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RNAi-based Gene Therapy for Blood Genetic Diseases

Mengyu Hu, Qiankun Ni, Yuxia Yang and Jianyuan Luo

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Abstract

Therapies for blood genetic diseases can be divided into different categories, including chemotherapy, radiotherapy, gene therapy, and hematopoietic stem cell transplantation. Among these treatments, gene targeting is progressively becoming a therapeutic alternative that offers the possibility of a permanent cure for certain blood genetic diseases. In recent years, gene therapy has played a more important role in curing genetic blood disorders. RNA interference (RNAi) is one of the directions for gene therapy, which was intensively studied in the past decades for its potentials in the treatment of diseases. In order to provide useful references and prospective directions for further studies concerning RNAi-based gene therapy for blood genetic diseases, current RNAi-based gene therapies for several typical blood genetic diseases have been summarized and discussed in this chapter.

Keywords: RNA interference, gene therapy, mechanism, therapeutic strategy, blood genetic diseases

1. Introduction

Conceived in the 1960s, gene therapy did not produce any meaningful results until recent reports of success appeared in clinical studies. Gene therapy attempts to treat inherited diseases using normal copies of the defective genes. Insertion and expression of specific exogenous genetic materials via the transfer of nucleic acids directly *in vivo*, or through modified cells *in vitro*, correct a cellular dysfunction or provide a new cellular function. It has the potential to cure any genetic disease with long-lasting therapeutic benefits [1, 2]. Gene therapy can be classified into (i) germ line and (ii) somatic line gene therapy types. In the former, genomes of germ cells (sperms or eggs) are integrated by exogenous functional genes, which can be carried onto the patient's offsprings. In the latter, therapeutic genes are introduced into somatic cells and the effects will only be limited to the individual patient [3].

According to the mechanisms, gene therapy may include three major categories: (a) direct modulation of the disease-causing gene, which can be applied to monogenic hereditary diseases; (b) indirect treatment through gene modulation, which can be applied to multifactorial diseases; and (c) immunotherapy by gene modulation (DNA vaccines), which leads to the synthesis of the relevant antigen or an adjuvant [1].

RNA interference (RNAi) can be used for gene therapy. It was intensively studied in the past few decades for its potential in the treatment of blood genetic diseases. RNAi-based gene therapy possesses several therapeutic advantages such as high efficiency, sequence specificity, and potentially less immunogenicity. Less immunogenicity is largely due to the use of non-protein-coding "gene products" to trigger RNAi, which makes gene therapy less likely to be potentially hampered by the host immune system [4, 5]. Compared to traditional small molecules and protein drugs, the target specificity and universal treatment spectrum make RNAi-based gene therapy an ideal treatment for blood genetic diseases. However, there are still obstacles that remain, such as barriers in the blood circulation system and the diseased tissues that block the actualization of the RNAi effects [6]. RNAi technology is a relatively new discovery, and it has already become a potent method for gene regulation. In order to provide useful references and prospective directions for further studies, current RNAi-based gene therapies for blood genetic diseases have been summarized and discussed in this chapter.

2. The mechanism of RNAi-based gene therapy

Being a well-described gene regulatory mechanism, RNAi not only suppresses transcription by transcriptional gene silencing (TGS) but also activates a homology-based mRNA degradation process by post-transcriptional gene silencing (PTGS). Both silencing pathways resulted in the decrease of the coding transcript level (mRNA) [7]. We will focus on PTGS due to its important role in RNAi-based gene therapy. Two distinct mechanisms regulate PTGS. The first one is the repression and degradation of mRNAs with imperfect complementarity. Endogenous microRNAs (miRNAs) belong to this category. They induce translational repression and mRNA degradation when the guide (antisense) strand has limited complementarity to the target mRNA. The second one is the sequence-specific cleavage of perfectly complementary mRNAs. Exogenous small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs) belong to this category. They have perfect or near-perfect base-pairings with the intended target mRNA. The miRNAs production and processing rely on host machinery that is guided by complementary miRNA strands to the target mRNA [8, 9]. During the process, double-stranded RNAs (dsRNA) of the target gene are produced and then processed into 21–24 non-coding small RNA duplexes with the help of RNaseIII enzyme dicer and its homologs. These siRNAs are then incorporated into a multi-subunit endonuclease silencing complex called RNA-induced silencing complex (RISC). siRNAs are associated with the defense against parasites, heterochromatin formation, transposon and transgene silencing, and PTGS. These siRNAs, loaded into the RISC, are used as the guide to recognize and degrade or suppress the complementary gene or mRNA utilizing the endonucleases activity of RISC. Gene silencing by RNAi can be used in different biological situations when sequence-specific knockdown of

gene expression is required, thus providing a convenient tool for analysis of gene function as well as gene therapy [10, 11, 12].

Recent reports have shown that off-targeting can commonly occur during RNAi, despite the belief that initial gene silencing through RNAi was thought to be specific. Because nonspecific hybridization of siRNAs with non-target transcripts can induce undesired effects, it should be guaranteed that the dsRNA and corresponding siRNA sequences do not exert off-target effects that negatively influence host physiology. Off-target silencing is determined by the similarity of sequences between siRNA and non-target genes, or mRNA sequences not selected for RNAi, as well as the size of siRNA and transitive RNAi. Thus, off-targeting effects can be avoided by using highly specific sequences in siRNA expressed under specific and inducible promoters. In addition, it is important to examine the off-targeting effects of RNAi at multiple levels, since undesired effects on the host may occur through the silencing of genes associated with regulatory functions and multiple metabolic pathways, such as transcription factors or signaling molecules [10].

3. RNAi-based gene therapy for blood genetic diseases

3.1. Blood diseases

Blood diseases refer to the disorders in the hematopoietic system or plasma components. The development of blood disorders is always thought to be related with inheritance, the environment, drugs, and biological factors where the changes in chromosome and/or genes play a critical role in some specific hemopathies. Therapeutic approaches for blood diseases can be divided into different categories, including chemotherapy, radiotherapy, RNAi-based gene therapy, and hematopoietic stem cell transplantation. Among these, RNAi-based gene therapy is progressively becoming a therapeutic alternative which offers the possibility of a permanent cure for some blood diseases [13].

3.2. Hemophilia

Hemophilia A and B are X-linked monogenic bleeding disorders resulting from deficiencies of factor VIII and IX, respectively. Gene therapy, utilizing both viral and non-viral delivery vectors *in vivo* and *ex vivo*, has been attempted for the treatment of both hemophilia A and B [14, 15]. Given its recent clinical success, adeno-associated vector (AAV)-mediated hepatic gene transfer could be primarily used for the treatment of hemophilia B. However, a number of problems, such as current immunosuppressive regimen and pre-existing neutralizing antibodies, limit the broad applicability of this approach. For hemophilia A, while AAV-mediated gene therapy has potential, a number of limitations reduce its desirability, such as packaging capacity and inefficient expression. While a number of transgene modifications have increased the expression levels, the vector doses require the corrective F.VIII expression to remain significantly higher than the F.IX. These expression limitations lead to further concerns about immune responses to both the capsid and, if expression levels are not sufficient, the transgene. As such, *ex vivo* gene transfer may be more effective for hemophilia A due to its

ability to enhance expression through cellular division. While a number of promising gene therapies for hemophilia have been elucidated, there are clearly numerous problems that still need to be addressed to develop approved gene therapies, especially RNAi, for both hemophilia A and B in humans [16]. RNAi-based gene therapy for hemophilia is still in its early stages of development.

3.3. β-Thalassemia

The globin chains have an extremely precise structure, ensuring their function of loading, delivering, and unloading oxygen. The globin chains are coded by genes in the chromosome 16 (α-gene) and 11(β-gene). The normal structure of globin is based on the balanced match between α-chains and β-chains. When the condition is not met, there will be a complete or partial defect in one or both allelic globin genes, such as β-thalassemia [17]. β-Thalassemia is a worldwide-distributed inherited hemoglobin disorder resulting in severe, chronic anemia [18, 19]. It is a heterozygous condition in which only a single β-globin gene is affected and results in the absence or reduced β-globin chain synthesis. The defects of β-globin synthesis lead to an excess of unmatched α-globin, which release free iron, non-heme iron, or hemichrome. These iron species promote a severe red cell membrane oxidative stress and lead to abnormal β-thalassemic red cell features. The abnormal red cells are finally removed by the macrophage system and results in anemia [20, 21].

Blood transfusion is a primary way to treat the most severe forms of β-thalassemia. Appropriate goals and optimal safety of transfused blood are necessary for routine administration of red blood cells to patients. The problem is that the high frequency of blood transfusion can lead to iron overload. In its less severe form, chronic transfusions are not required, but iron overload may still develop due to the chronic suppression of the synthesis of the iron regulatory hormone hepcidin by ineffective erythropoiesis. Untreated iron overload can be fatal, resulting in cardiac complications [3, 22]. Therefore, handling iron overload is a key factor for the successful treatment of this disease. TMPRSS6, a serine protease expressed predominantly in the liver, can inhibit an iron-responsive bone morphogenetic protein-mother against the decapentaplegic (BMP-SMAD) signaling pathway, resulting in the downregulation of hepcidin transcription. Researchers found that therapeutics with the lipid nanoparticle (LNP)-formulated RNAi targeting of TMPRSS6, in conjunction with oral deferiprone therapy, is superior to monotherapy with dietary iron deficiency and iron chelate for reducing hepatic iron storage [22].

3.4. B-lineage lymphoid malignancies

B-precursor acute lymphoblastic leukemia (BPL) is the most common form of cancer in children and adolescents. A dysfunctional CD22 is expressed in BPL cells due to the deletion of Exon 12 (CD22ΔE12) resulting from a splicing defect related to homozygous intronic mutations. CD22 is a negative regulator of multiple signal transduction pathways critical for the proliferation and survival of B-lineage lymphoid cells, and CD22ΔE12 leads to uncontrolled proliferation and the survival of B cells. Recently, researchers have found that CD22ΔE12 is especially associated with therapy-refractory clones in pediatric BPL, thus implicating the

impact of the CD22ΔE12 genetic defect on the aggressive biology of relapsed or therapy-refractory pediatric BPL. At the same time, forced expression of CD22ΔE12 in transgenic mice causes fatal BPL, demonstrating that CD22ΔE12 alone is sufficient as an oncogenic driver lesion for malignant transformation and clonal expansion of B-cell precursors [23].

Recent studies have demonstrated that B-lineage lymphoid malignancies in children and adults are characterized by a high incidence of the CD22ΔE12 genetic alterations. Moreover, the relationship between CD22ΔE12 and aggressive biology of BPL cells is also reported through the demonstration that siRNA-mediated knockdown of CD22ΔE12 in primary BPL cells is associated with a remarkable inhibition of their carcinogenicity. A unique polypeptide-based nanoparticle formulation of CD22ΔE12-siRNA is described as a first-in-class RNAi therapeutic candidate targeting of CD22ΔE12. This formulation is capable of delivering siRNA cargo into the cytoplasm of leukemia cells, leading to a remarkable inhibition of leukemic cell growth [24]. It is expected that further development of this nanoparticle may promote an effective therapeutic RNAi strategy of aggressive or chemotherapy-resistant B-lineage lymphoid malignancies [23].

3.5. Myeloid leukemia

Leukemia arising from genetic alterations in normal hematopoietic stem or progenitor cells results in the impaired regulation of proliferation, differentiation, and apoptosis, as well as the survival of malignant cells. Overall, the relative 5-year survival rate for various leukemias is only around 50% [25]. Leukemia is still a worldwide health problem, although various therapies have been explored to cure the disease. Among them, chemotherapy is always considered to be a frontline treatment, mainly containing a broad spectrum of cytotoxic agents and therapeutic molecules. Although leukemic cells respond well to chemotherapy at the onset of treatment, over a period of 6–12 months the drugs might lose effectiveness in a considerable fraction of patients. Moreover, significant side effects of traditional cytotoxic agents are inevitable at efficacious doses, which limit its function with the progression of the disease. For example, in chronic myeloid leukemia (CML), resistance to current frontline therapy of imatinib and failure to a complete cytogenetic response may occur in 24% of patients within 18 months. With a better understanding of molecular changes in leukemia, the treatment targeting tumor-specific changes, such as RNAi, is expected to make a difference in the therapeutic effectiveness of the disease [26].

Researchers have explored the suitability of RNAi in suppressing the growth and proliferation of myeloid leukemia cell lines including HL-60, U937, THP-1, and K562, which express c-raf and bcl-2 genes. The results were exciting, as the siRNA duplexes succeeded in significantly decreasing the level of target proteins by eliminating the expression of c-raf and bcl-2 genes. This led to the inhibition of the differentiation and programmed cell death suppression of myeloid leukemia cells. These results demonstrated the possibility of RNAi as a novel therapeutic approach to myeloid leukemia [27]. In recent years, based on the molecular alteration of CML, two clinical trials of RNAi therapy have been conducted to target the aberrantly expressed isoforms of the BCR-ABL fusion protein. In one case, there are no publishable outcomes. In the other case, silencing aberrant proteins with RNAi have been

found to be less prone to drug resistance [28]. In another *in vivo* application of targeted and non-virally delivered synthetic bcr-abl siRNA, a remarkable apoptosis of CML cells was found in a female patient with a recurrent Philadelphia chromosome with positive CML. The patient was resistant to imatinib and chemotherapy after hematopoietic stem cell transplantation. There were no clinically adverse events, implying feasibility and safety of the application of RNAi-based gene therapy for CML [29].

Furthermore, RNAi-based gene therapy also proves to be effective in acute promyelocytic leukemia (APL). APL is the M3 type of acute myeloid leukemia characterized by a clonal proliferation of abnormal promyelocytes in bone marrow and has a severe bleeding tendency. In 98% of APL patients, a balanced translocation between chromosomes 15 and 17 [t(15;17)(q22;q21)] was found, which leads to the formation and fusion of promelocytic leukemia protein (PML) and retinoic acid receptor alpha (RARα) [30, 31]. A variety of chromosomal aberrations have been identified in APL including t(11;17)(q23;q21), t(5;17)(q35;q12-21), t(11;17)(q13;q21), and der(17), in which the RARα gene is fused to the PLZF, NPM, NuMA, and STAT5b genes, respectively [32]. The differentiation of leukemic cells and complete remission of APL may occur after treatment with ATRA (all-trans retinoic acid). However, the alternative strategies of specific targeting of APL are required because of the ATRA resistance in patients with PLZF-RARα fusion mutation and a treatment of relapse. It has been found that the siRNA targeted knockdown of fused PML-RARα mRNA can induce differentiation and apoptosis of human APL cells; moreover, an injection of pretreated APL cells with anti-PML-RARα siRNA greatly inhibits the progression of APL in mice. Therefore, a targeted RNAi for PML-RARα fusion might be a promising treatment strategy for ATRA-resistant APL patients [33].

At present, several technological requirements and mechanistic challenges (i.e., targeted delivery of siRNAs) for efficient clinical trials have to be explored and overcome to make RNAi-based gene therapy readily applicable for the treatment of myeloid leukemia [26].

3.6. Multiple myeloma

Multiple myeloma (MM) of clonal plasma cells in bone marrow can cause damage to multiple organs. MM is the second most common hematological malignancy. Chromosomal abnormalities, oncogene activation, and growth factor dysregulation contribute to the development of MM. Like leukemia, chemotherapy is the most common treatment for MM right now. However, it is remarkably resistant to chemotherapy [34]. Molecular therapy became an alternative treatment, and the introduction of bortezomib has contributed to the improved survival of patients with MM. Resistance to this therapy inevitably occurs, and the clinical efficacy of bortezomib is significantly diminished. At present, RNAi is found to be helpful in sensitizing tumor cells to chemotherapy and radiation. Bmi-1, an oncogene, has been implicated in the pathogenesis of MM and might influence the response to bortezomib in MM patients. Bmi-1 has been silenced in two MM cell lines using shRNA targeting Bmi-1 (shBmi-1). The cell cycle progression and apoptosis of MM cells are evaluated. The prolonged G1 phase and enhanced apoptosis were observed that suggest RNAi-derived knockdown of Bmi-1 reducing the resistance to bortezomib. Therefore, Bmi-1-specific RNAi may serve as an important treatment strategy for MM [35].

4. Conclusions and future perspectives

As a powerful and novel treatment of blood genetic diseases, RNAi-based gene therapy has propelled the clinical testing of siRNAs for a variety of diseases at early stages. It is still too early to determine whether the RNAi-based therapeutics will be an efficient tool for the treatment of blood genetic diseases. Therefore, understanding the basic mechanisms of a targeted gene RNAi and its interconnection with genetic, biochemical, and physiological pathways, as well as "off-targets" and "side effects," are the most important factor for developing an efficient therapeutic RNAi strategy. It is highly possible that the RNAi-based gene therapy can be readily and efficiently used for clinical treatment in conjunction with other therapies. The power of sequence-specific suppression of gene expression without "off-targets" and undesired side effects makes RNAi-based gene therapy very promising. Although progress has been made [36], major obstacles, such as the development of methods for efficient targeted delivery of siRNAs in patients, still remain a critical task. In addition, uncovering target domains of genes related to blood genetic diseases and the discovery of more blood genetic disease-causing genes are also key research areas for developing RNAi-based gene therapy for important blood genetic diseases.

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References

[1] Linden R, Matte U. A snapshot of gene therapy in Latin America. Genet Mol Biol. 2014; 37(1 Suppl): 294–98.

[2]   Chodisetty S, Nelson EJ. Gene therapy in India: A focus. J Biosci. 2014; 39: 537–41.

[3]   Razi Soofiyani S, Baradaran B, Lotfipour F, Kazemi T, Mohammadnejad L. Gene therapy, early promises, subsequent problems, and recent breakthroughs. Adv Pharm Bull. 2013; 3: 249–55. DOI: 10.5681/apb

[4]   Liu J, Harper SQ. RNAi-based gene therapy for dominant limb girdle muscular dystrophies. Curr Gene Ther. 2012; 12: 307–14. DOI:

[5]   Liu J, Wallace LM, Garwick-Coppens SE, Sloboda DD, Davis CS, Hakim CH, Hauser MA, Brooks SV, Mendell JR, Harper SQ. RNAi-mediated gene silencing of mutant myotilin improves myopathy in LGMD1A mice. Nucleic Acids. 2014; 3: e160. DOI: 10.1038/mtna

[6]   Zhou Y, Zhang C, Liang W. Development of RNAi technology for targeted therapy — a track of siRNA based agents to RNAi therapeutics. J Controll Release. 2014; 193: 270–81. DOI: 10.1016/j.jconrel

[7]   Castanotto D, Rossi JJ. The promises and pitfalls of RNA-interference-based therapeutics. Nature. 2009; 457: 426-33. DOI: 10.1038/nature07758

[8]   Burnett JC, Rossi JJ. RNA-based therapeutics: current progress and future prospects. Chem Biol. 2012; 19: 60-71. DOI: 10.1016/j.chembiol

[9]   Kim D, Rossi J. RNAi mechanisms and applications. Biotechniques. 2008; 44: 613-6. DOI: 10.2144/000112792

[10]   Kola VS, Renuka P, Madhav MS, Mangrauthia SK. Key enzyme and protein of crop insects as candidate for RNAi based gene silencing. Front Physiol. 2015; 6: 119. DOI: 10.3389/fphys

[11]   Presloid JB, Novella IS. RNA viruses and RNAi: quasispecies implications for viral escape. Viruses. 2015; 7: 3226–40. DOI: 10.3390/v7062768

[12]   Makkonen KE, Airenne K, Ylä-Herttulala S. Baculovirus-mediated gene delivery and RNAi applications. Viruses. 2015; 7: 2099–25. DOI: 10.3390/v7042099

[13]   Porada CD, Stem C, Almeida-Porada G. Gene therapy: the promise of a permanent cure. N C Med J. 2013; 74: 526–9.

[14]   Kay MA, Manno CS, Ragni MV, Larson PJ, Couto LB, McClelland A, Glader B, Chew AJ, Tai SJ, Herzog RW, Arruda V, Johnson F, Scallan C, Skarsgard E, Flake AW, High KA. Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. Nat Genet. 2000; 24: 257–61.

[15]   Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, Ozelo MC, Hoots K, Blatt P, Konkle B, Dake M, Kaye R, Razavi M, Zajko A, Zehnder J, Rustagi PK, Nakai H, Chew A, Leonard D, Wright JF, Lessard RR, Sommer JM, Tigges M, Sabatino D, Luk A, Jiang H, Mingozzi F, Couto L, Ertl HC, High KA, Kay MA. Successful trans-

duction of liver in hemophilia by AAV-factor IX and limitations imposed by the host immune response. Nat Genet. 2006; 12: 342–7.

[16] Rogers GL, Herzog RW. Gene therapy for hemophilia. Front Biosci (Landmark Ed). 2015; 20: 556–603.

[17] Meo A, Cassinerio E, Castelli R, Bignamini D, Perego L, Cappellini MD. Effect of hy-droxyurea on extramedullary haematopoiesis in thalassaemia intermedia: case re-ports and literature review. Int J Lab Hematol. 2008; 30: 425–31. DOI: 10.1111/j.1751-553

[18] Parthasarathy V. A search for beta thalassemia trait in India. Turk J Haematol. 2012; 29: 427–9. DOI: 10.5505/tjh.2012.21703

[19] Hagag AA, Elfrargy MS, Gazar RA, El-Lateef AE. Therapeutic value of combined therapy with deferasirox and silymarin on iron overload in children with Beta thalas-semia. Mediterr J Hematol Infect Dis. 2013; 5: e2013065. DOI: 10.4084/MJHID

[20] Kalpravidh RW, Tangjaidee T, Hatairaktham S, Charoensakdi R, Panichkul N, Sirita-naratkul N, Fucharoen S. Glutathione redox system in beta-thalassemia/Hb E pa-tients. Sci World J. 2013; 7: 543973. DOI: 10.1155/2013/543973

[21] De Franceschi L, Bertoldi M, Matte A, Santos Franco S, Pantaleo A, Ferru E, Turrini F. Oxidative stress and beta-thalassemic erythroid cells behind the molecular defect. Oxid Med Cell Longev, 2013; 985210. DOI: 10.1155/2013/985210

[22] Schmidt PJ, Racie T, Westerman M, Fitzgerald K, Butler JS, Fleming MD. Combina-tion therapy with a *Tmprss6* RNAi-therapeutic and the oral iron chelator deferiprone additively diminishes secondary iron overload in a mouse model of β-thalassemia in-termedia. Exp Toxicol Pathol. 2015; 90: 310–3. DOI: 10.1002/ajh.23934

[23] Uckun FM, Qazi S, Ma H, Yin L, Cheng J. A rationally designed nanoparticle for RNA interference therapy in B-lineage lymphoid malignancies. EBioMedicine. 2014; 1: 141–55.

[24] Uckun FM, Ma H, Cheng J, Myers DE, Qazi S. CD22ΔE12 as a molecular target for RNAi therapy. Br J Haematol. 2015; 169: 401–14. DOI: 10.1111/bjh.13306

[25] National Cancer Institute Surveillance Research Program. Fast Stats: An interactive tool for access to SEER cancer statistics. 2013. Available from: http://seer.cancer.gov/faststats [Accessed: 2013-02-02]

[26] Landry B, Valencia-Serna J, Gul-Uludag H, Jiang X, Janowska-Wieczorek A, Brand-wein J, Uludag H. Progress in RNAi-mediated molecular therapy of acute and chron-ic myeloid leukemia. Mol Ther Nucleic Acids. 2015; 12: 4:e240. DOI: 10.1038/mtna

[27] Cioca DP, Aoki Y, Kiyosawa K. RNA interference is a functional pathway with thera-peutic potential in human myeloid leukemia cell lines. Cancer Gene Ther. 2003; 10: 125–33.

[28] Sawyers CL. Perspective: combined forces. Nature. 2013; 498:S7. DOI: 10.1038/498S7a

[29] Koldehoff M. Targeting bcr-abl transcripts with siRNAs in an imatinib-resistant chronic myeloid leukemia patient: challenges and future directions. Methods Mol Biol. 2015; 1218: 277–92. DOI: 10.1007/978-1-4939-1538-5_17

[30] Marchwicka A, Cebrat M, Sampath P, Snieżewski L, Marcinkowska E. Perspectives of differentiation therapies of acute myeloid leukemia: the search for the molecular basis of patients' variable responses to 1,25-dihydroxyvitamin D and vitamin D analogs. Front Oncol. 2014; 4: 125. DOI: 10.3389/fonc.2014.00125

[31] Zhou GB, Zhang J, Wang ZY, Chen SJ, Chen Z. Treatment of acute promyelocytic leukaemia with all-trans retinoic acid and arsenic trioxide: a paradigm of synergistic molecular targeting therapy. Philos Trans R Soc Lond B Biol Sci. 2007; 362: 959–71.

[32] Pagnano KB, Rego EM, Rohr S, Chauffaille Mde L, Jacomo RH, Bittencourt R, Firmato AB, Fagundes EM, Melo RA, Bernardo W. Guidelines on the diagnosis and treatment for acute promyelocytic leukemia: Associacao Brasileira de Hematologia, Hemoterapia e Terapia Celular Guidelines Project: Associacao Medica Brasileira-2013. Rev Bras Hematol Hemoter. 2014; 36: 71–92. DOI: 10.5581/1516-8484.20140018

[33] Guo J, Cahill MR, McKenna SL, O'Driscoll CM. Biomimetic nanoparticles for siRNA delivery in the treatment of leukaemia. Biotechnol Adv. 2014; 32: 1396–409. DOI: 10.1016/j.biotechadv

[34] Mutlu P, Kiraz Y, Gündüz U, Baran Y. An update on molecular biology and drug resistance mechanisms of multiple myeloma. Crit Rev Oncol Hematol. 2015; S1040-8428: 30003-2. DOI: 10.1016/j.critrevonc

[35] Wu SQ, Xu ZZ, Niu WY, Huang HB, Zhan R. ShRNA-mediated Bmi-1 silencing sensitizes multiple myeloma cells to bortezomib. Int J Mol Med. 2014; 34: 616–23. DOI: 10.3892/ijmm.2014.1798

[36] Tiemann K, Rossi JJ. RNAi-based therapeutics–current status, challenges and prospects. EMBO Mol Med. 2009; 1: 142–51. DOI: 10.1002/emmm.200900023