Medical
Research
Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: September 30, 2022

Citation: Momparler RL, Côté S, et al., 2022. Eradication of leukemia stem cells by inhibitors of DNA methyltransferase, EZH2 and G9a histone methyltransferases, Medical Research Archives, [online] 10(9). https://doi.org/10.18103/mra.v10i9.3020

Copyright: © 2022 European Society of Medicine. This is an open- access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

DOI
https://doi.org/10.18103/mra.v10i9.3020

ISSN: 2375-1924

RESEARCH ARTICLE

Eradication of leukemia stem cells by inhibitors of DNA methyltransferase, EZH2 and G9a histone methyltransferases

Richard L. Momparler[1,2]*, Sylvie Côté [2] and Louise F. Momparler [2]

[1]Département de pharmacologie-physiologie, Université de Montréal; [2] Centre de recherche, Service d'hématologie-oncologie, CHU Sainte-Justine, Montreal, Quebec H3T 1C5, Canada

* richard.l.momparler@umontreal.ca

ABSTRACT

Complete eradication of leukemic stem cells (LSCs) in patients with acute myeloid leukemia (AML) is required for curative therapy. Epigenetic alterations that involve gene-silencing by DNA methylation by DNMT1, methylation of H3K27 by EZH2 histone methyltransferase (HMT) and methylation of H3K9 by G9a HMT may play a major role in the development of AML. The major action of these epigenetic alterations is the silencing of the genes that program differentiation of AML cells. Inhibitors of DNA and histone methylation have the potential to reverse this block in differentiation. If tumor suppressor genes (TSGs) contain two gene-silencing markers, such as DNA methylation and H3K27me3, they may not be fully reactivated with only an inhibitor of DNA methylation, such as 5-aza-2'-deoxycytidine (5-AZA-CdR), but may also require an inhibitor of EZH2. In support of this model is the synergistic antileukemic action as shown by a colony assay on AML cells using 5-AZA-CdR in combination with 3-deazaneplanocin A (DZNep), a potent inhibitor of EZH2. A similar type of interaction can occur when TSGs are silenced by DNA methylation and the G9a methylation of H3K9me2, a second gene-silencing marker. Treatment of these AML cells with 5-AZA-CdR and BIX01294, an inhibitor of G9a, also results in a synergistic antileukemic action. Leukemic cells that contain 3 different gene-silencing markers: DNA methylation, H3K27me3 and H3K9me2 may require 3 different inhibitors for maximal antineoplastic activity. This result was observed when the AML cells were treated in with 5-AZA-CdR, DZNep and BIX01294. The aim of this study was to demonstrate that epigenetic agents that target DNA and histone methylation have remarkable antineoplastic activity against myeloid leukemia cells. The second aim was to propose a dose-schedule for these epigenetic agents that can be evaluated in a clinical trial in patients with advanced AML for its potential to eradicate LSCs. One of the most sensitive targets for chemotherapeutic intervention in LSCs is the block in differentiation due to gene-silencing by DNA and histone methylation. Epigenetic agents that have the potential to reverse this block merit clinical investigation with high priority.

Keywords: epigenetic therapy, leukemia, DNA methylation, histone methylation, differentiation

INTRODUCTION

Epigenetic alterations may play an important role in the transformation of normal hematopoietic stem cell (HSC) to leukemic stem cell (LSC).[1,2] Investigations in this area can provide insight on the molecular mechanisms involved in leukemogenesis and identify potential targets for antileukemic therapy. DNA methylation is a major mechanism that silences the genes that program differentiation in LSCs.[1,2] The importance of DNA methylation in leukemogenesis is shown by the antileukemic action of the inhibitor of DNA methylation, 5-aza-2'-deoxycytidine (5-AZA-CdR, decitabine). 5-AZA-CdR can induce complete remissions in patients with acute myeloid leukemia (AML).[3,4] The antileukemic action of 5-AZA-CdR is due to its re-activation of tumor suppressor genes (TSGs).[5]

Mutations in enzymes that lead to an increase in DNA methylation give insight into the molecular mechanisms involved in the transformation of normal HSC to LSCs. Loss-of-function mutations of TET2 are common in AML.[6] TET2 catalyzes the demethylation of DNA by converting methylcytosine to hydroxymethyl-cytosine. Loss of TET2 function leads to aberrant DNA hypermethylation and progression of leukemogenesis.[6] Another important epigenetic mechanism leading to DNA hypermethylation is mutations of isocitrate dehydrogenase 1 and 2 (IDH1, IDH2) which alter their enzymatic activity.[7] The result of this mutation is the production from isocitrate of the oncometabolite 2-hydroxyglutarate (2-HG). Inhibition of TET enzymatic activity by 2-HG results in DNA hypermethylation. Inhibitors of mutant IDH1 or IDH2 induce terminal differentiation of the mutant leukemic blasts and provide durable clinical responses in AML patients with the IDH mutations.[7] This important observation indicates that aberrant DNA methylation plays a major role in the development of AML.

Histone methylation is another important epigenetic mechanism of gene-silencing. One example is the key role of EZH2 histone methyltransferase for the conversion of H3K27 to H3K27me3, a gene-silencing marker.[8] Importance of H3K27 methylation in leukemogenesis is shown by the antileukemic action of 3-deazaneplanocin A (DZNep), a potent inhibitor of EZH2.[9] DZNep was also shown to induce the differentiation of AML cells.[9] Gain of function mutations in EZH2 have been shown to favor malignant transformation.[8] An additional histone mechanism for gene silencing is the methylation of H3K9 to H3K9me2 by G9a histone methyltransferase.[10] This action leads to a reduction in the expression of genes that program differentiation. Human AML cells are sensitive to the inhibition of G9a.[11] The Inhibitor of G9a, BIX01294, was shown to inhibit the proliferation and induce differentiation of HL-60 myeloid leukemia cells.[11]

Therapy of AML using only 5-AZA-CdR can induce complete remisisons, but most patients will relapse.[3] One possible explanation why the 5-AZA-CdR did not cure the AML patients is that the target LSCs contain two different epigenetic gene-silencing mechanisms, such as DNA methylation and H3K27me3. In order to eradicate these LSC, two different drugs are required: an inhibitor of DNA methylation and an inhibitor of EZH2.[12] We reported previously that 5-AZA-CdR in combination with DZNep exhibited a synergistic action against the HL60 myeloid leukemia cells.[13,14] The combination of an inhibitor of DNA methylation and an inhibitor of EZH2 also exhibits synergistic antineoplastic activity against tumors.[15]

The curative potential of AML by combination of 5-AZA-CdR and DZNep may also be limited by the presence of a third epigenetic gene-silencing marker, such as H3K9me2.[10]
Inhibition of the methylation of H3K9 by G9a was reported to enhance the antitumor action of 5-AZA-CdR.[16] In this report we demonstrate that the addition of BIX01294 (BIX), an inhibitor of G9a,[11] can increase further the antileukemic action of 5-AZA-CdR and DZNep. This novel epigenetic therapy using three different agents that target three different gene-silencing mechanism merits investigation for the therapy of AML.

METHODS

Cells: HL-60 human myeloid leukemia cells were obtained from ATCC and maintained in RPMI-1640-HEPES medium (Invitrogen) with 10% fetal bovine serum (FBS). The doubling time of the HL-60 cells was about 20 h.

Preparation of drugs: 5-AZA-CdR was provided by Dr Alois Piskala, Czechoslovak Academy of Sciences, Prague. DZNep was provided by Dr Victor E. Marquez, Chemical Biology Laboratory NIH (Frederick, MD). BIX01294 was obtained from Sigma-Aldrich. The drugs were dissolved in 0.5 PBS, pH6.8 and sterilized by 0.22 micron filtration. 5-AZA-CdR was stored at -70C. DZNep and BIX were stored at -20C.

Preparation of culture media: RPMI-1640 powdered media containing glutamine and HEPES buffer, 1 L size (GIBCO) was dissolved in a bottle containing 800 ml sterile water; 220 mg sodium pyruvate and 2.2 g sodium bicarbonate were

added and the media filtered 0.22 micron. Fetal bovine serum (FBS) 20 ml was added to 80 of media to give a final concentration of 20%.

Preparation of tubes for colony assay: Place 0.1 ml containing 1,000 cells/ml (100 cells total) in Falcon screw cap tube 13 x 100 mm (#2027).

Preparation of 0.3% agarose RPMI medium: Place 180 mg SeaPlaque Low Melting Agarose in 100 ml bottle with screw cap, add 10 ml sterile water, heat mixture in microwave at medium power for about 1 min until first sign of boiling to dissolve agarose. Add immediately 4 ml of hot agarose media to 20 ml RPMI media + 20 % FBS (room temperature). Pipet 2 ml agarose-RPMI media in tubes containing cells and place in ice water for 2 min. Flush tubes with 5% CO_2 and place in CO_2 incubator at 37C for 16-18 days. Count colonies using indirect illumination. The cloning efficiency of the HL-60 leukemia cells was in the range of 60–70%. Statistical analysis of the data was performed using Prism GraphPad software and Tukey's multiple comparison test.

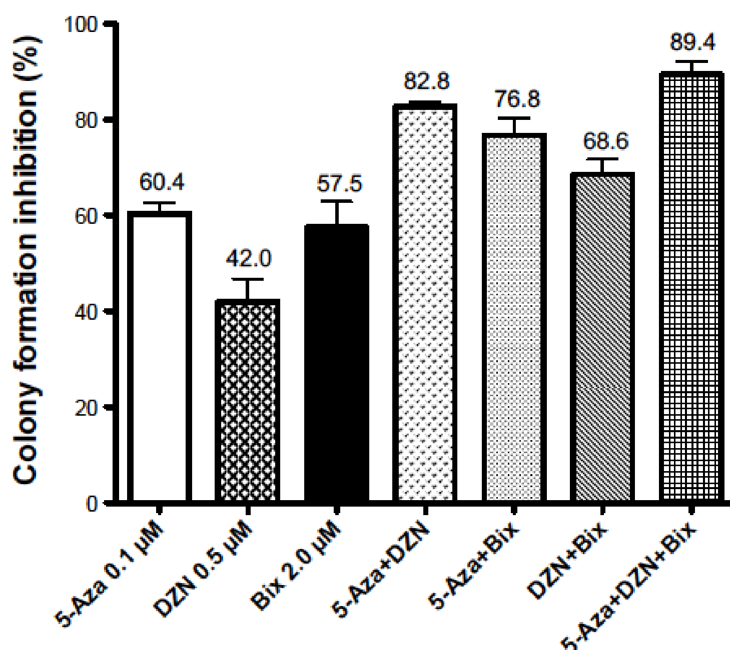

Figure 1. The antileukemic action of 5-AZA-CdR, DZNep and BIX01294 (BIX), alone and in combination on HL-60 myeloid leukemia cells. The leukemia cells were treated for 24h with 0.1 µM 5-AZA-CdR (5-Aza), 0.5 µM DZNep (DZN) and/or 2.0 µM BIX. Cell counts were made at the end of the treatment and cells were placed in soft-agar medium to determine cell survival using a colony assay. The values shown are the inhibition of colony formation (mean ± S.E. n = 3). Statistical analysis: 5-Aza+DZN > 5-Aza or DZN $p < 0.01$; 5-Aza+BIX > 5-Aza or BIX $p < 0.05$; DZN+BIX > DZN $p < 0.01$; DZN+BIX > BIX n.s.; 5-Aza+DZN+BIX > 5-Aza+BIX $p < 0.05$; 5-Aza+DZN+BIX > DZN+BIX $p < 0.01$. 5-Aza+DZN+BIX > 5Aza + DZN n.s.

RESULTS

The antineoplastic action on HL-60 myeloid leukemia cells of 5-AZA-CdR, DZNep, and/or BIX was evaluated by a colony assay. The leukemic cells were treated with these inhibitors for 24 h and the cells were then placed in drug-free soft agar medium. After 18-20 days incubation the number of colonies were counted. The results of this assay are shown in **Figure 1.** 5-AZA-CdR (5Aza) at 0.1 µM inhibited colony formation by 60.4 ± 2.3% (mean ± SE). DZNep (DZN) at 0.5 µM inhibited colony formation of the leukemic cells by 42.0 ± 4.8%. BIX. at 2 µM inhibited colony formation by 57.5% ± 5.3 %. The reduction in colony formation was greater when these agents were used in combination. The inhibition of colony formation for: 5Aza + DZN was 82.8 ± 0.9%; 5Aza + BIX was 76.8 ± 3.6 %; DZN + BIX was 68.6 ± 3.1 %; 5Aza + DZN + BIX was 89.4 ± 2.8%. The method of Valeriote and Lin was used to evaluate the drug interactions.[17] The interaction of 5-AZA-CdR and DZNep and the interaction of 5-AZA-CdR and BIX were synergistic. The synergistic antileukemic action of 5AZA-CdR and DZNep is in accord with our results published previously.[13,14] The interaction of DZNep and BIX was additive. The interaction between 5-AZA-CdR,

DZNep and BIX was also additive. A summary of the action of the three gene-silencing mechanisms and their reversal by specific inhibitors of the target enzymes: DNMT1, EZH2, and G9a is shown in **Figure 2**. A good understanding of these interactions can lead to the development of more effective therapy of leukemia.

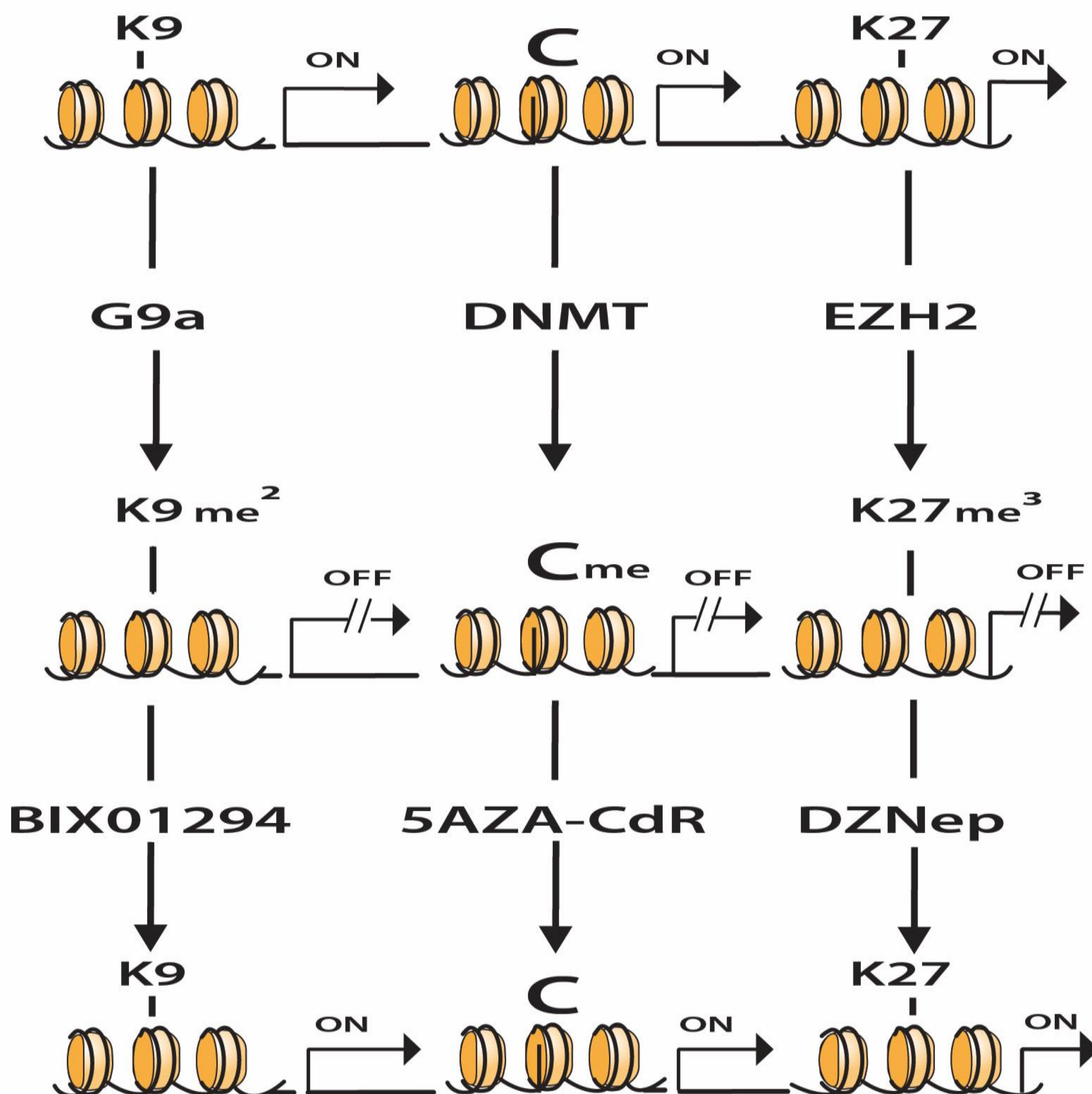

Figure 2. Epigenetic gene-silencing by DNA methylation by DNMT, methylation of H3K27 by EZH2 histone methyltransferase (HMT) and methylation of H3K9 by G9a HMT. Reversal of gene-silencing by DNA methylation by 5-aza-2'-deoxycytidine (5AZA-CdR), by H3K27me3 by 3-deazaplanocin A (DZNep) and by H3K9me2 by BIX01294 (BIX). C, cytosine; Cme, 5-methylcytosine; ON, transcription of mRNA; OFF, no transcription of mRNA; DNMT, DNA methyltransferase.

DISCUSSSION

Curative therapy of AML requires the complete eradication of the LSC. A major hallmark of the LSC is the block in their differentiation.[1] One key mechanism responsible for this block is epigenetic gene-silencing by DNA methylation.[2] The LSC genome display a large increase in the number of 5-methylcytosine molecules as compared to the genome of normal white blood cells.[20] The major role of DNA methylation is the silencing of genes that program differentiation. The importance of DNA methylation is shown by the induction of

complete remissions in AML patients by the potent and specific inhibitor of DNA methylation, 5-AZA-CdR. However, most patients induced into complete remission with 5-AZA-CdR will relapse.[3] Possible explanations for this observation are that the LSC that survive treatment are drug resistant, the dose-schedule used for 5-AZA-CdR was suboptimal or the LSC contained TSGs that were silenced by epigenetic mechanisms different than DNA methylation. These latter mechanisms could be gene-silencing by histone methylation. EZH2 is the HMT that methylates H3K27 to H3K27me3, a gene-silencing marker.[8] LSC that contain both DNA methylation and H3K27me3 may escape therapy with only 5-AZA-CdR.[16] One approach to overcome this impediment is to use a combination of inhibitors target DNA methylation and the methylation of H3K27. The results shown in **Figure 1** support this hypothesis since the combination of 5-AZA-CdR and DZNep exhibit a synergistic interaction to suppress colony formation of the HL60 myeloid leukemia cells. This latter result confirms our previous reports.[13,14]

Epigenetic gene-silencing can also occur by methylation of H3K9 to H3K9me2 by G9a HMT.[10] This third histone gene-silencing mechanism may also limit the potential of 5-AZA-CdR for curative therapy. Inhibition of G9a by BIX and DNMT by 5-AZA-CdR also results in a synergistic antileukemic action (**Figure 1**). The most potent epigenetic therapy against myeloid leukemia cells may be the combination of 5-AZA-CdR, DZNep and BIX which target all three epigenetic gene-silencing mechanisms. This triple combination of epigenetic agents exhibited the greatest reduction in colony formation by the leukemic cells and merits clinical investigation in patients with AML. Preclinical studies in mice with AML should be done to confirm that the in vitro results on the antineoplastic activity are also observed in vivo.

A pilot clinical trial on patients with advanced AML is suggested using a continuous 60 h i.v. infusion of 5-AZA-CdR and DZNep at a dosage of these agents that gives a steady-state plasma concentration of 1 μM for both drugs. Depending on the response and adverse events, the duration of the continuous infusion can be increased in a step wise manner. We performed a pilot clinical trial in patients with advanced AML using an i.v. infusion with a duration of 60 h with acceptable hematopoietic toxicity.[18] The duration of the infusion of 5-AZA-CdR needs to be optimized.[19] 5-AZA-CdR was selected because it is the most potent inhibitor of DNA methylation and is a more potent antileukemic agent than cytosine arabinoside (ARA-C)[21], the most active drug for the treatment of AML. The major adverse event induced by 5-AZA-CdR is hematopoietic toxicity.[3]

DZNep was chosen because it is the most potent inhibitor of EZH2, much more effective than tazemetostat which is used clinical therapy.[22] In addition, the combination of DZNep and 5-AZA-CdR exhibits a synergistic antineoplastic action against myeloid leukemia cells (**Figure 1**). However, DZNep requires FDA approval for clinical use. For initial studies it is possible to replace DZNep with another inhibitor of EH2, such as tazemetostat, which is approved for clinical use, but is not as potent as DZNep.[22]

If a combination of inhibitors that target DNMT1 and EZH2 cannot induce complete remissions of long duration or curative therapy in AML patients, consideration should be given to investigate if the addition of a third epigenetic drug can achieve this objective. A drug that targets G9a is a good choice for this option. The addition of BIX, an inhibitor of G9a,[11] enhanced the antileukemic action of the combination of inhibitors that target DNMT1 and EZH2 (**Figure 1**). Novel inhibitors of G9a are under development, some of which may be more effective than BIX and should also become available for clinical use.

Epigenetic alterations that involve gene-silencing by DNA methylation by DNMT1, methylation of H3K27 by EZH2 histone methyltransferase (HMT) and methylation of H3K9 by G9a HMT may play a major role in the development of AML. The major action of these epigenetic alterations is the silencing of the genes that program differentiation of AML cells. Inhibitors of DNA and histone methylation have the potential to reverse this block in differentiation. If tumor suppressor genes (TSGs) contain two gene-silencing markers, such as DNA methylation and H3K27me3, they may not be fully reactivated with only an inhibitor of DNA methylation, such as 5-aza-2'-deoxycytidine (5-AZA-CdR), but may also require an inhibitor of EZH2. In support of this model is the synergistic antileukemic action as shown by a colony assays on AML cells using 5-AZA-CdR in combination with DZNep, a potent inhibitor of EZH2. A similar type of interaction can occur when TSGs are silenced by DNA methylation and the G9a methylation of H3K9me2, a second gene-silencing marker. Treatment of these AML cells with 5-AZA-CdR and BIX01294, an inhibitor of G9a, also results in a synergistic antileukemic action. Leukemic cells that contain 3 different gene-silencing markers:

DNA methylation, H3K27me3 and H3K9me2 may require 3 different inhibitors for maximal antineoplastic activity. This result was observed when the AML cells were treated in with 5-AZA-CdR, DZNep and BIX01294. The aim of this study was to demonstrate that epigenetic agents that target DNA and histone methylation have remarkable antineoplastic activity against myeloid leukemia cells. The second aim was to propose a dose-schedule for these epigenetic agents that can be evaluated in a clinical trial in patients with advanced AML for its potential to eradicate LSCs. One of the most sensitive targets for chemotherapeutic intervention in LSCs is the block in differentiation due to gene-silencing by DNA and histone methylation. Epigenetic agents that have the potential to reverse this block merit clinical investigation with high priority. These epigenetic alterations may be one of the most sensitive targets for intervention using epigenetic chemotherapy and merit intensive investigation.

A natural mechanism exists in cells that has the potential to prevent or reverse neoplastic transformation.[23] The ten eleven translocation (TET) pathway of DNA hydrolases can remove 5-methylcytosine from DNA and reverse epigenetic gene-silencing. Loss of function mutations of TET were observed in some patients with AML indicating an important role of TET in the suppression of leukemogenesis.[6] The importance of this natural mechanism in leukemogenesis remains to be determined.

Nutritional agents, such as Vitamin C, have the potential to increase the effectiveness of the proposed epigenetic therapy.[6] We reported previously that vitamin C increases the antineoplastic activity of 5-AZA-CdR and DZNep against myeloid leukemia cells.[24] The mechanism responsible for this action of vitamin C is due to its function as a cofactor of alpha-ketoglutarate-dependent dioxygenases (KGDD). The enhancement by Vitamin C of the catalytic activity of KGDD of the TET pathway, as well as of the Jumonji C histone demethylases (JHDMs), was shown to result in demethylation of DNA and histones, leading to reactivation of tumor suppressor genes and an enhancement of its antineoplastic effect.[6]

CONCLUSION

Curative therapy of leukemia can be facilitated by identifying the most sensitive targets for chemotherapeutic intervention. During the process of development, HSCs have to activate the specific program of differentiation that leads to white blood formation. This task is accomplished by activation of the expression of the developmental genes responsible for white blood cell formation by enzymatic DNA and histone demethylation. In premature HSCs the hematopoietic development genes are silenced by DNA methylation and histone methylation by the presence of H3K27me3.[25,26] When HSCs cannot reverse this epigenetic gene-silencing, they are transformed to LSCs. Reversal of this process provides one of the most sensitive targets for chemotherapeutic intervention in leukemia cells using epigenetic agents. A good example of this interaction is the synergistic antineoplastic and gene-activation action on AML cells induced by 5-AZA-CdR, an inhibitor of DNA methylation, and DZNep, an inhibitor of EZH2. This observation provides support of the hypothesis that gene-silencing by DNA and histone methylation plays a major role in the transformation of HSCs to LSCs and merits clinical investigation with high priority in patients with AML using epigenetic agents that reverse gene-silencing.

Funding: This research was funded by a grant from the Faculty of Medicine, University of Montreal.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wouters BJ, Delwel R. Epigenetics and approaches to targeted epigenetic therapy in acute myeloid leukemia. *Blood*. 2016; 127:42-52.
2. Bates SE. Epigenetic therapies for cancer. *N Engl J Med*. 2020;383(7):650-663.
3. Cashen AF, Schiller GJ, O'Donnell MR, DiPersio JF. Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia. *J Clin Oncol*. 2009;28:556–561.
4. Lübbert M, Grishina O, Schmoor C, Schlenk RF, Jost E, Crysandt M, et al. DECIDER study team. Valproate and retinoic acid in combination with decitabine in elderly nonfit patients With acute myeloid leukemia: Results of a multicenter, randomized, 2 × 2, phase II trial. *J Clin Oncol*. 2020; 38:257-270.
5. Momparler RL, Bovenzi V. DNA methylation and cancer. *J Cell Physiol*. 2000;183(2):145-154.
6. Cimmino L, Dolgalev I, Wang Y, Yoshimi A, Martin GH, Wang J, at al. Restoration of TET2 function blocks aberrant self-renewal and leukemia progression. *Cell*. 2017;170(6):1079-1095
7. Wang F, Morita K, DiNardo CD, Furudate K, Tanaka T, Yan Y, et al. Leukemia stemness and co-occurring mutations drive resistance to IDH inhibitors in acute myeloid leukemia. *Nat Commun*. 2021;12(1):2607.
8. Kim KH, Roberts CWM. Targeting EZH2 in cancer. *Nature Med* .2016;22:128-134.
9. Fiskus W, Wang Y, Sreekumar A. et al. Combined epigenetic therapy with the histone methyltransferase EZH2 inhibitor 3-deazaneplanocin A and the histone deacetylase inhibitor panobinostat against human AML cells. *Blood*. 2009;114:2733-2743.
10. Lehnertz B, Pabst C, Su L, Miller M, Liu F, Yi L, et al. The methyltransferase G9a regulates HoxA9-dependent transcription in AML. *Genes Dev*. 2014;28:317–327.
11. Savickiene J, Treigyte G, Stirblyte I, Valiuliene G, Navakauskiene R. Euchromatic histone methyltransferase 2 inhibitor, BIX-01294, sensitizes human promyelocytic leukemia HL-60 and NB4 cells to growth inhibition and differentiation. *Leuk Res*. 2014;38(7):822-829.
12. Si J, Boumber YA, Shu J, Qin T, Ahmed S, He R, at al. Chromatin remodeling is required for gene reactivation after decitabine-mediated DNA hypomethylation. *Cancer Res*. 2010;70:6968-6977.
13. Momparler RL, Idaghdour Y, Marquez VE, Côté S, Momparler LF, et al., Synergistic antileukemic action of inhibitors of DNA methylation and histone methylation. *Leuk Res*. 2012;36:1049-1054.
14. Momparler RL, Côté S, Momparler LF, Idaghdour Y. Inhibition of DNA and histone methylation by 5-aza-2'-deoxycytidine (Decitabine) and 3-deazaneplanocin-A on antineoplastic action and gene expression in myeloid leukemic cells. *Front Oncol*. 2017; 15:7,19.
15. Takeshima H, Wakabayashi M, Hattori N, Yamashita S, Ushijima T. Identification of coexistence of DNA methylation and H3K27me3 specifically in cancer cells as a promising target for epigenetic therapy. *Carcinogenesis*. 2015;36:192-201.
16. Sato T, Cesaroni M, Chung W, Panjarian S, Tran A, Madzo J, et al. Transcriptional selectivity of epigenetic therapy in cancer. *Cancer Res*. 2017;77:470-481.
17. Valeriote F, Lin H. Synergistic interaction of anticancer agents: a cellular perspective. *Cancer Chemother Rep*. 1975;59:895–899.
18. Momparler RL, Rivard GE, Gyger M. Clinical trial on 5-aza-2'-deoxycytidine in patients with acute leukemia. *Pharmacol Ther*. 1985;30:277-286.
19. Lemaire M, Chabot GG, Raynal NJ, Momparler LF, Hurtubise A, Bernstein ML, Momparler RL. Importance of dose-schedule of 5-aza-2'-deoxycytidine for epigenetic therapy of cancer. *BMC Cancer*. 2008;2:8,128.
20. The Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Eng J Med*. 2013;368:2059-2074.
21. Momparler RL, Côté S, Momparler LF. Epigenetic action of decitabine (5-aza-2'-deoxycytidine) is more effective against acute myeloid leukemia than cytotoxic action of cytarabine (ARA-C). *Leuk Res*. 2013;37:980-984.
22. Momparler RL, Coȋté S, Marquez VE, Momparler LF. Comparison of the antineoplastic action of 3-deazaneplanocin-A and inhibitors that target the catalytic site of EZH2 histone methyltransferase. *Cancer Rep Rev*. 2020;3:1-4.

23. Momparler RL, Côté S, Momparler LF. Epigenetic modulation of self-renewal capacity of leukemic stem cells and implications for chemotherapy. *Epigenomes*. 2020;4,3.
24. Momparler RL, Côté S, Momparler LF. Enhancement of the antileukemic action of the Inhibitors of DNA and histone methylation: 5-aza-2'-Deoxycytidine and 3-deazaneplanocin-A by Vitamin C. *Epigenomes*. 2021;24:5,7.
25. Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, et al. Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell*. 2006;125(2):301-313.
26. Schlesinger Y, Straussman R, Keshet I, Farkash S, Hecht M, Zimmerman J, et al. Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. *Nat Genet*. 2007;39(2):232-236.