

# Molecular and cellular aspects of extramedullary manifestations of acute myeloid leukemia

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## ABSTRACT

The myeloid extramedullary tumor is a solid tumor formed by infiltration of immature myeloid cells in various tissues of the body. This tumor is also identified as chloroma or myeloid sarcoma (MS). MS is a manifestation of acute myeloid leukemia (AML) occurring at presentation or during treatment or relapse. MS is associated with multiple chromosomal abnormalities and molecular mutations since patients with these disorders bear a high potential for MS manifestation. There is a high incidence of extramedullary infiltration (EMI) in AML. AML patients with EMI have a worse prognosis than patients without it. Hematopoietic stem cells and leukemic stem cells reside in a special bone marrow microenvironment called niche, which is essential for their normal functions. Cancers are exploited dysfunctional cell-cell and matrix-cell interactions, which convert a normal niche into a neoplastic niche. This study summarizes the current knowledge on the molecular and cellular characteristics of AML with EMI and extramedullary niches in AML patients.

**Key words:** Acute myeloid leukemia; extramedullary infiltration; niche

## INTRODUCTION

Acute myeloid leukemia (AML) is an aggressive myeloid neoplasm characterized by maturation arrest of myelopoiesis leading to an accumulation of myeloblasts in the blood and bone marrow (BM).<sup>[1]</sup> AML is a complex and heterogeneous disease strongly associated with genetic and epigenetic changes in the hematopoietic progenitors.<sup>[2]</sup> These changes lead to disruption of several signaling pathways that result in increased proliferation, survival and accumulation of leukemic cells.<sup>[3]</sup>

Normal hematopoietic stem cells (HSCs) reside in a specialized area of the BM microenvironment known as niche, which regulates their survival and function. Two distinct niches exist in the BM: Vascular and endosteal/osteoblastic niche. The vascular niche is localized in close proximity to the osteoblastic niche, at the inner surface of bone cavity with abundant bone-forming osteoblasts. The vascular niche is composed of sinusoidal endothelial cells

lining blood vessels, and it promotes the proliferation and differentiation of short-term HSCs. The endosteal niche includes osteoblasts, osteoclasts, glial non-myelinating Schwann cells and regulatory T-cells, and it is located in the endosteum. The vascular niche contains CXCL12-abundant reticular cells, nestin-positive mesenchymal stem cells and leptin receptor-positive cells.<sup>[4]</sup> HSC niches are present in different tissues during development, first in the aorta-gonad-mesonephros (AGM) region and yolk sac, then in the placenta, fetal liver, spleen and BM. After birth, the BM is the primary site of HSC maintenance and hematopoiesis, but the niche can shift to extramedullary sites in response to hematopoietic stress.<sup>[5]</sup>

AML may present with extramedullary-AML at initial diagnosis or in relapse. Myeloid sarcoma (MS) is defined as an extramedullary mass composed of myeloid blasts occurring in anatomic sites other than BM.<sup>[6]</sup> Extramedullary

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infiltration (EMI) is fairly common in AML patients. In addition, MS has been observed in all age groups, and may occur anywhere in the body. The most common tissues include soft tissues, bone, peritoneum, lymph nodes and gastrointestinal tract. Other occasional sites include male and female urogenital system and central nervous system (CNS).<sup>[7]</sup> Moreover, several studies have found a worse prognosis in cases of acute leukemia with EMI, which may be explained by a poor response to chemotherapy and disease relapse.<sup>[8]</sup> Evaluation of the cellular and molecular structures of extramedullary niches, as well as the migration and homing of leukemic cells, may help in designing diagnostic and therapeutic techniques and preventing relapse. However, there is still little information in this regard. The aim of this study was to investigate the characteristics of leukemic cells and the changes in their microenvironment that promote to EMI.

## GENETIC AND MOLECULAR FEATURES OF EXTRAMEDULLARY INFILTRATION IN AML

Extramedullary leukemia (EML) is also called MS, granulocyte sarcoma and chloroma. In the WHO classification, MS is an important subgroup of myeloid neoplasia and acute leukemia. MS may occur simultaneously with, before or after the diagnosis of AML.<sup>[9]</sup> Genetic mutations and molecular aberrations are an important tool for the evaluation of acute leukemia and assessment of prognosis. However, there is very limited information on the role of genetic mutations in MS.<sup>[10]</sup> Although the overall incidence of MS in AML has been reported at 1.4-9%, it is particularly high in some subtypes of AML, reaching 18-24% in AML patients with t(8:21) and 25% in pediatric AML.<sup>[11]</sup> Other genetic abnormalities diagnosed in EML patients include t(15:17), t(9:11), t(1:11), t(8:17), del(16q), del(5q), del(20q), monosomy 7, trisomy 4 and trisomy 8.<sup>[12]</sup> Moreover, according to the French-American-British classification, some AML types are associated with EML, including M4 and M5 monocytic leukemias and the M2 subtype.<sup>[13]</sup>

The t(8:21) has been reported as the most common cytogenetic abnormality associated with EML, occurring both at presentation and upon relapse, and is associated with orbital involvement in infants.<sup>[10]</sup> Inv(16) is another abnormality associated with EML; it is rarer than t(8:21). According to studies, the bowel may be a target organ in men with inv(16) while breast and ovary tend to develop EML in female patients with inv(16).<sup>[14]</sup> AML with trisomy 8 is found in nearly 5% of AML cases with a genetic abnormality. According to a study, trisomy or tetrasomy of chromosome 8 is observed in 35-65% of AML cases with leukemia cutis as a type of MS. Although confirmation of this relationship requires further evaluation, based on numerous reports, we can suggest that trisomy 8 is a risk factor for skin infiltration in AML.<sup>[15]</sup>

Acute promyelocytic leukemia (APL) is another subtype of AML defined as having a translocation between chromosomes 15 and 17 and generation of promyelocytic leukemia/retinoic acid receptor alpha fusion protein.<sup>[16]</sup> This fusion protein causes a block at the promyelocytic differentiation stage.<sup>[17]</sup> APL can occur in extramedullary form, and EMI is responsible for 3-8% of cases in relapse. The most common target tissues are CNS and skin.<sup>[18]</sup> Some studies indicate a relationship between 11q23 mixed-lineage leukemia rearrangement (MLLr) and EML. According to some studies, the involvement in this type of cytogenetic abnormalities has been limited to chest and uterus.<sup>[19,20]</sup> Furthermore, another study suggests a link between MLLr and lymph node involvement.<sup>[21]</sup> More studies are needed to confirm these observations.

Molecular abnormalities associated with EML have not been systematically defined; however, a well-documented molecular abnormality is a mutation in the nucleophosmin (NPM-1) gene.<sup>[12]</sup> Nucleolar phosphoprotein or NPM-1 is localized in nuclear foci and is a multifunctional protein expressed in various cells.<sup>[22]</sup> *NPM-1* gene mutation is the most common molecular genetic abnormality in AML, particularly AML with normal karyotype.<sup>[23]</sup> *NPM-1* is mutated in almost 15% of cases of MS.<sup>[24]</sup> In a survey conducted on 89 AML patients, 15 patients (18%) had extramedullary manifestation at diagnosis, and 13 of them (87%) had mutated *NPM-1*.<sup>[25]</sup>

FMS-related tyrosine kinase 3-internal tandem duplication (FLT3-ITD) mutation is observed in 28-34% of cases of AML with normal cytogenetics. It plays an important role in cell proliferation, survival and differentiation of hematopoietic progenitor cells.<sup>[26]</sup> Some studies have found an association between FLT3-ITD mutation and EML, so that in one study, 15% of MS patients have this mutation.<sup>[27,28]</sup>

CD56, a neural cell-adhesion molecule, is expressed in normal, natural killer cells. Aberrant expression of CD56 in AML blasts, particularly AML with translocation t(8:21) correlates with a worse prognosis than CD56-negative cases.<sup>[29]</sup> An association has been described between the expression of CD56 and EMI, especially in lymph nodes (lymphadenopathy).<sup>[21,30,31]</sup> CD56 gene is in the 11q23.1 locus.<sup>[32]</sup> Due to this fact and to the connection between 11q23 mutation and EMI, MLLr is likely associated with aberrant expression of CD56 in EMI. Some case reports and studies support this hypothesis.<sup>[20,21,33]</sup>

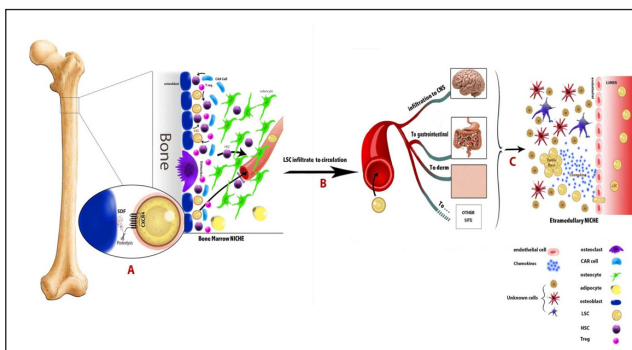
Minimal residual disease (MRD) assessment is an important feature of therapy management, especially in cases whose recurrence risk is high. There is not much information on MRD in MS patients, and only one study has evaluated the correlation between continuous detection of AML1-MTG8 chimeric transcripts in BM and peripheral blood, and extramedullary relapse in t(8:21) AML.<sup>[34]</sup>

Available information indicates that the prognosis of EML is poor with short overall survival.<sup>[11]</sup> In an evaluation, the 5-year survival rate for patients with MS was 21%. Patients treated with chemotherapy showed longer survival than untreated patients.<sup>[35]</sup> Although the mortality rate of acute leukemia patients has been reduced with the emergence of new therapies, many patients still suffer from refractory disease or relapse, and EMI is one of the main causes of poor prognosis in these patients.<sup>[8]</sup>

## EXTRAMEDULLARY NICHE IN AML

During development, HSCs are initially present in AGM and then migrate into the fetal liver and embryonic bone, which remains the only active site of hematopoiesis in adult life. Movement and homing of HSCs in the BM is associated with CXCL12 chemokine and its receptor CXCR4.<sup>[36]</sup> Cancer subverts cell-cell and matrix-cell interactions and converts the normal niche to a neoplastic one.<sup>[37]</sup>

ITD-FLT3 mutation, which is common in AML and MS patients, leads to deregulation of CXCR4 in AML leukemic cells since CXCR4 signaling is markedly decreased in patients with ITD-FLT3 compared with patients without it. It is thought that this mutation facilitates the infiltration of leukemic cells into visceral organs by reducing the homing of leukemic cells.<sup>[38]</sup> Infiltration of leukemic cells in other organs is likely associated with chemokine receptor expression and different adhesion molecules. For example, NCAM1 or CD56, which is associated with a high incidence of MS, is highly expressed in the breast, testicular tissue, ovary and gut. This molecule is responsible for homing of leukemic cells in these tissues.<sup>[11]</sup> Moreover, AML blasts isolated from skin show a group of specific chemokine receptors including CCR5, CXCR4, CXCR7, and CX3CR1 compared with AML blasts isolated from blood and BM. These cytokine-cytokine receptor interactions enable homing and survival of AML blasts in skin [Figure 1].<sup>[39]</sup>



**Figure 1:** Extramedullary infiltration formation process in acute myeloid leukemia. (A) Within deregulation of LSCs located in BM, cell links with niche will be cut, and then they will enter into the circulation via BM sinusoids; (B) disseminated LSCs circulate in blood vessels and based on their special characteristics, enter specific tissue, like skin; (C) leukemic cells, along with new, distinct niche, initiate new tumor in metastatic tissue. The exact molecular and cellular characteristics have not been defined completely. SDF-1: stromal cell-derived factor 1; BM: bone marrow; LSC: leukemic stem cells; HSC: hematopoietic stem cell; CAR cell: CXCL12-abundant reticular cell

Expression of matrix metalloproteases may contribute to the increased incidence of EMI in some subtypes of AML. For example, in SHI-1 cells, a highly invasive human acute monocytic leukemia cell line, there is a high expression level of matrix metalloproteinase 2 (MMP-2), membrane-type 1 MMP and tissue inhibitor of metalloproteinase, which facilitate cell invasion.<sup>[40]</sup> Moreover, it has been suggested that the specific binding of MMP-9 via its procatalytic domain to leukocyte surface I domains of beta-2 integrins is essential for precellular proteolysis and migration of AML-derived cells<sup>[41]</sup> [Table 1].

## MICRORNAS' SIGNIFICANCE IN EXTRAMEDULLARY AML

MicroRNAs (miRNAs) are small, 18-25 nucleotide non-coding RNA molecules, which regulate gene expression by hybridizing to their complementary messenger RNA. Each miRNA has the potential to regulate several different transcripts through partially complementary target sequences. miRNAs participate in cell differentiation, proliferation and carcinogenesis.<sup>[56]</sup> Several studies have shown that miRNAs play key roles in normal hematopoiesis and various hematological malignancies. Different miRNAs are also known in myelopoiesis and myeloid neoplasias like AML.<sup>[57]</sup> Functional studies have shown that miRNAs play an important role in the pathogenesis of AML as either oncogenes or tumor suppressors. It has also been shown that distinct miRNA expression signatures are associated with response to chemotherapy and clinical outcomes.<sup>[58]</sup> Based on our literature and database searches, there has been no study describing miRNA signatures in MS. However, some studies show a link between miRNAs and mutation-induced MS. MiR-100 is aberrantly expressed in a number of cancer cells, including AML cells. Increased expression of miR-100 in AML is associated with maturation block. *In vitro* studies indicate that increased expression of miR-100 in AML cells inhibits retinoblastoma 1 serine phosphates from human chromosome 3 and causes the release of E2F in addition to increased levels of phosphorylated retinoblastoma. These events induced proliferation and inhibited the differentiation of granulocyte/monocyte cells.<sup>[59]</sup> In a study performed on 106 pediatric AML patients, it showed that this miRNA was associated with AML with extramedullary manifestation.<sup>[60]</sup>

High expression level of miR-10 family is associated with AML with mutated NPM-1.<sup>[25,61]</sup> Furthermore, miR-424 in AML patients with NPM-1 mutation is down-modulated.<sup>[62]</sup> In AML patients with the FLT3-ITD mutation, miR-451 and miR-144 were down-regulated while miR-155 was overexpressed.<sup>[63]</sup> As previously mentioned, NPM-1 and FLT3-ITD mutations, as well as some other cytogenetic abnormalities, are associated with increased risk of EMI.<sup>[25,27,28]</sup> In summary, deregulated miRNAs in these disorders can be considered as candidate markers for future studies in MS patients [Table 2].

**Table 1:** Evaluation of AML patients with extramedullary infiltration

Age (years), sex	Cytogenetics	Molecular test	CD markers	Extramedullary site	Subtype	Prognosis	References
51, female	46XX (50%)/45XX del (5)(q13q33), -7, add (15)(q22), -18	WT1+	MPO+, CD3-, CD20-, TdT-	Genital area	AML with multi-lineage dysplasia	Poor	[42]
16, female	PML-RAR at (15;17)		CD13+(85%), CD15+, CD33+, CD117+, CD34±, HLA-DR-	Right humerus, right proximal femur and distal tibia	APL		[43]
19, male		11q23 (MLL-AF10) rearrangement, low-level	HLA-DR+, CD4+, CD11c+, CD13+, CD15+, CD33+, CD117+, CD56+, CD45±	Pulmonary	M5		[44]
42, female	t(8;21)-RUNX1-RUNX1T1	FLT3 inhibition FLT3-ITD	MPO+, CD34+, Ki67 (60-70%)	Auditory canal	M2	Second morphologic CR	[45]
29, female	47, XX, +8, t(9;11)(p22; q23)	MLL-AF9 fusion gene	CD117+, CD33+, CD38+, CD15+, CD64+, CD4+, CD56+	Left and right breast	M4	CR	[20]
28, female	No overt cytogenetic aberration was shown	MLL-AF9 fusion gene	CD117+, CD13+, CD33+, CD34+, CD38+, MPO+, HLA-DR+	Left breast	M4	CR	[20]
12, female	t(9;11)(p22;q23)	MLL gene rearrangement	CD45+, CD33+, CD4+, alpha-1-antitrypsin+, muramidase+	Intra-abdominal and presacral	M5	CR	[46]
15, female			HLA-DR+, CD33+, CD15+, CD4+, CD11c+, CD11b+, CD9+, CD7+, CD56+, CD14+	Abdomen	M5a	Poor	[47]
3-month, female	normal female karyotype - t(9;11)(p22; q23)	MLL gene rearrangement	CD45+, CD33+, CD117+, CD4+, CD1a+	Skin	M5	CR	[47]
10, male	45, X,-Y, del(2)(p21), t(8;21)(q22;q22)		CD13+, CD34+, CD33+, MPO+, HLA-DR+	Appendix	M2	CR	[48]
38, female	t(6;21)		CD13+, CD33+, CD34+, CD15+, CD117+, CD64+, CD65+, MPO+, CD56+	Gastric	M4	Poor	[33]
57, male	47, XY, +8, t(9;11)(p22; q23)	11q23, tetrasomy 8	CD4+, CD13+, CD16+, CD33+, CD56+ and	Forearm and thigh	M5a	No relapse during chemotherapy	[33]
69, female			HLA-DR+	Eye	M1	Poor	[49]
47, male	t(8;17), t(17;17)		MPO+, CD43+, CD33+, CD34+, CD117+	Pancreas	M2		[50]
1, male	t(15;17)	PML-RARA	CD33+, CD65+, MPO+	Mandible	APL		[51]
13, female	47, XX, +21 and 46, del(x)(q22)		CD45+, CD117+, CD34+, CD43+, CD68+	Cardiac	M5	Poor	[52]

Contd...

**Table 1: Contd...**

Age (years), Cytogenetics sex	Molecular test	CD markers	Extramedullary site	Subtype	Prognosis	References
59, male	46, XY, dup (1) (q21; q32) in 2/20 cells and 46, XY 18/20 cell	FLT3-ITD mutation	CD34+, MPO+, CD25+	Epidural	M2	[53]
64, male	Trisomy 8		CD45+, CD68+ (KP-1), CD4+, CD56+	Skin	M5	[15]
24, male	46, XY and t (8;21) (q22; q22)		MPO+, CD56+	Stomach	M2	[54]
47, female	Deletion 17q21		CD43+, CD68+, CD56-	Eye	M4 Poor	[55]

MPO: myeloperoxidase; AML: acute myeloid leukemia; APL: acute promyelocytic leukemia; CR: complete remission; FLT3-ITD: FMS-related tyrosine kinase 3-internal tandem duplication; MLL: mixed-lineage leukemia; PML-RARA: promyelocytic leukemia/retinoic acid receptor alpha; HLA-DR: human leukocyte antigens-DR; TdT: terminal deoxynucleotidyl transferase

**Table 2: MiR changes in AML with abnormalities associated with prevalence of myeloid sarcoma**

Abnormality	Down-regulate	Up-regulate	References
11q23, MLL rearrangement	miR-34b, miR-15a, miR-29a, miR-29c, miR-372, miR-30a, miR-29b, miR-30e, miR-196a, miR-196b, let-7f, miR-102, miR-331, miR-299, miR-193	miR-326, miR-219, miR-194, miR-301, miR-324, miR-339, miR-99b, miR-328, miR-150, miR-17-92 cluster	[64-66]
FIT3-ITD	miR-451, miR-144	miR-155 (3.1-fold), miR-10a (2.5-fold) and miR-10b (2.27-fold)	[63,65]
NPM-1	miR-424	miR-10	[61,62]
APL	miR-181b	miR-15a, miR-15b, miR-16-1, let-7a-3, let-7c, let-7d, miR-223, miR-342 and miR-107, miR-125b	[67,68]
+8 AML	miR-496, miR-493	miR-34b, miR-370, miR-107, miR-342-3p, miR-96	[69]

AML: acute myeloid leukemia; APL: acute promyelocytic leukemia; NPM-1: nucleophosmin-1; MLL: mixed-lineage leukemia

## CONCLUSION

EMI is a relatively common manifestation of AML, with increased incidence in specific subtypes.<sup>[7]</sup> Despite advances in the diagnosis and treatment of myeloid leukemias, there is insufficient information on the diagnosis, treatment and pathogenesis of EML.<sup>[38]</sup> Molecular and cellular studies of EML cases, as well as evaluation of the differences between AML patients with and without EMI, have revealed some features of EML. Elucidating the relationship between genetic abnormalities and sites prone to infiltration may contribute to the prevention and early detection of EML in target tissues. In many cases, MS is misdiagnosed at first, with the most common alternative diagnoses being lymphoma, melanoma, extramedullary hematopoiesis and inflammation. Given the aggressive nature of MS, early diagnosis with sensitive and specific tests is vital to these patients.<sup>[9]</sup> Available information suggests that ITD-FLT3 mutations, which are prevalent in patients with EML, may play an important role in the pathogenesis of disease. Therefore, ITD-FLT3 mutation scan should be evaluated as a diagnostic and prognostic factor in patients. Moreover, *NPM-1* mutation, which also has a high prevalence in EML, should be evaluated as a prognostic test.

According to case report studies, common CD markers in EML include CD13, CD33, CD34, CD117, myeloperoxidase (MPO), CD56 and CD68; these should be considered in immunophenotype assessment of the disease [Table 1]. In a study conducted on MS patients, similar results were indicated, and CD68/KP1 was the most common positive

marker in 100% of patients. Other markers, in order of positive frequency, were: MPO (83.6%), CD117 (80.4%), CD99 (54.3%), CD68/ PG-M1 (51%), CD34 (43.4%), terminal deoxynucleotidyl transferase (31.5%), CD56 (13%), CD61 (2.2%), CD30 (2.2%) and CD4 (1.1%).<sup>[70]</sup> These data can be useful to develop a diagnostic immunophenotyping panel for MS patients. Allogeneic hematopoietic stem cell transplantation (HSCT) is increasingly used as treatment procedure for AML patients, but there are no standard procedures for EML therapy. Furthermore, HSCT not only is not an effective procedure for EML, but it can also increase the risk of EML relapse in AML patients.<sup>[71]</sup> Studies reviewed in this article suggest that cases of AML that have blasts with relatively specific characteristics have a high-risk for non-hematopoietic tissue infiltration. These features may be very helpful in distinguishing patients susceptible to EMI. Further studies are needed to develop diagnostic and therapeutic standards for patients with EMI as well as sensitive and specific prognostic biomarkers.

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## Conflicts of interest

There are no conflicts of interest.



41. Stefanidakis M, Karjalainen K, Jaalouk DE, Gahmberg CG, O'Brien S, Pasqualini R, Arap W, Koivunen E. Role of leukemia cell invadosome in extramedullary infiltration. *Blood* 2009;114:3008-17.
42. Pulgar F, Vélez D, Valdeolivas N, García J, Cabrera A, Pericet L, Trasobares L, Medina S, García M. Post-bone-marrow-transplant leukemia cutis. *Dermatol Online J* 2013;19:6.
43. Worch J, Ritter J, Frühwald MC. Presentation of acute promyelocytic leukemia as granulocytic sarcoma. *Pediatr Blood Cancer* 2008;50:657-60.
44. Hoffman LM, Gore L, Maloney KW. Pulmonary presentation of relapsed acute myeloid leukemia. *J Pediatr Hematol Oncol* 2014;36:228-30.
45. Di Veroli A, Micarelli A, Cefalo M, Ceresoli E, Nasso D, Cicconi L, Mauramati S, Ottaviani F, Venditti A, Amadori S. Recurrence of at(8;21)-positive acute myeloid leukemia in the form of a granulocytic sarcoma involving cranial bones: a diagnostic and therapeutic challenge. *Case Rep Hematol* 2013;2013:245395.
46. Johansson B, Fioretos T, Kullendorff CM, Wiebe T, Békássy AN, Garwicz S, Forestier E, Roos G, Akerman M, Mitelman F, Billström R. Granulocytic sarcomas in body cavities in childhood acute myeloid leukemias with 11q23/MLL rearrangements. *Genes Chromosomes Cancer* 2000;27:136-42.
47. Lin CH, Wu KH, Lin WC, Tsai JD, Peng CT, Chen AC. Granulocytic sarcoma of the colon in a child with acute myeloid leukemia presenting as hematochezia. *J Pediatr Hematol Oncol* 2008;30:981-3.
48. Choi ER, Ko YH, Kim SJ, Jang JH, Kim K, Kang WK, Jung CW, Kim DH. Gastric recurrence of extramedullary granulocytic sarcoma after allogeneic stem cell transplantation for acute myeloid leukemia. *J Clin Oncol* 2010;28:e54-5.
49. Méndez-Cepeda P, Millán-Rodríguez AC, Dios E, Alvarez C, Sevillano C. Conjunctival myeloid sarcoma in acute myeloblastic leukemia-M1. *Arch Soc Esp Ophthalmol* 2012;87:79-81.
50. Tabriz N, Tannapfel A, Griesinger F, Weyhe D. Chloroma of pancreas-initial manifestation of a secondary leukemia after stem cell transplantation case report and review of the literature. *J Gastrointest Surg* 2013;17:1331-5.
51. Yamashita Y, Isomura N, Hamasaki Y, Goto M. Case of pediatric acute promyelocytic leukemia presenting as extramedullary tumor of the mandible. *Head Neck* 2013;35:E310-3.
52. Cash T, Becton D, Mian A. Cardiac myeloid sarcoma: a case report and review of literature. *J Pediatr Hematol Oncol* 2011;33:e330-2.
53. Isshiki Y, Ohwada C, Togasaki E, Shimizu R, Hasegawa N, Yamazaki A, Sugita Y, Kawaguchi T, Tsukamoto S, Sakai S, Takeda Y, Takeuchi M, Sakaida E, Shimizu N, Ota S, Yokote K, Iseki T, Nakaseko C. Acute myeloid leukemia concurrent with spinal epidural extramedullary myeloid sarcoma accompanied by a high CD25 expression and the FLT3-ITD mutation. *Intern Med* 2014;53:1159-64.
54. Ando T, Mitani N, Matsui K, Yamashita K, Nomiya J, Tsuru M, Yujiri T, Tanizawa Y. Recurrent extramedullary relapse of acute myelogenous leukemia after allogeneic hematopoietic stem cell transplantation in a patient with the chromosomal abnormality t(8;21) and CD56-positivity. *Int J Hematol* 2009;90:374-7.
55. Kiratli H, Demiroğlu H, Emeç S. Ocular relapse in acute myeloid leukemia (M4) with normal bone marrow. *Int Ophthalmol* 2009;29:243-5.
56. Svensson AM, Hu Y. Molecular diagnostics in adult acute myeloid leukemia. *North Am J Med Sci* 2012;5:29-37.
57. Undi RB, Kandi R, Gutti RK. MicroRNAs as haematopoiesis regulators. *Adv Hematol* 2013;2013:695754.
58. Ranganathan P, Garzon R. Clinical implications of MicroRNAs in AML. In: Andreeff M, editor. Targeted Therapy of Acute Myeloid Leukemia. Berlin: Springer; 2015. p. 699-705.
59. Zheng YS, Zhang H, Zhang XJ, Feng DD, Luo XQ, Zeng CW, Lin KY, Zhou H, Qu LH, Zhang P, Chen YQ. MiR-100 regulates cell differentiation and survival by targeting RBSP3, a phosphatase-like tumor suppressor in acute myeloid leukemia. *Oncogene* 2011;31:80-92.
60. Bai J, Guo A, Hong Z, Kuai W. Upregulation of microRNA-100 predicts poor prognosis in patients with pediatric acute myeloid leukemia. *Onco Targets Ther* 2012;5:213-9.
61. Havelange V, Ranganathan P, Geyer S, Nicolet D, Huang X, Yu X, Volinia S, Kornblau SM, Andreeff M, Croce CM, Marcucci G, Bloomfield CD, Garzon R. Implications of the miR-10 family in chemotherapy response of NPM1-mutated AML. *Blood* 2014;123:2412-5.
62. Faraoni I, Laterza S, Ardiri D, Ciardi C, Fazi F, Lo-Coco F. MiR-424 and miR-155 deregulated expression in cytogenetically normal acute myeloid leukaemia: correlation with NPM1 and FLT3 mutation status. *J Hematol Oncol* 2012;5:26.
63. Whitman SP, Maharry K, Radmacher MD, Becker H, Mrózek K, Margeson D, Holland KB, Wu YZ, Schwind S, Metzler KH, Wen J, Baer MR, Powell BL, Carter TH, Kolitz JE, Wetzler M, Moore JO, Stone RM, Carroll AJ, Larson RA, Caligiuri MA, Marcucci G, Bloomfield CD. FLT3 internal tandem duplication associates with adverse outcome and gene-and microRNA-expression signatures in patients 60 years of age or older with primary cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *Blood* 2010;116:3622-6.
64. Mi S, Li Z, Chen P, He C, Cao D, Elkahloun A, Lu J, Pelloso LA, Wunderlich M, Huang H, Luo RT, Sun M, He M, Neilly MB, Zeleznik-Le NJ, Thirman MJ, Mulloy JC, Liu PP, Rowley JD, Chen J. Aberrant overexpression and function of the miR-17-92 cluster in MLL-rearranged acute leukemia. *Proc Natl Acad Sci U S A* 2010;107:3710-5.
65. Garzon R, Volinia S, Liu CG, Fernandez-Cymering C, Palumbo T, Pichiorri F, Fabbri M, Coombes K, Alder H, Nakamura T, Flomenberg N, Marcucci G, Calin GA, Kornblau SM, Kantarjian H, Bloomfield CD, Andreeff M, Croce CM. MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. *Blood* 2008;111:3183-9.
66. Jiang X, Huang H, Li Z, Li Y, Wang X, Gurbuxani S, Chen P, He C, You D, Zhang S, Wang J, Arnovitz S, Elkahloun A, Price C, Hong GM, Ren H, Kunjamma RB, Neilly MB, Matthews JM, Xu M, Larson RA, Le Beau MM, Slany RK, Liu PP, Lu J, Zhang J, He C, Chen J. Blockade of miR-150 maturation by MLL-fusion/MYC/LIN-28 is required for MLL-associated leukemia. *Cancer Cell* 2012;22:524-35.
67. Garzon R, Pichiorri F, Palumbo T, Visentini M, Aqeilan R, Cimmino A, Wang H, Sun H, Volinia S, Alder H, Calin GA, Liu CG, Andreeff M, Croce CM. MicroRNA gene expression during retinoic acid-induced differentiation of human acute promyelocytic leukemia. *Oncogene* 2007;26:4148-57.
68. Zhang H, Luo XQ, Feng DD, Zhang XJ, Wu J, Zheng YS, Chen X, Xu L, Chen YQ. Upregulation of microRNA-125b contributes to leukemogenesis and increases drug resistance in pediatric acute promyelocytic leukemia. *Mol Cancer* 2011;10:108.
69. Becker H, Maharry K, Mrózek K, Volinia S, Eisfeld A, Radmacher MD, Kohlschmidt J, Metzler KH, Schwind S, Whitman SP, Mandler JH, Wu YZ, Nicolet D, Paschka P, Powell BL, Carter TH, Wetzler M, Kolitz JE, Carroll AJ, Baer MR, Caligiuri MA, Stone RM, Marcucci G, Bloomfield CD. Prognostic gene mutations and distinct gene-and microRNA-expression signatures in acute myeloid leukemia with a sole trisomy 8. *Leukemia* 2014;28:1754-8.
70. Pileri SA, Ascani S, Cox MC, Campidelli C, Bacci F, Piccioli M, Piccaluga PP, Agostinelli C, Asioli S, Novero D, Bisceglia M, Ponzoni M, Gentile A, Rinaldi P, Franco V, Vincelli D, Pileri A Jr, Gasbarra R, Falini B, Zinzani PL, Baccarani M. Myeloid sarcoma: clinicopathologic, phenotypic and cytogenetic analysis of 92 adult patients. *Leukemia* 2006;21:340-50.
71. Harris AC, Kitko CL, Couriel DR, Braun TM, Choi SW, Magenau J, Mineishi S, Pawarode A, Yanik G, Levine JE. Extramedullary relapse of acute myeloid leukemia following allogeneic hematopoietic stem cell transplantation: incidence, risk factors and outcomes. *Haematologica* 2013;98:179-84.