinical hematology. The journal is owned by the Ferrata Storti Foundation, a non-profit organization, and serves the scientific community with strict adherence to the principles of open access publishing (www.doaj.org). In addition, the journal makes every paper published immediately available in PubMed Central (PMC), the US National Institutes of Health (NIH) free digital archive of biomedical and life sciences journal literature. Haematologica is the

Human acute myeloid leukemia CD34+CD38- stem cells are susceptible to allorecognition and lysis by single KIR-expressing natural killer cells

Ulrich Langenkamp,[1] Uwe Siegler,[1,2] Simon Jörger,[1] Stefan Diermayr,[1] Alois Gratwohl,[2] Christian P. Kalberer,[1] and Aleksandra Wodnar-Filipowicz[1]

[1]Experimental Hematology, Department of Biomedicine, University Hospital Basel, Basel, and [2]Division of Hematology, University Hospital Basel, Basel, Switzerland

ABSTRACT

The concept of tumor immunosurveillance has raised prospects for natural killer (NK) cell-based immunotherapy of human cancer. The cure of acute myeloid leukemia (AML) may depend on eradication of leukemic stem cells (LSCs), the self-renewing component of leukemia. Whether NK cells can recognize and lyse LSCs is not known. To develop strategies that effectively target AML-LSCs, we investigated anti-leukemic effects of human alloreactive single KIR+ NK cells. NK effectors with KIR specificity mismatched with respect to HLA class I allotype of target cells effectively recognized AML-LSCs defined phenotypically as CD34+CD38-, while healthy bone marrow-derived CD34+CD38- hematopoietic stem cells were spared, as demonstrated by cytotoxicity and hematopoietic colony-forming assays. The HDAC inhibitor valproic acid augmented the activating NKG2D ligand-dependent lysis of AML-CD34+CD38- LSCs. These results show that alloreactive NK cells have the potential to detect and target LSCs, and thus to improve the treatment outcome in AML.

Key words: ¿¿¿¿

Citation: Langenkamp U, Siegler U, Jörger S, Diermayr S, Gratwohl A, Kalberer CP, and Wodnar-Filipowicz A. Human acute myeloid leukemia CD34+CD38- stem cells are susceptible to allorecognition and lysis by single KIR-expressing natural killer cells. Haematologica 2009. doi: 10.3324/haematol.005967

Introduction

Leukemia-initiating cells, also termed leukemic stem cells (LSCs), are implicated in sustaining the malignancy and thus a poor treatment outcome.[1] Relapse may occur because of the resistance of quiescent LSCs to cell cycle-dependent cytotoxic chemotherapies. Consequently, specific targeting of LSCs has emerged as a novel therapeutic goal.[2] Natural killer (NK) cells are the innate immunity lymphocytes designated to recognize and kill malignant cells.[3] This property has been clinically verified in acute myeloid leukemia (AML) by graft-versus-leukemia effect improving the outcome of recipients of stem cells from haploidentical donors.[4,5] The alloreactivity of NK cells is based on the absence of inhibitory killer immunoglobulin-like receptors (KIRs) engagement with human leukocyte antigen (HLA) class I molecules, and is triggered by cognate recognition of cell surface ligands by activating NK cell receptors.[6] NKG2D ligands (NKG2D-L) serve as tumor-specific antigens initiating NKG2D receptor-dependent activation of NK cells.[7,8] While numerous studies have characterized the cytolytic potential of human NK cells against leukemic blasts,[9-12] their ability to target LSCs has not been examined. Although the presize phenotypic identity of human LSCs remains elusive, AML-initiating LSCs were shown to reside within the CD34+CD38- population, the phenotype of which corresponds to a healthy bone marrow population containing the hematopoietic stem cells (HSCs).[13-15] Here, we demonstrate that AML-CD34+CD38- LSCs are efficiently recognized and destroyed by single KIR+ NK cells with predicted mismatch with respect to HLA class I specificity of the AML patient. This study provides arguments for exploiting immunotherapy with alloreactive NK cells to target LSCs.

Design and Methods

Patients and healthy controls

Peripheral blood (PB) from AML patients (n=8) and normal bone marrow (N-BM) or normal G-CSF mobilized PB (N-mPB) from healthy donors (n=8) were obtained with informed consent, in agreement with the guidelines of the Ethical Committee of University Hospital Basel. Patients' selection criteria were: primary untreated AML, high blast content (79%±16%) with predominantly CD34+ phenotype (76%±20%), and HLA class I allotype enabling a KIR mismatch (*Online Supplementary Table S1*).

This paperr contains Supplementary Appendix. Acknowledgments: we thank Amgen for aULBP1,2,3 and aNKG2D mAbs, and V. Jäggin and E. Traunecker for cell sorting. Funding: this work was supported by grants from the Swiss National Science Foundation 3100-110511, Oncosuisse 01664-02-05 and 02175-02-2008, Freie Akademische Gesellschaft, and Stiftung für Hämatologische Forschung. Manuscript received on January 14, 2009. Revised version arrived on May 19, 2009. Manuscript accepted on June 3, 2009. *Correspondence: Aleksandra Wodnar-Filipowicz, Department of Biomedicine, University Hospital Basel, Hebelstrasse 20, CH-4031 Basel, Switzerland. Email: aleksandra.wodnar-filipowicz@unibas.ch*

Flow cytometry (FACS)

Fluorochrome-conjugated monoclonal antibodies (mAbs) against human CD45, CD34, CD38, and control IgG1 (BD Biosciences, San Jose, CA) were used. Unconjugated mAbs against ULBP1 (M295), ULBP2 (M311), ULBP3 (M551) (D. Cosman, Amgen, WA, USA), and MICA/B (BD Biosciences), all at 10 mg/mL, were revealed with goat a-mouse IgG-FITC (Jackson ImmunoResearch, West Grove, PA). 100,000 events were acquired using CyAn ADP Flow Cytometer (DAKO Cytomation, Glostrup, Denmark) and analyzed with FlowJo software (Tree Star, Standford, CA, USA).

Purification and culture of AML and N-BM cells

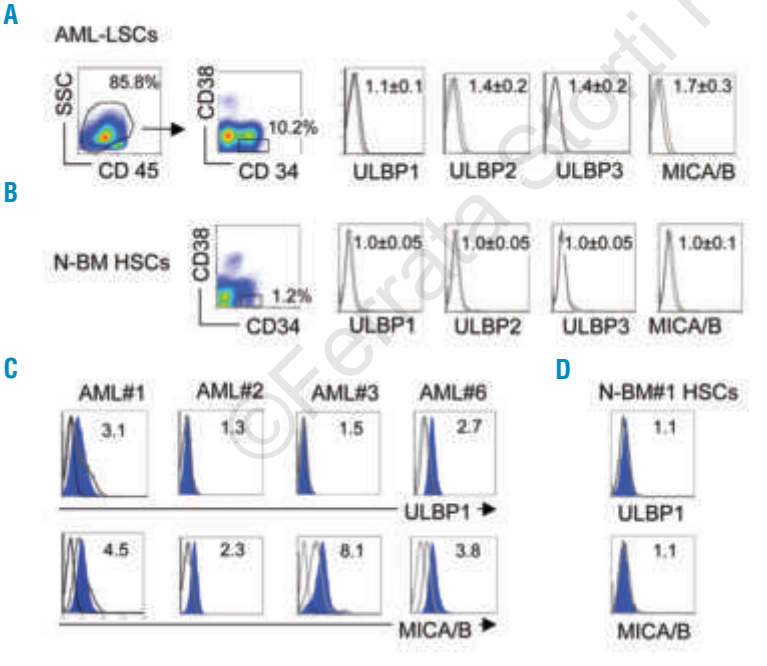
Mononuclear cells (MNCs) from AML PB and N-BM were prepared and $CD34^+CD38^-$ and $CD34^+CD38^+$ subpopulations were purified by FACS-sorting (Cytopeia Influx and Spigot 6.1.4 software; Seattle, WA; *Online Supplementary Figure S1*). MNCs (1×10^6/mL) or purified cell populations ($1\text{-}2\times10^5$/200 mL) were cultured for 2 days in serum-free X-Vivo 10 medium (Lonza, Basel, Switzerland), 20% BIT9500 (Stem Cell Technologies, Vancouver, Canada) and growth factors.[12] Valproic acid (VA) was at 1 mM (Orfiril; Desitin, Liestal, Switzerland).

NK cell lines

Single KIR^+ NK cell lines were obtained from PB $CD56^+CD3^-$ NK cells by FACS-sorting of $CD158a^+$, $CD158b^+$, or $CD158e^+$ cells and culture for 14-21 days in IL-2 containing medium.[12,16] NK cell lines were 95%-99% pure with respect to CD158a, b or e expression (*Online Supplementary Figure 2S and Table S1*).

Cellular cytotoxicity and colony-forming unit (CFU) assays

The cytotoxic activity of single KIR+ NK cells against K562 erythroleukemia, and FACS-sorted AML PB, N-BM and N-mPB cell subpopulations was tested by chromium-release assay with $2\text{-}5\times10^3$ targets/well at indicated effector to target (E:T) ratios.12 For blocking experiments, NK effectors were preincubated with anti-NKG2D mAb (M585; D. Cosman) or mouse IgG1k (BD Biosciences) at 10 mg/mL for 1 hour at 37oC. For CFU assays, FACS-sorted AML (1 x 105) and N-BM (1 x 103) cell subpopulations were seeded into 1% methylcellulose,[17] either immediately or after 2 day culture in medium or with VA, and additional 4 hrs incubation without or with NK cells at E:T ratio of 5:1. Primary CFUs were counted after 14 days in an inverted microscope, harvested, and all cells were replated into secondary methylcellulose cultures.

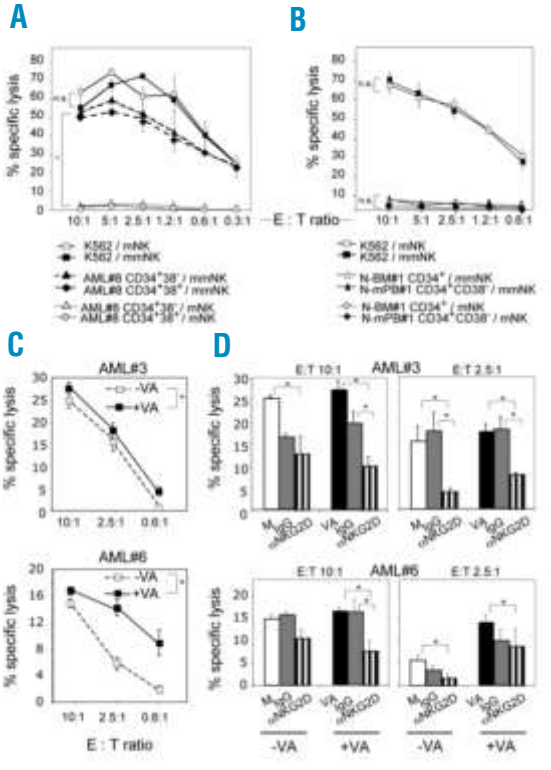


E

			ULBP1*	ULBP2*	ULBP3*	MICA/B*
AML	n=8	d0	1.1±0.1 (1.0-1.4)	1.4±0.2 (1.0-2.5)	1.4±0.2 (1.0-2.1)	1.7±0.3 (1.0-3.5)
		M	1.6±0.3 (1.0-3.4)	1.8±0.7 (1.0-3.9)	2.9±1.3 (1.1-5.4)	2.7±0.8 (1.0-6.5)
		VA	2.2±0.3 (1.3-3.8)	1.8±0.3 (1.5-2.9)	2.9±1.1 (1.5-5.0)	3.3±0.8 (2.0-8.1)
		p VA/d0**	p=0.01	n.s.	n.s.	p=0.04
		p VA/M***	p=0.05	n.s.	n.s.	p=0.05
N-BM	n=5	d0	1.0 (1.0-1.1)	1.0 (1.0)	1.0 (1.0)	1.0 (1.0)
		M	1.1 (1.0-1.2)	1.0 (1.0-1.2)	1.0 (1.0-1.1)	1.1 (1.0-1.1)
		VA	1.1 (1.0-1.3	1.1 (1.0-1.1)	1.0 (1.0)	1.0 (1.0-1.1)
		p VA/M	n.s.	n.s.	n.s.	n.s.

* MFI-R, mean±SEM (range)
** p value of NKG2D-L levels in response to VA at 2 days as compared to d0
*** p Value of NKG2D-L levels in response to VA as compared to M at 2 days
n.s.; non significant
d0; day 0 of culture
M, medium; VA, Valproic Acid

Figure 1. Expression of NKG2D-L by AML-LSCs and N-BM HSCs. (A) FACS analysis of AML-LSCs. Gating was performed sequentially on AML cells identified as CD45-dim and on the $CD34^+CD38^-$ cells. (B) FACS analysis of N-BM HSCs gated on $CD34^+CD38^-$ cells. Expression levels of ULBP1-3 and MICA/B were defined as the mean fluorescence intensity ratio (MFI-R) of values obtained with specific mAbs divided by values given by secondary or control mAbs; MFI-R±SEM for n=8 in A, and n=6 in B are given. NKG2D-L, black line; isotype control, grey line. (C) FACS analysis of the effect of VA on ULBP1 and MICA/B expression levels by AML-$CD34^+CD38^-$ LSCs from 4 individual AML patients. (D) FACS analysis of the effect of VA on ULBP1 and MICA/B expression levels by N-BM $CD34^+CD38^-$ HSCs from a representative donor. Cells were cultured for 2 days with VA, filled histograms; in medium alone, grey line; staining with isotype control, black line. MFI-R of ULBP1 and MICA/B levels after VA treatment are given. (E) Summary of ULBP1-3 and MICA/B expression levels by AML (n=8) and N-BM (n=6) $CD34^+CD38^-$ cells before (d0) and after 2 days exposure to medium alone (M) or VA; *$p<0.05$, statistical significance of NKG2D-L expression in response to VA, as compared to d0, and to M.



Statistical analysis

Expression of NKG2D-L and cytolysis by NK cells were analysed using Student's t-test.

Results and Discussion

The AML-LSCs, defined phenotypically as the CD45^{dim}CD34^{+}CD38^{-} population and analysed in de novo untreated AML, expressed low/undetectable levels of NKG2D-L, including UL-16 binding proteins (ULBP1-3) and MHC-related MICA/B molecules (Figure 1A). Ligands were also at background levels on N-BM CD34vCD38- HSCs (Figure 1B). The paucity of NKG2D-L on AML-LSCs supports earlier findings with leukemic myeloblasts.[12] The absence of NKG2D receptor-dependent interactions is known to accelerate cancer progression,[18] and conversely, tumors which up-reg-

Figure 2 (*left*). Cytotoxicity of NK cells against AML-LSCs and N-HSCs. (A-D) Single-KIR^{+} NK cells were used as effectors in chromium-release assays. (A) AML-CD34^{+}CD38^{-} LSCs and CD34^{+}CD38^{+} blasts from patient AML#8 or control K562 cells were exposed to NK cells, matched (mNK, KIRe^{+} NK-1) or mismatched (mmNK, KIRa+ NK-1), at the indicated E:T ratios. (B) N-BM CD34^{+} and N-mPB CD34+CD38- HSCs from two healthy donors, or control K562 cells were exposed to NK cells, mNK (KIRe^{+} NK-2 for N-BM; KIRa^{+} NK-3 for N-mPB#1) or mmNK (KIRa^{+} NK-2 for N-BM; KIRb^{+} NK-3 for N-mPB), at the indicated E:T ratios. Availability of N-BM samples was limited and this did not allow selection for CD34^{+}CD38- cells in numbers sufficient for the cytotoxicity assay. (C) AML-CD34^{+}CD38- LSCs from patients AML#3 and AML#6, either non-treated (-VA) or incubated for 2 days with VA (+VA) were exposed to mmNK cells (KIRe^{+} NK-1) at the indicated E:T ratios. (D) AML-CD34^{+}D38- LSCs from patients AML#3 and AML#6 were incubated for 2 days with medium alone (M; o), or with VA (■) and exposed to mmNK cells (as in C) which were blocked by preincubation with control mouse IgG (■) or aNKG2D mAb (□) for 1 hr at 37oC, at 10:1 E:T ratio. All experiments were performed in triplicates; mean±SEM values are shown. *p<0.05. The NK cell lines are defined in *Online Supplementary Table S1*.

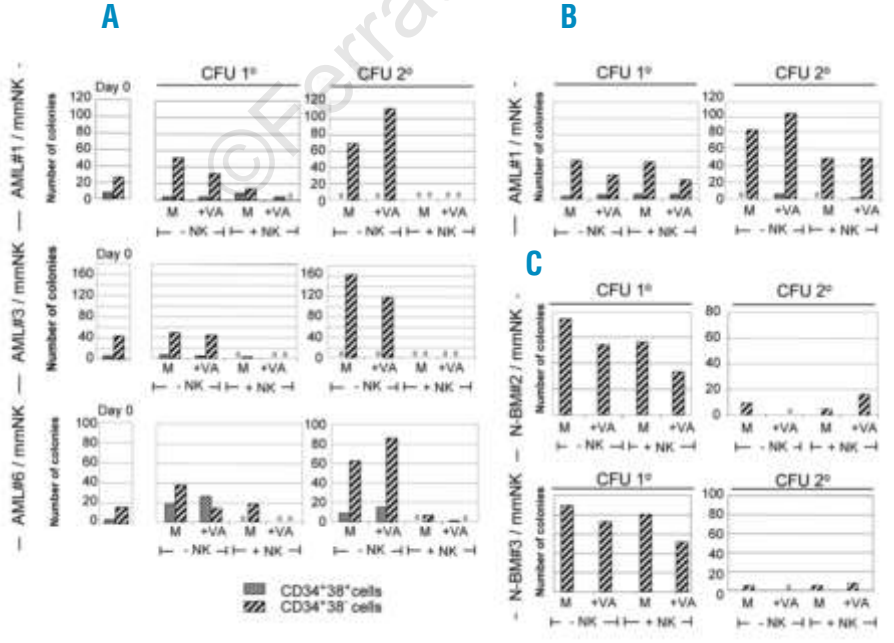


Figure 3. Effect of NK cells on hematopoietic colony formation by AML-LSCs and N-BM HSCs. (A-C) Single KIR+ NK cells were used as effectors and their effect on growth of CFU from AML-LSCs and N-BM HSCs was tested in 1% methylcellulose cultures containing erythropoietin (3U/mL), interleukin (IL)-3 and -6, granulocyte- and granulocyte-macrophage-colony stimulating factor (20 ng/mL, each), stem cell factor and flt3 ligand (100 ng/mL, each). AML-CD34^{+}CD38- LSCs (1x10^{5}) and CD34^{+}CD38^{+} blasts (1x10^{5}) from 3 patients (AML#1, AML#3 and AML#6), or N-BM CD34+CD38- HSCs (1x10^{3}) from 2 healthy donors (N-BM#2 and #3) were incubated for 2 days with medium alone (M) or VA (+VA) and plated in methylcellulose directly (-NK) or after exposure to NK cells (+NK), either matched (mNK) or mismatched (mmNK), for 4 hrs at E:T ratio of 5:1. In A, CFU numbers by AML cells prior to incubation (Day 0) are shown. In A-C, numbers of CFU, primary 1o or secondary 2o, are shown. In A, mmNK were KIRe^{+} NK-1. In B, mNK were KIRa^{+} NK-1. In C, mmNK were KIRa^{+} NK-1. The NK cell lines are defined is *Online Supplementary Table S1*. Results are shown as mean of duplicate analyses. Cultures in which no CFU-derived colonies were seen are indicated with "0".

ulate cell surface NKG2D-L in response to cellular stress, DNA damage or pharmacological treatment are rendered susceptible to killing by NK cells.[19,20] We have recently demonstrated that NKG2D-L levels increased in response to histone deacetylase (HDAC)-inhibitor VA, a drug with antineoplastic activities, and this increase enhanced the cytolysis of AML blasts.12 Here, we observed a VA-dependent up-regulation of NKG2D-L on CD34$^+$CD38- LSCs (Figure 1C), whereas no response to VA was seen with N-BM HSCs (Figure 1D). The VA effect on AML-LSCs was modest, but apparent with ULBP1 and MICA/B, the expression of which increased 2.0±0.8 and 1.9±1.5 fold, respectively (p<0.05; Figure 1E).

To test whether AML-LSCs are susceptible to NK cell-mediated lysis, NK effectors carrying single KIR specificities were selected according to patients' HLA class I allotypes (*Online Supplementary Table S1*). HLA-mismatched, but not HLA-matched, single KIR$^+$ NK cells were able to lyse purified AML-CD34$^+$CD38$^-$ LSCs, with an efficiency comparable to killing of leukemic blasts, phenotypically defined as CD34$^+$CD38$^+$ (Figure 2A). Matched NK cells effectively lysed control HLA class I-negative K562 cells, confirming the requirement for HLA-KIR mismatch in LSC detection (Figure 2A). There were interindividual differences in susceptibility of purified AML-CD34$^+$CD38$^-$ cells from individual patients (n=3, range 15%-55% at E:T ratio of 10:1) but lysis was always seen using HLA-mismatched and not HLA-matched effectors, in accordance with our earlier studies on unfractionated leukemic blasts.[12] Single KIR$^+$ NK cells expressed also the inhibitory receptor NKG2A on 30%-60% of cells, but NKG2A ligand HLA-E on AML targets was low or absent (MFI-R<10), in comparison with high levels of KIR ligands HLA-ABC (MFI-R 100-300). Unlike with AML targets, neither HLA-mismatched nor HLA-matched NK cells lysed purified N-BM CD34+ cells or N-mPB CD34+CD38- cells (Figure 2B), indicating that normal progenitors are protected, and underlining a specificity of alloreactive single KIR+ NK cells towards leukemic targets. Treatment of AML-CD34$^+$CD38$^-$ LSCs with VA resulted in an increase in susceptibility to alloreactive single KIR+ NK cells which was consistently observed at 10:1 to 0.6:1 E:T ratios, but the extend of which varied dependent on AML targets (Figure 2C). This was likely linked to VA-mediated up-regulation of cell-surface NKG2D-L (Figure 1C). The cytolysis of LSCs was indeed partly NKG2D-dependent, as it was specifically reduced in the presence of anti-NKG2D mAbs, the blocking effect of which was particularly pronounced with VA-treated AML cells (Figure 2D). To define the effect of NK cells on the colony-forming properties of LSCs,[21] serial replating CFU assays were performed and colony numbers generated from purified AML and N-BM subpopulations in response to VA and single-KIR$^+$ NK cells were monitored (Figure 3A-C). AML-CD34$^+$CD38- LSCs displayed higher clonogenicity than AML-CD34$^+$CD38$^+$ blasts when plated directly (day 0) or after 2 days incubation with VA in primary (1o) CFU, and gave efficiently rise to colonies in secondary (2o) CFU assays (Figure 3A) due to aberrant self-renewal. With all 3 tested AML patients' LSCs, exposure to HLA class I-KIR mismatched NK cells strongly reduced the capacity to form 1o and 2o CFU. Preincubation of AML-LSCs with VA potentiated this effect, since 1o CFUs were fully eradicated (Figure 3A). Matched NK cells did not affect 1o and 2o CFU numbers generated from AML-LSCs (Figure 3B), in agreement with absent killing in cytotoxicity assays (Figure 2A). To test whether NK cells can discriminate between normal and leukemic CFUs, the N-BM CD34$^+$CD38$^-$ HSCs were used; in contrast to AML, HSC-derived colonies do not support serial replatings (Figure 3C). The 1o CFU numbers from N-BM HSCs were unaffected by exposure to single-KIR$^+$ mismatched NK cells, and were preserved after VA treatment,[22] indicating that allorecognition by single KIR$^+$ NK cells is specific towards malignant colony-forming LSCs.

In this first report addressing the susceptibility of AML-LSCs to NK cells, we demonstrate that selection of NK effectors with a predicted KIR-HLA class I mismatch is prerequisite for targeting AML-LSCs. Allorecognition can be augmented by VA which up-regulates NKG2D-L, thus priming the AML for the cytotoxic effectors and underlying the importance of interventions which enhance the NKG2D axis for tumor recognition. VA-induced epigenetic modifications may also promote the entry of LSCs into the cell cycle rendering them more accessible to chemo- and immunotherapies.[23] Importantly, we show that healthy CD34$^+$CD38$^-$ cells containing the HSCs are not targeted by HLA-mismatched NK cells and do not respond to VA, indicating that normal hematopoietic functions will be spared and arguing for the specificity in eradication of malignant CD34$^+$CD38$^-$ cells containing the LSCs. Our data reinforce the concept of alloreactive NK cell-based adoptive immunotherapy,[24] in combination with antineoplastic drugs to enhance the tumor reactivity, as a rational strategy towards curing leukemia.

Authorship and Disclosures

UL and AW-F were the principal investigators and take primary responsability for the paper. UL, US, SJ, and SD performed research, analysed data, and edited the paper, AG provided clinical data. AW-F and CPK designed research, analyzed data, wrote and edited the paper.

The authors reported no potential conflicts of interest.

References

1. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994;367:645-8.
2. Krause DS, Van Etten RA. Right on target: eradicating leukemic stem cells. Trends Mol Med 2007;13:470-81.
3. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol 2001;22:633-40.
4. Velardi A, Ruggeri L, Alessandro, Moretta, Moretta L. NK cells: a lesson from mismatched hematopoietic transplantation. Trends Immunol 2002;23:438-44.
5. Ruggeri L, Mancusi A, Capanni M, Urbani E, Carotti A, Aloisi T, et al. Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. Blood 2007; 110:433-40.
6. Parham P, McQueen KL. Alloreactive killer cells: hindrance and help for haematopoietic transplants. Nat Rev Immunol 2003; 3:108-22.
7. Lanier LL. On guard--activating NK cell receptors. Nat Immunol 2001;2:23-7.
8. Raulet DH. Roles of the NKG2D immunoreceptor and its ligands. Nat Rev Immunol 2003;3:781-90.
9. Salih HR, Antropius H, Gieseke F, Lutz SZ, Kanz L, Rammensee HG, Steinle A. Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. Blood 2003;102:1389-96.
10. Nowbakht P, Ionescu MC, Rohner A, Kalberer CP, Rossy E, Mori L, et al. Ligands for natural killer cell-activating receptors are expressed upon the maturation of normal myelomonocytic cells but at low levels in acute myeloid leukemias. Blood 2005;105:3615-22.
11. Pende D, Spaggiari GM, Marcenaro S, Martini S, Rivera P, Capobianco A, et al. Analysis of the receptor-ligand interactions in the natural killer-mediated lysis of freshly isolated myeloid or lymphoblastic leukemias: evidence for the involvement of the Poliovirus receptor (CD155) and Nectin-2 (CD112). Blood 2005;105:2066-73.
12. Diermayr S, Himmelreich H, Durovic B, Mathys-Schneeberger A, Siegler U, Langenkamp U, et al. NKG2D ligand expression in AML increases in response to HDAC inhibitor valproic acid and contributes to allorecognition by NK-cell lines with single KIR-HLA class I specificities. Blood 2008;111:1428-36.
13. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997;3:730-7.
14. Hope KJ, Jin L, Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. Nat Immunol 2004;5:738-43.
15. Ishikawa F, Yoshida S, Saito Y, Hijikata A, Kitamura H, Tanaka S, et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. Nat Biotechnol 2007;25:1315-21.
16. Siegler U, Kalberer CP, Nowbakht P, Sendelov S, Meyer-Monard S, Wodnar-Filipowicz A. Activated natural killer cells from patients with acute myeloid leukemia ary cytotoxic against autologous lekemic blasts in NOD/SCID mice. Leukemia 2005;19:2215-22.
17. Bridenbaugh S, Kenins L, Bouliong-Pillai E, Kalberer CP, Shklovskaya E, Gratwohl A, et al. Clinical stem-cell sources contain CD8+CD3+ T-cell receptor-negative cells that facilitate bone marrow repopulation with hematopoietic stem cells. Blood 2008;111:1735-8.
18. Guerra N, Tan YX, Joncker NT, Choy A, Gallardo F, Xiong N, et al. NKG2D-deficient mice are defective in tumor surveillance in models of spontaneous malignancy. Immunity 2008;28:571-80.
19. Gasser S, Raulet DH. The DNA damage response arouses the immune system. Cancer Res 2006;66:3959-62.
20. Lopez-Larrea C, Suarez-Alvarez B, Lopez-Soto A, Lopez-Vazquez A, Gonzalez S. The NKG2D receptor: sensing stressed cells. Trends Mol Med 2008;14:179-89.
21. Somervaille TC, Cleary ML. Identification and characterization of leukemia stem cells in murine MLL-AF9 acute myeloid leukemia. Cancer Cell 2006;10:257-68.
22. Bug G, Gul H, Schwarz K, Pfeifer H, Kampfmann M, Zheng X, et al. Valproic acid stimulates proliferation and self-renewal of hematopoietic stem cells. Cancer Res 2005; 65:2537-41.
23. De Felice L, Tatarelli C, Mascolo MG, Gregorj C, Agostini F, Fiorini R, et al. Histone deacetylase inhibitor valproic acid enhances the cytokine-induced expansion of human hematopoietic stem cells. Cancer Res 2005;65:1505-13.
24. Ljunggren HG, Malmberg KJ. Prospects for the use of NK cells in immunotherapy of human cancer. Nat Rev Immunol. 2007;7:329-39.