Exp Oncol 2021
43, 3, 229–233

Experimental ONCOLOGY

11q23/*MLL* REARRANGEMENTS IN ADULT ACUTE LEUKEMIA

*O.V. Zotova[1, *], A.S. Lukianova[2], M.O.Valchuk[1], Yu.S. Karol[3], O.O. Shalay[1], V.L. Novak[1], V.E. Loginsky[1]*
[1]State Institute "Institute of Blood Pathology and Transfusion Medicine", National Academy of Medical Sciences of Ukraine, Lviv 79057, Ukraine
[2]Medical Biology Centre "Genom", Kyiv 04216, Ukraine
[3] Communal Noncommercial Enterprise "Municipal Clinical Hospital № 5", Lviv 79057, Ukraine

Aim: **To detect the frequency, diagnostic and prognostic significance of 11q23/*MLL* rearrangements and to determine the chromosomes that are most frequently involved in 11q23/*MLL* abnormalities in adult acute leukemia (AL).** ***Materials and Methods*****: Cytogenetic investigations of bone marrow and/or peripheral blood cells from 140 patients with acute myeloid leukemia (AML) and 57 patients with acute lymphoblastic leukemia (ALL) were performed. The methods of conventional cytogenetics (GTG-banding) and fluorescence *in situ* hybridization were used.** ***Results*****: Chromosomal abnormalities in leukemia cells were found by conventional cytogenetic methods in 80 (57%) and 37 (65%) adult patients with AML and ALL, respectively. 11q23/*MLL* rearrangements were found in 7 (5%) and 8 (14%) patients with AML and ALL, respectively. Among them, 8 (53.4%) patients had translocations, 2 (13.3%) — had deletions and 5 (33.3%) patients had trisomies or tetrasomies of chromosome 11. With respect to the distribution of partner chromosomes involved in 11q23/*MLL* translocations chromosome 4 was found to participate in 3 (37.5%) cases of 11q23/*MLL* translocations, 9 — in 2 (25%) cases and chromosomes 10, 14 and non-identified chromosome were involved in 1 (12.5%) case each. Nine patients (60%), besides abnormal ones, had 9–86% normal metaphases in their karyotypes. Of 15 patients with 11q23/*MLL* rearrangements, 5 (33%) patients had only 11q23/*MLL* rearrangements, whereas other 10 (67%) — had additional cytogenetic abnormalities, besides 11q23/*MLL* rearrangements.** ***Conclusions*****: Chromosomal abnormalities of various kinds were found in 57% and 65% adult patients with AML and ALL, respectively. The frequency of 11q23/*MLL* rearrangements in patients with AML and ALL was 5% and 14%, respectively. Since AL patients with 11q23/*MLL* rearrangements are attributed to cytogenetic categories of AL with a poor or intermediate risk prognosis, cytogenetic methods should be included in the standard examination of AL patients for diagnosis, prognosis and selection of the optimal treatment strategy.**
Key Words**: acute leukemia, karyotype, cytogenetic abnormalities, 11q23/*MLL* rearrangements, diagnosis, prognosis.**

DOI: 10.32471/exp-oncology.2312-8852.vol-43-no-3.16495

Acute leukemia (AL) is a heterogeneous disease characterized by diverse clinical course and varied sensitivity to therapy. A key event in the development of AL is the restructuring of the progenitor cell genome, causing the disruption in molecular control of cell cycle, transcription and translation of major protein regulators. Damage to the leukemic cells genome is mainly represented by chromosomal rearrangements of proto-oncogenes or suppressor genes (translocations, inversions, deletions, loss of chromosomes, extra copies of chromosomes, etc.). The cloning of the genes located at the breakpoints of chromosomal translocations has resulted in the identification of new genes involved in carcinogenesis [1–3]. Molecular studies of the breakpoint of several translocations involving chromosomal band 11q23 led to the cloning of the *MLL* gene (mixed-lineage leukemia or myeloid-lymphoid leukemia). Based on literature data, 11q23/*MLL* rearrangements were detected in 5% of acute myeloid leukemia (AML) and 10–20% of acute lymphoblastic leukemia (ALL). More than 100 different rearrangements involving band 11q23 have so far been identified, of which the most frequently observed are t(4;11)(q21;q23), t(9;11)(q22;q23), t(11;19)(q23;p13.1), t(11;19)(q23;p13.3) [4–6].

Despite the large variety of rearrangements involving the *MLL* gene, the presence of distinct 11q23/*MLL* rearrangements is an independent dismal prognostic factor, while very few 11q23/*MLL* rearrangements display an intermediate outcome. Therefore, detection of 11q23/*MLL* disruption or amplification is much needed for treatment decision. Studying the wide variety of fusion genes involving *MLL* could also lead to a better understanding of leukemogenesis.

The aim of the study was to detect the frequency, diagnostic and prognostic significance of 11q23/*MLL* rearrangements in adult AL and to determine the most common chromosomes involved.

MATERIALS AND METHODS

Cytogenetic investigations of bone marrow (BM) and/or peripheral blood (PB) cells from 197 adult patients with AL [age range, 18–85 years, 113 (57%) males and 84 (43%) females] were performed. AL was diagnosed according to the WHO definition of > 20% blasts in the BM or PB and based on French-American-British (FAB) classification and immunophenotype. 140 patients had AML and 57 patients had ALL. All patients were hospitalized at Hematology Departments of the Institute of Blood Pathology and Transfusion Medicine (Lviv, Ukraine) or Municipal Clinical Hospital № 5 (Lviv, Ukraine). Compliance of the study with bioethical standards was approved by the Ethics

Submitted: February 25, 2021.
*Correspondence: E-mail: lnkzotova@gmail.com
Abbreviations used: AL – acute leukemia; ALL – acute lymphoblastic leukemia; AML – acute myeloid leukemia; BM – bone marrow; CK – complex karyotype; FAB – French-American-British classification; FISH – fluorescence *in situ* hybridization; MK – monosomal karyotype; MLL – mixed-lineage leukemia or myeloid-lymphoid leukemia; PB – peripheral blood.

Committee of the State Institution "Institute of Blood Pathology and Transfusion Medicine" (Lviv, Ukraine), protocol № 02/01, February 25, 2021. All patients provided their consent to the participation in the study.

The cytogenetic analysis of BM/PB blast cells was performed after 24-h unstimulated culture. The methods of conventional cytogenetics (GTG-banding) and fluorescence *in situ* hybridization (FISH) were used. Conventional cytogenetic methods were carried out using standard techniques [7–10], which included exposure to colchicines, hypotonic treatment, fixation and samples preparation. The samples were analyzed at a ×1000 magnification under the light microscope Olympus BX41 (Olympus, Japan) using a system for chromosomal analysis CytoVision (Applied Imaging, UK) at the Laboratory of Immunology and Genetics of Blood Neoplasms. At least 20 metaphase plates were analyzed. The karyotypes were described according to the International System for Human Cytogenetic Nomenclature [11]. Only clonal abnormalities were considered as positive results. Abnormalities were considered clonal if ⩾ 2 metaphases had the same aberration in the case of a structural abnormality or an extra chromosome, or if ⩾ 3 metaphases shared the same aberration in the case of a monosomy. Complex karyotype (CK) was defined as 3 or more clonal abnormalities. Additionally FISH technique with appropriate probes was used. Samples preparation and hybridization procedure was carried out according to Pinkel *et al*. [12] taking into consideration the recommendations of the probe manufacturer under a microscope Olympus BX41 (Olympus, Japan) using a system for chromosomal analysis CytoVision (Applied Imaging, UK) at the Laboratory of Immunology and Genetics of Blood Neoplasms. At least 200 cells were analyzed. The results of the FISH analysis were described according to the International System for Human Cytogenetic Nomenclature [11].

RESULTS AND DISCUSSION

Chromosomal aberrations of various kinds were found in 80 (57%) and 37 (65%) cases of AML and ALL, respectively. The most common abnormalities were: monosomies of 5 and 7, –Y, trisomy of 8, deletions of 5q and 7q, rearrangements of 3q, 12p, 17p and 11q23, translocations t(8;21)(q22;q22), t(9;22)(q34;q11), t(15;17)(q22;q11-21), t(16;16)(p13;q22), inversion inv(16)(p13q22), marker and ring chromosomes, acentric structures, CK, monosomal karyotype (MK) for AML patients; trisomy of 8, rearrangements of 7q, 17p and 11q23, translocations t(4;11)(q21;q23), t(9;22)(q34;q11), marker chromosomes, acentric structures, hypodiploidy, hyperdiploidy, CK for ALL patients. Some genetic abnormalities (*BCR/ABL*, *PML/*RARA, *AML/ETO*, *CBFβ/MYH11*, *MLL/*AF4, *MLL/AF10* fusion genes) were detected by FISH.

Among the detected chromosomal aberrations, 11q23/*MLL* rearrangements were found in 7 (5%) and 8 (14%) cases of AML and ALL, respectively. The presence of *MLL/*AF4 and *MLL/AF10* fusion genes was confirmed by FISH in 2 cases (Table).

Of 15 AL patients with 11q23/*MLL* rearrangements, 8 (53.4%) patients had translocations (cases 1–8) (Fig. 1, 2), 2 (13.3%) — had deletions (cases 9, 10) (Fig. 3) and 5 (33.3%) patients had trisomies or tetrasomies of chromosome 11 (cases 11-15). With respect to the distribution of partner chromosomes involved in 11q23/*MLL* translocations: chromosome 4 was found to participate in 3 (37.5%) cases of 11q23/*MLL* translocations (cases 1–3) (Fig. 1), 9 — in 2 (25%) cases (4, 5) (Fig. 2) and chromosomes 10, 14 and non-identified chromosome were involved — in 1 (12.5%) case each (cases 6, 7, 8, respectively). Nine patients (60%), besides abnormal ones, had 9–86% normal metaphases in their karyotypes. Of 15 patients with 11q23/*MLL* rearrangements, 5 (33%) had only 11q23/*MLL* rearrangements, whereas others 10 (67%) — had additional cytogenetic abnormalities, besides 11q23/*MLL* rearrangements. Among them, the presence of one additional karyotype abnormality was established in 3 (30%) cases, multiple structural and/or numerical changes (⩾ 3) — in 7 (70%) cases. Spectrum of additional chromosomal aberrations associated with 11q23/*MLL* rearrangements was as follows: t(2;9)(q11.2;q34), del(3)(q21q26), r(3)(p26q29), t(9;22)(q34;q11), add(14)(q32), +der(22)t(9;22)(q34;q11), -5, marker chromosomes (mar), hypodiploidy, hyperdiploidy (Table).

According to the literature data, 11q23/*MLL* rearrangements are detected in 5% of AML and ~10–20% of ALL. Differences in the frequency and the distribution of translocations were noted according to the type

Table. Results of cytogenetic investigations of leukemic cells from patients with 11q23/*MLL* rearrangements

Case	Sex	Age	Subtype of AL	Karyotype	FISH
1	F	23	B-ALL	46,XX,t(4;11)(q21;q23)[7]/46,XX[13]	–
2	F	33	B-ALL	46,XX,t(4;11)(q21;q23)[10]/46,XX[10]	*MLL/AF4*
3	M	82	B-ALL	77,XXY,t(4;11)(q21;q23)×2,+13,+16,+17,+18,+19,+20,+21,+22[20]	–
4	M	37	AML M5	46,XY,t(9;11)(p21-22;q23)[21]	–
5	F	63	AML M2	46,XX,del(3)(q21q26),t(9;11)(p21-22;q23)[21]/46,XX[2]	–
6	F	39	AML M1	46,XX,t(10;11)(p11-15;q13-23)[14]/46,XX[8]	*MLL/AF10*
7	M	33	B-ALL	46,XY,t(2;9)(q11.2;q34),t(11;14)(q23;q32)[17]/46,XY[3]	–
8	M	69	AML M4	46,XY,r(3)(p26q29),-5,t(11;?)(q23;?),+mar[17]/46,XY[7]	–
9	M	35	B-ALL	46,XY,del(11)(q23),add(14)(q32)[20]	–
10	F	51	AML M5	46,XX,del(11)(q23)[10]/46,XX[10]	–
11	F	29	B-ALL	57,XX,+X,+2,+4,+6,+8,t(9;22)(q34;q11),+11,+14,+17,+21,+21,+der(22)t(9;22)[20]	–
12	F	53	B-ALL	62~67,XX,+2,+3,+3,+4,+5,+6,+7,+10,+11,+11,+12,+12,+13,+14,+15,+16,+17,+18,+19,+19,+20,+20,+21,+22[cp3]/46,XX[18]	–
13	F	20	B-ALL	60~61,XX,+X,+1,+3,+4,+6,+8,+9,+10,+11,+14,+15,+17,+18,+21,+mar[cp3]/46,XX[18]	–
14	F	46	AML M5	43~46,XX,-5,-8,-10,+11,-16,-17,-17,+1~4mar[cp20]	–
15	M	71	AML M5	46~57,XY,+Y,+1,-5,+6,+8,+9,+11,+12,+1~2mar1,+mar2,+mar3,+mar4[cp20]	–

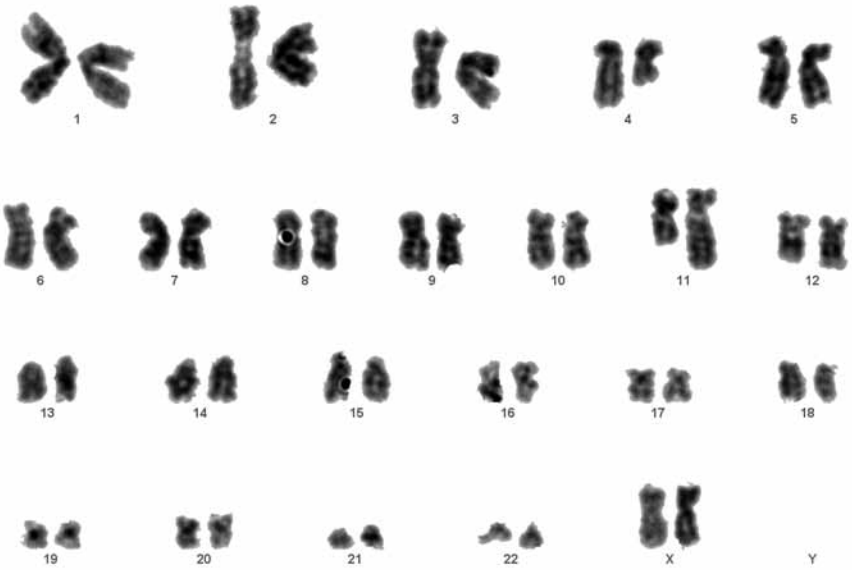

Fig. 1. Karyotype of leukemic cell from ALL patient with 11q23/*MLL* rearrangements (Table, case 1) — 46,XX,t(4;11)(q21;q23)

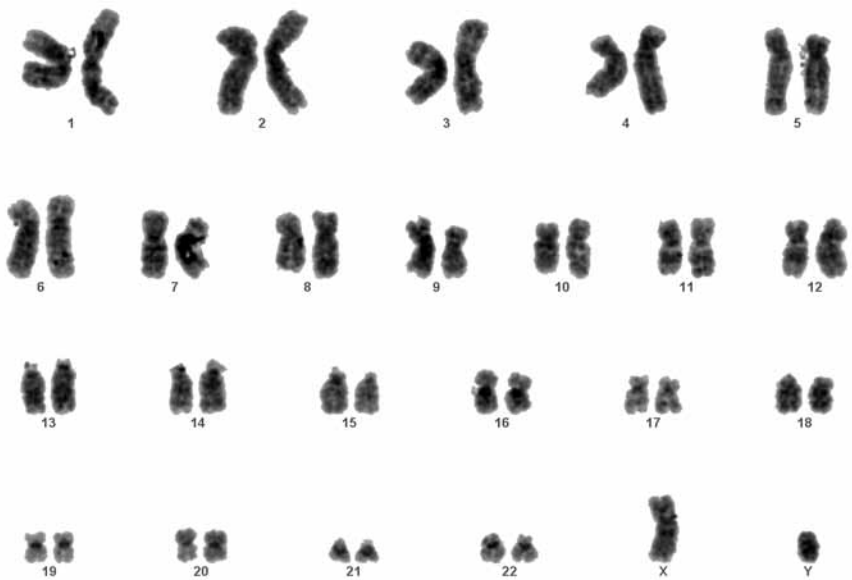

Fig. 2. Karyotype of leukemic cell from AML patient with 11q23/*MLL* rearrangements (Table, case 4) — 46,XY,t(9;11)(p21-22;q23)

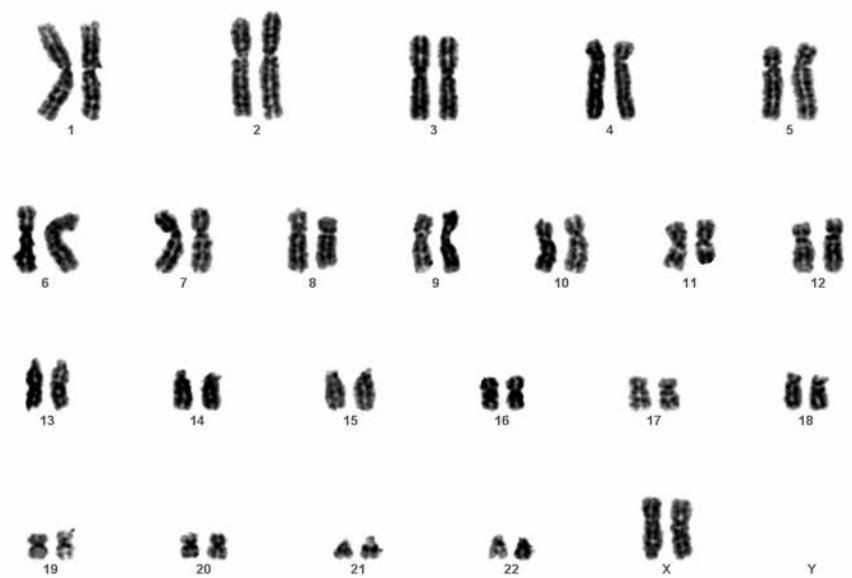

Fig 3. Karyotype of leukemic cell from AML patient with 11q23/*MLL* rearrangements (Table, case 10) — 46,XX,del(11)(q23)

of AL and age of the patients [2, 4, 13]. In our study, the frequency of 11q23/*MLL* rearrangements was 5% for AML and 14% for ALL, which is comparable to the data reported in the literature. Of the cases with 11q23/MLL rearrangements, 33% patients had only 11q23/*MLL* rearrangements, whereas other 67% patients — had also additional cytogenetic abnormalities of various kinds, besides 11q23/*MLL* rearrangements. In our investigation of 15 cases with 11q23/*MLL* rearrangements, 40% patients had only abnormal metaphases in their karyotypes, whereas other 60% — had also 9–86% normal metaphases, besides abnormal ones.

11q23/*MLL* abnormalities can be divided in two categories. The first category consists of *MLL* rearrangements, usually as translocations or insertions, some of them cryptic, leading to fusion genes with a large number of partners. In addition, self-fusion of two parts of *MLL* within the breakpoint cluster region, leading to internal rearrangements called partial tandem duplication, have also been described in several cases. A second category of abnormalities is the amplification of the 11q23 region resulting in multiple copies of *MLL* gene, located either intrachromosomally as homogeneously staining region, or extrachromosomally in double minutes (dmin). The numerical abnormalities of chromosome 11, such as trisomies or tetrasomies, also result in additional copies of *MLL* gene [4–6].

MLL is known to be required for the expression of posterior *HOXA* (homeobox A cluster) genes in the hematopoietic cell lineage. The expression of the latter is highest at the immature progenitor stage, such as multipotent progenitors, but then gradually declines. Therefore, *MLL* is required for the proliferation of immature hematopoietic progenitors while MLL fusion proteins constitutively activate genes that promote self-renewal of hematopoietic stem cells [14, 15].

The differences in the frequency and the distribution of translocations were noted according to the form of AL and age of the patients. The prognostic effect of 11q23/*MLL* aberrations may depend on *MLL* partner genes. Many studies have shown that the translocations t(6;11)(q27;q23), t(10;11)(p12;q23) in AML and translocation t(4;11)(q21;q23) in ALL are associated with an unfavorable prognosis; however, the t(9;11)(p22;q23) translocation is accompanied with a significantly longer patient survival rate in AML. However, none of the 11q23/*MLL* aberrations has a favorable prognostic value [4–6].

All patients with 11q23/*MLL* rearrangements are usually classified into cytogenetic categories of AL with a poor or intermediate risk prognosis. Distribution of patients into risk groups according to the identified cytogenetic prognostic markers allows us to choose the most appropriate treatment approach for them, namely the intensity of therapy, the necessity of BM transplantation at the first remission, the necessity of the prescription of tyrosine kinase or all-trans retinoic acid [16, 17]. Thus, the modern diagnosis of AL should include the study of cytogenetic and molecular genetic features of blast cells together with cytomorphological, cytochemical and immunophenotype peculiarities of these cells. The combination of these techniques allows not only individualizing treatment according to the prognostic risk factors, but also it is the basis for understanding the pathogenesis and biological nature of AL.

In our study, chromosomal abnormalities in leukemia cells were found by conventional cytogenetic methods in 57% and 65% adult patients with AML and ALL, respectively and 11q23/*MLL* rearrangements were established in 5% and 14% adult patients with AML and ALL, respectively. Among the cases with 11q23/*MLL* rearrangements, 33% patients had only 11q23/*MLL* rearrangements, whereas other 67% — had also additional cytogenetic abnormalities, besides 11q23/*MLL* rearrangements.

Since AL patients with 11q23/MLL rearrangements are referred to cytogenetic categories of AL with a poor or intermediate risk prognosis, cytogenetic investigations should be included in the standard examination of these patients for diagnosis, prognosis and selection the optimal treatment strategy.

REFERENCES

1. **Bain BJ**. Leukaemia Diagnosis. 5 ed. NY: Wiley-Blackwell, 2017. 559 p. https://doi.org/10.1111/bjh.16351.

2. **Heim S, Mitelman F**. Cancer Cytogenetics. 4 ed. NY: Wiley-Blackwell, 2015. 648 p. https://doi.org/10.1002/9781118795569.

3. **Liang K, Volk A, Haug J, *et al***. Therapeutic targeting of *MLL* degradation pathways in MLL-rearranged leukemia. Cell 2017; **168**: 59–72. https://doi.org/10.1016/j.cell.2016.12.011.

4. **Cox M, Panetta P, Lo-Coco F, *et al***. Chromosomal aberration of the 11q23 locus in acute leukemia and frequency of *MLL* gene translocation. Am J Clin Pathol 2004; **122**: 298–306. https://doi.org/10.1309/RX27R8GJQM330C22.

5. **Meyer C, Burmeister T, Groger D, *et al***. The *MLL* recombinome of acute leukemias in 2017. Leukemia 2018; **32**: 273–84. https://doi.org/10.1038/leu.2017.213.

6. **Ilencikova D, Kolenova A**. *MLL* gene alterations in acute myeloid leukemia (11q23/*MLL*+ AML). In: Siregar Y, ed. Oncogene and Cancer — From Bench to Clinic. IntechOpen, 2013: 225–46. https://doi.org/10.5772/55141.

7. **Andreyeva SV, Drozdova VD**. Standard of Analysis of Chromosome Preparations in Hematopoietic Neoplasms (Guidelines). Kyiv, 2007. 44 p. (in Ukrainian).

8. **Wan TSK**. Cancer Cytogenetics. Methods and Protocols. Methods in Molecular Biology. NY: Humana Press, 2017. 340 p. https://doi.org/10.1007/978-1-4939-6703-2.

9. **Haferlach C, Rieder H, Lillington D, *et al***. Proposals for standardized protocols for cytogenetic analyses of acute leukemias, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic myeloproliferative disorders, and myelodysplastic syndromes. Genes Chromosomes Cancer 2007; **46**: 494–9. https://doi.org/10.1002/gcc.20433.

10. **Rack KA, van den Berg E, Haferlach C**. Cytogenetics and molecular genetics European recommendations and quality assurance for cytogenomic analysis of hematological neoplasms. Leukemia 2019; **33**: 1851–67. https://doi.org/10.1038/s41375-019-0378-z.

11. **McGowan-Jordan J, Simons A, Schmid M**. An International System for Human Cytogenetic Nomenclature. Basel: S. Karger, 2016. 140 p. https://doi.org/10.1159/isbn.978-3-318-06861-0.

12. **Pinkel D, Straume T, Gray JW**. Cytogenetic analysis using quantitative high sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 1986; **83**: 2934–8. https://doi.org/10.1073/pnas.83.9.2934.

13. **Olshanskaya YuV, Domracheva EV**. Chromosomal Rearrangements in Acute Leukemias. Moscow: MEDpress-inform, 2006. 122 p. (in Russian).

14. **Yokoyama A**. Molecular mechanisms of MLL-associated leukemia. Int J Hematol 2015; **101**: 352–61. https://doi.org/10.1007/s12185-015-1774-4.

15. **Wang KC, Helms JA, Chang HY**. Regeneration, repair and remembering identity: the three Rs of *Hox* gene expression. Trends Cell Biol 2009; **19**: 268–75. https://doi.org/10.1016/j.tcb.2009.03.007.

16. **Arber DA, Orazi A, Hasserjian R, *et al***. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016; **127**: 2391–405. https://doi.org/10.1182/blood-2016-03-643544.

17. **Mrozek K, Marcucci G, Nicolet D, *et al***. (2012). Prognostic significance of the European LeukemiaNet standardized system for reporting cytogenetic and molecular alterations in adults with acute myeloid leukemia. J Clin Oncol 2012; **30**: 4515–23. https://doi.org/10.1200/JCO.2012.43.4738.

ПЕРЕБУДОВИ 11q23/*MLL* ПРИ ГОСТРИХ ЛЕЙКЕМІЯХ У ДОРОСЛИХ

О.В. Зотова[1, *], А.С. Лук'янова[2], М.О. Вальчук[1], Ю.С. Кароль[3], О.О. Шалай[1], В.Л. Новак[1], В.Є. Логінський[1]

[1]ДУ "Інститут патології крові та трансфузійної медицини НАМН України", Львів 79057, Україна

[2]Медико-біологічний центр "Геном", Київ 04216, Україна

[3]Комунальне некомерційне підприємство "Муніципальна клінічна лікарня № 5", Львів 79057, Україна

Мета: Визначити частоту, діагностичну та прогностичну значущість перебудов 11q23/*MLL,* а також визначити, які хромосоми найчастіше залучені до аномалій 11q23/*MLL* у дорослих хворих на гостру лейкемію. ***Матеріали та методи:*** Проводили цитогенетичне дослідження клітин кісткового мозку та/або периферичної крові у 140 хворих на гостру мієлоїдну лейкемію (ГМЛ) та 57 пацієнтів з гострою лімфобластною лейкемією (ГЛЛ) із застосуванням традиційного методу диференційного забарвлення (GTG), а також флуоресцентної гібридизації *in situ*. ***Результати:*** Хромосомні аномалії в лейкемічних клітинах за допомогою традиційного методу цитогенетичного аналізу виявили у 80 (57%) та 37 (65%) хворих на ГМЛ та ГЛЛ відповідно. Перебудову 11q23/*MLL* виявили у 7 (5%) та 8 (14%) хворих на ГМЛ та ГЛЛ відповідно. Серед них у 8 (53,4%) хворих визначали транслокації, у 2 (13,3%) — делеції і у 5 (33,3%) — трисомію або тетрасомію за хромосомою 11. Стосовно розподілу хромосом-партнерів, залучених до транслокацій 11q23/*MLL*, у 3 (37,5%) випадках це була хромосома 4, у 2 (25%) — хромосома 9; було виявлено ще по одному випадку залучення хромосом 10 та 14, в одному випадку хромосому-партнера не було ідентифіковано. У 9 (60%) хворих поруч з аномальними метафазними пластинками виявляли від 9 до 86% метафаз з нормальним каріотипом. Серед 15 хворих з перебудовами 11q23/*MLL* у 10 (67%), окрім перебудов 11q23/*MLL,* виявляли також додаткові цитогенетичні аномалії. ***Висновки:*** Різноманітні хромосомні аномалії виявляли у 80 (57%) та 37 (65%) дорослих хворих на ГМЛ та ГЛЛ відповідно. Частота перебудови 11q23/*MLL* у дорослих хворих на ГМЛ та ГЛЛ станови відповідно 5 та 14%. Оскільки хворі на гостру лейкемію з перебудовами 11q23/*MLL* належать до категорії високого ризику з несприятливим або проміжним прогнозом, цитогенетичні методи дослідження мають бути обов'язково включені до стандартного дослідження хворих на гостру лейкемію з метою точної діагностики, прогнозування та вибору оптимальної стратегії лікування.

Ключові слова: гостра лейкемія, каріотип, цитогенетичні аномалії, перебудови 11q23/*MLL*, діагностика, прогноз.