Genetic abnormalities in leukemia secondary to treatment in patients with Hodgkin’s disease

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ARTÍCULO DE REVISIÓN

ABSTRACT

Hodgkin’s disease has been treated mainly with two chemotherapy schedules, MOPP (nitrogen mustard, Oncovin, procarbazine and prednisone), which includes alkylating agents, and ABVD (adriamycin, bleomycin, vinblastine and dacarbazine), which includes topoisomerase II inhibitors, either with or without radiation therapy. Due to the types of agents used, patients with Hodgkin’s disease often develop secondary leukemias. The alkylating agents included in the MOPP scheme were the first drugs associated with the development of therapy-related myelodysplastic syndrome (t-MDS) and acute myeloid leukemia (t-AML); both entities are the result of the clonal selection of cells with accumulated genomic lesions induced by antineoplastic therapy. In patients who developed t-MDS and t-AML, eight alternative routes with specific cytogenetic and molecular changes have been identified, and the routes are related to the type of therapy, alkylating agents or DNA topoisomerase II inhibitors. At the cytogenetic level, patients treated with alkylating agents show deletion 5q/monosomy 5 and deletion 7q/monosomy 7; in contrast, those who were treated with topoisomerase II inhibitors show 11q23 translocations involving the MLL gene. At the molecular level, there are two types of mutations: Class I, which alter the RAS-BRAF signal transduction pathways and increase cell proliferation; Class II, which disrupt genes that encode transcription factors and NPM1 that are involved in cell differentiation, and the inactivation of p53 tumor suppressor gene. Knowledge of the genetic alterations in these conditions is important for the classification, treatment and prognosis of patients as well as essential for increasing the knowledge of the biology of these diseases, which leads to identifying potential therapeutic targets.
INTRODUCTION

Advances in cancer treatment have increased patient survival; currently, there are 12 million cancer survivors in the US, representing 4% of the population.\(^1,2\) More than 80% of children who have been diagnosed with Hodgkin’s disease (HD) will survive for ten years or more; however, antineoplastic therapy for HD is not specific to tumor cells and affects normal cells. Thus, the majority of these survivors are at risk for developing one or more long-term sequelae of their therapy.\(^3\) The development of secondary cancer is a complication that is often associated with cancer therapy in HD survivors.\(^4\) The risk of developing a secondary cancer may be related to several factors such as lifestyle, environmental exposure and their interaction with the genetic background of the patient. In particular, the risk related to the specific treatment for the first cancer is one of the most important factors.\(^5\)

Therapy-related myeloid neoplasms (t-MN) are recognized by the World Health Organization (WHO) as a distinct entity that includes myelodysplastic syndrome (t-MDS) and acute myeloid leukemia (t-AML).\(^6\) From the mid-1960s to early 2000s, the most widely used chemotherapy regimen for the treatment of HD was the MOPP scheme (nitrogen mustard, Oncovin, procarbazine and prednisone), which included alkylating substances such as nitrogen mustard and procarbazine that are recognized as potent mutagenic and clastogenic agents.\(^7\) In the early 70s, another combination of chemotherapy drugs, ABVD (adriamycin, bleomycin, vinblastine and dacarbazine) was developed with increased potency and less toxicity in comparison to MOPP. While it contains fewer alkylating agents, this combination includes other mutagens and an inhibitor of topoisomerase II, adriamycin.\(^8,9,10\)

After treatment regimens of chemotherapy and radiotherapy, it has been observed that 20% of HD survivors develop three types of secondary tumors: different solid tumors (58%), acute leukemia (25%) and non-Hodgkin lymphoma (17%) (Figure 1). In this work, we will refer only to acute leukemia since it represents the most frequent group.\(^11\)

The alkylating agents included in the MOPP scheme were the first drugs found to be related to the development of secondary malignancies, especially t-MDS and t-AML, with a risk of occurrence of 2% that increases to 12.4% when radiotherapy is also used.\(^4,8,9\) Patients may develop t-MDS 2 to 5 years after treatment with these agents, and t-MDS can often progress into t-AML. ABVD, which includes topoisomerase II inhibitors, has also been linked with the development of t-AML without a prior t-MDS state.\(^12,13\)

The cytogenetic and morphological traits of t-MDS/t-AML are related to the type of therapy received for the primary tumor, and both occur within 5 to 7 years after chemotherapy and radiotherapy and confer a poor prognosis.\(^14\) Both entities are well-recognized clinical syndromes\(^12\) that may arise as a result of the clonal selection of cells with accumulated genomic lesions due to defects in DNA repair mechanisms, defects in detoxification systems designed to limit oxidative damage to DNA\(^15\) and changes in factors for chromatin assembly, resulting in the accumulation of double-strand breaks in DNA.

In addition, increased frequencies of the following gene polymorphisms in t-AML patients have been observed compared with healthy individuals: human...
homeobox *HLX1* gene (HLX1-C/T 3 ‘UTR), which is essential for hematopoietic development; the promoter region of the DNA repair gene *RAD51* (-135 G/C); and the DNA repair gene *XRCC3*(18067 C/T).16,17 These findings suggest a direct relationship between these polymorphisms and the risk of developing t-AML,16,18 as all of these alterations are assumed to increase susceptibility for developing secondary cancer.15-19

The risk of developing t-MDS and t-AML is up to 100-fold higher in HD patients after treatment than in the general population, indicating that at least 99% of cases of secondary cancer should be considered as induced by treatment and changes dependent on the type and dose of drug administered, in addition to patient age at the time of the therapy.20 Previous studies have found that the risk of t-MDS and/or t-AML increases with respect to the square of the patient’s age at the time of receiving treatment for primary cancer and directly with the cumulative dose of alkylating agents used.21,22

There are few studies on genetic risk factors for developing secondary cancers in HD, but it has been observed that 75% of patients with HD who are treated with alkylating agents such as cyclophosphamide or procarbazine and have developed t-AML have a G>A polymorphism in position -93 of the promoter of the *MLH1* repair gene. Thus, it follows that this polymorphism confers a high risk of developing t-AML.4,23 Because of this, it is important to associate the genetic characteristics of individuals with secondary leukemia and specific treatment to identify individuals at high risk of t-AML and monitoring to detect the emergence of a second cancer.

SECONDARY LEUKEMIAS according to the type of chemotherapy

Secondary leukemias are classified in two types according to the chemotherapy used to treat the primary cancer:

- Classical t-MDS/t-AML, which is secondary to exposure to alkylating agents and
- t-AML, which is secondary to the use of topoisomerase II inhibitors.

Classical t-MDS/t-AML

Treatment with alkylating agents has been directly linked with t-MDS/t-AML. It is known that alkylating agents can induce various types of alterations in the cells such as the following:

![Figure 2. Alterations induced by alkylating agents. The chemotherapeutic agent results in methylation of DNA bases, thus activated repair mechanisms can produce different alterations such as: a) Inter-strand cross-linking of DNA that prevents replication and transcription; b) chemical modification of bases, such as the generation of methylguanine N7 or O6, which tends to pair with thymine instead of cytosine and generates transition-type mutations; and c) double-strand breaks, which are extremely dangerous lesions because they can induce chromosome alterations such as deletions and translocations that are directly involved with progression toward cancer.](image-url)
• DNA inter-strand cross-links, which interfere with replication and transcription.
• Chemically modified DNA bases, which generate point mutations; and
• Double-stranded breaks, which lead directly to chromosome aberrations2,20,24 (Figure 2).

In most patients with the classical form of t-AML, total or partial deletion of chromosomes 5 and 7 are identified together with hypodiploid karyotypes and complex structural chromosomal abnormalities such as unbalanced chromosomal rearrangements. In general, these alterations are not restricted to specific genes or chromosomal bands, therefore the predominance of cases with alterations in chromosomes 5 and 7, probably reflects a selection of cells, due to a proliferative advantage.12-14,20,25,26 In t-MDS, balanced aberrations are rare, while unbalanced aberrations, such as the deletion of the long arm of chromosome 5 [del(5q)] or loss of chromosome 5 (monosomy 5), and the deletion of the long arm of chromosome 7 [del(7q)] or loss of chromosome 7 (monosomy 7) (Figure 3A), were observed in 50 to 70% of patients, and the presence of normal karyotypes were observed in 5 to 10% of cases. In t-AML, balanced aberrations (translocations or inversions) (Figure 3B) occur in 15 to 20% of the cases, unbalanced changes are common (70%) and normal karyotypes were found in 10 to 15% cases.27

In order of frequency, the most common chromosomal abnormality is monosomy 7 followed by del(5q) and monosomy 5.28,29 The same alterations were observed in de novo MDS and AML, especially in adult patients and in those occupationally exposed to carcinogens such as benzene.12

- Mitoxantrone, etoposide and adriamycin, among others, are related to the induction of t-AML and are characterized by blocking the rejoining of double-stranded breaks in DNA and induce illegitimate mitotic recombination between the broken ends of two different chromosomes, resulting in translocation3 (Figure 4). t-AML can occur as early as 2 years after initial treatment for primary cancer and is associated with balanced chromosomal aberrations as the only abnormality, mainly 11q23 translocations resulting in MLL gene rearrangements that generate fusion proteins and prevent methylation of the histone H3 lys4, which is related to the transformation of hematopoietic cells into leukemic cells.13,19,25-27,30,31

Less commonly, there may be alterations in 21q22, 16q22, 11p15.5, 17q21 and less frequently in 3q26. Unlike classic t-AML, these secondary leukemias have a short latency period from the beginning of the use of chemotherapy for primary cancer to their development and are rarely preceded by t-MDS.12,14,20

**IMPACT OF CYTOGENETIC ABNORMALITIES ON THE PROGNOSIS OF t-AML**

In follow-up studies of patients with HD that covered the start of treatment with alkylating agents and/or topoisomerase II inhibitors through the development of t-MDS and t-AML,11 it has been observed that the presence of particular chromosomal aberrations are important factors for leukemic transformation and are associated directly with clinical features and response to treatment (Table 1).
Table 1. Cytogenetic characteristics in EH patients with t-MDS/t-AML.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of EH patients (Cycles)</th>
<th>Treatment</th>
<th>Number of patients with t-AML</th>
<th>Time to develop t-AML (months)</th>
<th>Type of secondary leukemia</th>
<th>Cytogenetic alterations</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>761 (CR) 9 RT+MOPP (3-6)</td>
<td>13</td>
<td>12-123</td>
<td>11 ANLL</td>
<td>t(1;11) (p32;q23)</td>
<td>t(4;11) (q22;q23).</td>
</tr>
<tr>
<td></td>
<td>4 ABVD (3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>6</td>
<td>1 RT</td>
<td>41-254</td>
<td>2 ANLL</td>
<td>46, XY</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 RT +MOPVCbx</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 RT +VPCCNU,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 RT+OVAEtopPClb,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 RT+MOPCblb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 RT+MOPP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>1 RT+MOPP/VBM (3/6)</td>
<td>3</td>
<td>25-76</td>
<td>M0-M1</td>
<td>Normal karyotype</td>
</tr>
<tr>
<td></td>
<td>2 MOPP/ABVD (8/8)</td>
<td></td>
<td></td>
<td>t-AML M1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>4</td>
<td>1 RT+MOPP (6)</td>
<td>4</td>
<td>24-540</td>
<td>t-AML M4</td>
<td>46, XY</td>
</tr>
<tr>
<td></td>
<td>1 MOPP +CbxAVP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 MOPP(6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

In a study at the University of Chicago, 306 patients with t-AML were analyzed to determine the impact of cytogenetic findings on prognosis of the disease. Twenty-four patients (8%) with normal karyotypes survived for 3 years after diagnosis, and patients with abnormalities of chromosomes 5 and 7 had the shortest survival time (7-9 months), compared with 11 months for those with balanced translocations.\textsuperscript{12,32} In another study by the German Cooperative Group (AMLCG) in 93 patients with t-AML and 1091 patients with de novo AML, it was found that overall survival (defined from the time of diagnosis of secondary leukemia to death) was 18 months for those with favorable karyotypes (normal karyotype and balanced chromosomal rearrangements) and 6 months for those with unfavorable karyotypes (alterations of chromosomes 5 and 7). In both t-AML and de novo AML, unfavorable karyotypes were associated with short survival (6 months in both entities).

In contrast, patients with balanced alterations such as t(15;17), t(8;21) or inversion of chromosome 16 [inv(16)] have a better prognosis, similar to that presented by patients with de novo AML and the same chromosome rearrangement.\textsuperscript{12,33} In fact, in two series of patients with exposure to topoisomerase II inhibitors, it was observed that 33 of 39 patients (85%) with t-AML and inv(16) and 24 of 35 (69%) patients with t(15;17), achieved complete remission, and patients with t(8;21) had better prognosis compared with those who had other rearrangements involving the 21q22 region, such as t(3; 21) and t(16;21).\textsuperscript{12,30,34}

In a study of 511 patients with t-MDS/t-AML, 32% had rearrangements in 11q23, and their survival time was 8 months, which was significantly lo-
lower than the survival times of patients with 21q22 abnormalities, inv(16) and t(15;17) of 14, 28 and 29 months, respectively.12,33,35

In conclusion, patients with chromosomal abnormalities such as unbalanced alterations on chromosomes 5 and 7 have a lower survival time than patients with balanced rearrangements; among these, patients with 21q22 abnormalities, inv(16), and t(15;17) have a better prognosis compared with those with rearrangements involving 11q23.

MOLECULAR CHARACTERISTICS OF t-MDS AND t-AML

The clinical and cytogenetic features observed in the two forms of t-AML may reflect the type of damage induced by the different therapies used to treat the primary cancer. Chromosomal deletions can cause that one normal allele of tumor suppressor genes may be inactivated; the evidence suggests haploinsufficiency of the suppressor gene EGR1 or promoter methylation of the gene encoding α-catenin (CTNNA1), both located on 5q31, as the only alteration.12,27

However, loss of both alleles of a tumor suppressor gene may not be sufficient to confer a malignant phenotype; as described in a model of colorectal tumorigenesis, multiple genetic alterations may be required to transform a cell, and this series of changes require a long period of time, explaining the long latency between the initial antineoplastic therapy to alkylating agent-induced t-AML12. In contrast, balanced chromosome translocations that follow treatment with topoisomerase II inhibitors cause the activation of cellular oncogenes in a dominant fashion, and although the fusion gene alone may not be sufficient for the complete transformation of a hematopoietic progenitor cell, relatively few genetic events are required to progress to the leukemic phenotype.12 At the molecular level, three classes of mutations have been proposed to be involved in t-MDS and t-AML (Table 2):

- **Class I mutations** alter RAS-BRAF signal transduction pathways, stimulating cell proliferation.
- **Class II mutations** occur in transcription factors genes and NPM1, causing altered cellular differentiation. Here are also included mutations that inactivate the p53 tumor suppressor gene (sometimes are classified in a third category Class III mutations).14,26,36

One of the most important works at the molecular level was the Copenhagen series, which was conducted in 140 patients, including 89 with t-MDS and 51 with t-AML,23,24,26,27 on cryopreserved bone marrow cells upon the diagnosis of secondary cancer. They searched for Class I mutations in tyrosine kinase receptor genes such as FLT3, cKIT, cFMS and intracellular JAK2 and in signal transduction genes involved in the RAS-BRAF-MEK-ERK pathway such as KRAS, NRAS, BRAF and PTPN11; Class II mutations in the transcription factors AML1, CBFB, MLL, EVI1 and CEBPA, the transcription regulator NPM1 and the retinoic acid receptor RARA; and mutations in the tumor suppressor gene p53. The results of this study showed that some mutations occur with high frequency. In particular, 34 and 22 patients had mutations in p53, and AML1, respectively. Seventeen patients had mutations in FLT3, cKIT and JAK2, and 20 patients had mutations in genes downstream of the RAS/BRAF signal transduction pathway. RAS mutations were associated with the progression from t-MDS to t-AML. Finally, 61 mutations in transcription and hematopoietic differentiation genes were observed in 59 patients with t-MDS or t-AML. The chimeric rearrangements of AML1, CBFB, MLL, RARA and EVI1 were related to previous therapy with topoisomerase II inhibitors.26,27

Another finding observed in therapy-related leukemia are epigenetic changes, such as promoter methylation of CTNN1A and promoter methylation of p15INK4B on 9p21, which is involved in the G1 checkpoint.23,24,26,27,29,37 were observed in 58-68% of t-MDS/t-AML patients.38 Gene inactivation by homozygous deletion or hypermethylation of CpG sites in the promoter region of p16INK4A and p15INK4B, both located in a small region (2.5 kb) on chromoso-

<table>
<thead>
<tr>
<th>Class</th>
<th>Type of Mutation</th>
<th>Involved Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tyrosine-kinase</td>
<td>KRAS, NRAS, BRAF</td>
</tr>
<tr>
<td></td>
<td>Transcription</td>
<td>AML1, CEBPA, NPM1, p53</td>
</tr>
<tr>
<td></td>
<td>RAS/BRAF pathway</td>
<td>FLT3, cKIT, cFMS, JAK2</td>
</tr>
</tbody>
</table>

Table 2. Classification of mutations related with the involved genes.
Table 3. Genetic pathways identified in t-MDS and t-AML patients.

<table>
<thead>
<tr>
<th>GENETIC PATHWAYS</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presentation</td>
<td>t-MDS</td>
<td>t-MDS/ t-AML</td>
<td>t-AML</td>
<td>t-AML</td>
<td>APL</td>
<td>t-MDS/ t-AML</td>
<td>t-MDS/ t-AML</td>
<td></td>
</tr>
<tr>
<td>Type of previous therapy</td>
<td>Alkylating agents</td>
<td>Alkylating agents</td>
<td>Topo II inhibitors</td>
<td>Topo II inhibitors</td>
<td>Topo II inhibitors</td>
<td>Without specific inhibitors</td>
<td>Without specific inhibitors</td>
<td></td>
</tr>
<tr>
<td>Chromosome alterations</td>
<td>7q deletions and/or monosomy 7</td>
<td>5q deletion and/or monosomy 5</td>
<td>11q23 balanced alterations</td>
<td>21q22 inv(16)</td>
<td>17q21</td>
<td>11p15</td>
<td>Normal</td>
<td>Some cases with trisomy B</td>
</tr>
<tr>
<td>Genes involved</td>
<td>MLL</td>
<td>AML1</td>
<td>RARA</td>
<td>NUP98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutations</td>
<td>AML1 point mutations with t-AML development</td>
<td>p53</td>
<td>NRAS, KRAS or BRAF</td>
<td>C-kit and PTPN11</td>
<td>FLT3, RAS or AML1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other alterations</td>
<td>Deletion 7q and/or monosomy 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epigenetic modifications</td>
<td>p15INK4B</td>
<td>CTNNAL</td>
<td>p16INK4B</td>
<td>methylated</td>
<td>p15INK4B</td>
<td>C6orf40</td>
<td>methylated</td>
<td>with FLT3 tandem duplication</td>
</tr>
</tbody>
</table>

APL: Acute promyelocytic leukemia. Topo II. Topoisomerase II.
me 9p21, are common in acute lymphoblastic leukemia. In t-MDS or t-AML patients, studies have been conducted to examine the methylation status of the p14, p15 and p16 gene promoters and relate it with previous treatment, clinical and cytogenetic characteristics and prognosis of the disease.\textsuperscript{39,40} Au, et al.,\textsuperscript{32,40} in 17 patients with secondary leukemia, found that 58% had methylation of p15\textsuperscript{NK}\textsuperscript{48} associated with del(7q), and in 3 of 6 patients with bone marrow samples obtained before the diagnosis of t-MDS/t-AML, methylation of p15 was observed 2 years before leukemia development, demonstrating that in some patients, this disturbance is an early event in the development of leukemia.

In another study, in 81 patients with t-MDS or t-AML, Christiansen, et al.,\textsuperscript{39} found that 68 and 6% had methylation of p15 and p16, respectively, unrelated to age, previous therapy (alkylating agent, topoisomerase II inhibitors or radiotherapy) or the period from the start of therapy for primary cancer to the development of secondary leukemia; however, survival time from the diagnosis of secondary leukemia was significantly shorter in patients with p15 methylation.\textsuperscript{41}

In general, it has been observed that the development of t-AML after treatment of the primary tumor was significantly shorter in methylated than in unmethylated patients.\textsuperscript{38} There was a strong association between p15 methylation and chromosomal abnormalities, including monosomy 7, del(7q) and tandem duplication in 13q12 (FLT3) and 11q23 (MLL), but there was no association with p53 mutations.\textsuperscript{37,42}

Finally, it is clear that there are combinations of genetic alterations at different levels (molecular, epigenetic and chromosomal) that lead to the appearance of genetic pathways directly related to the development of t-MDS and/or t-AML.

**GENETIC PATHWAYS IN t-MDS AND t-AML**

In 1995, Pedersen-Bjergaard, et al., analyzed 140 patients with t-MDS or t-AML\textsuperscript{13} and proposed eight alternative genetic pathways with specific genetic characteristics directly related to the pathogenesis of t-MDS and t-AML (Table 3), with important implications for the classification of patients:

- **Pathway I.** Comprises patients with 7q deletions and/or monosomy 7 and normal chromosome 5 without balanced aberrations. They presented with t-MDS following therapy with alkylating agents. These patients frequently have AML1 point mutations that are associated with the progression of t-MDS to t-AML. Of 140 patients with t-MDS or t-AML, 39 were on pathway I, and 15 (38%) had mutations of AML1. A few patients had p53 and RAS mutations, and none had mutations of FLT3.
- **Pathway II.** Patients with 5q deletions and/or monosomy 5 without balanced aberrations. They presented with t-MDS or t-AML. Most of these cases have p53 mutations and complex unbalanced chromosome rearrangements. These alterations are generated by the use of alkylating agents and occasionally also involve 7q deletions and/or monosomy 7, deletion of 17p (loss of heterozygosity of p53), derivative chromosomes and complex karyotypes composed of material of at least three different chromosomes and amplifications or duplications in 11q23 and 21q22.
- **Pathway III.** t-AML patients. These patients do not have previous t-MDS do present unbalanced translocations involving band 11q23 (MLL) associated with topoisomerase II inhibitors (etoposide and cisplatin). In such patients, mutations of NRAS, KRAS or BRF are common.
- **Pathway IV.** Patients with t-AML and balanced aberrations, frequently balanced translocations in chromosome band 21q22 (AML1) or inv(16) (16q22) with CBFB rearrangements. They are associated with the use of anthracyclines and presented t-AML, except for patients with t(3;21) with previous t-MDS. The second most common alteration in this group of patients is deletion of 7q/monosomy 7, and in some patients, mutations of cKIT and PTPN11.
- **Pathway V.** Comprises patients with acute promyelocytic leukemia and rearrangements of RARA gene at 17q21. Only one patient had a tandem duplication of FLT3.
- **Pathway VI.** Patients with t-MDS or t-AML and rearrangements of the NUP98 gene on 11p15. They are found in cases treated with topoisomerase II inhibitors.
- **Pathway VII.** Patients with t-MDS and t-AML, normal karyotypes and no association with a specific type of therapy. In a study of 24 patients who had normal karyotypes, 50% had a mutation in at least one of the following genes: FLT3, RAS or AML1.
- **Pathway VIII.** Some cases only have one chromosome aberration (trisomy 8) that is not related to any specific type of therapy.\textsuperscript{12,14,20,23,27}
CONCLUSIONS

In general, in patients with secondary leukemia, three cytogenetic subtypes are observed. The first subtype is represented by patients with unbalanced aberrations, mainly del(5q) and/or monosomy 5 or del(7q) and/or monosomy 7, which are associated with exposure to alkylating agents. Patients with recurrent balanced aberrations, such as translocations or inversions, are the second subtype, and in cases related to therapy, these aberrations are the result of the activity of topoisomerase II inhibitors that generate double-strand breaks and aberrant rejoining of chromosome segments. Finally, the third subtype includes patients with normal karyotypes. There are also two types of point mutations: Class I, mutations in the RAS-BRAF pathway leading to increased cell proliferation; Class II, inactivating mutations of genes encoding transcription factors leading to disturbed cell differentiation and mutations of p53 that can occur in patients with any type of karyotype, unbalanced, balanced or normal.

When patients are studied from the point of view of the existence of cytogenetic alterations associated with molecular alterations, at least eight different genetic pathways can be defined that are characterized by specific alterations. Some abnormalities are associated with t-MDS and others with t-AML directly. t-MDS has great potential for transformation to t-AML, which is associated with point mutations of AML1 and RAS. Knowledge of the genetic alterations in these conditions is important because it can directly help with proper classification, treatment, and prognosis of patients and can increase the knowledge of the biology of the disease, which leads to identifying potential therapeutic targets.

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