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Review Article

Therapy-related acute myeloid leukemia and its prevention

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Abstract: Background: Secondary tumors, including therapy-related acute myeloid leukemia (t-AML), represent one of the most undesirable side effects of chemotherapy, which arise several years after primary cancer treatment. This review aims to analyze the current data on molecular pathogenesis of t-AML revealing potential criteria for predicting predisposition to the disease. Another objective is to analyze the information on promising approaches for t-AML prevention. Methods: We analyzed studies regarding t-AML and possible approaches for cancer prevention of drug-induced tumors. Publications in the databases, such as SciVerse Scopus (948), PubMed (1837) and Web of Science (935) were used. Among 92 the most important publications cited in the review, 79 were published during the last decade. Results: The review provides the information concerning t-AML pathogenesis, molecular markers of primary cancer patients with high risk of t-AML. The role of the bone marrow niche in clonal hematopoiesis and t-AML pathogenesis is discussed. Current approaches for t-AML prevention both at the stage of therapy and at the latent period are described. Inhibition effects of polyphenols on cell proliferation and on the appearance of hemopoetic clones of indeterminate potential are proposed for t-AML prevention. Conclusion: The problem of the t-AML, a cancer induced by genotoxic chemotherapeutic drugs, is considered from the point of view of the fundamental mechanisms of chemical carcinogenesis, highlighting initiation and promotion stages. It enables to reveal the possible markers for the group of patients with high risk for t-AML and to demonstrate perspectives for the use of plant polyphenols for t-AML prevention.

Keywords: Therapy-related cancer, leukemia, stem cells, metastatic niches, cytotoxic drugs, prevention of secondary AML, plant polyphenols

Introduction

Chemotherapy of malignancies, along with smoking and occupational cancer, is one of the huge chemical carcinogenesis experiments that humans have been conducting on themselves. Treating cancer through trial and error has revealed that cytostatics have a broad range of adverse effects. Secondary malignancies of different origins are among the long-term sequelae of chemotherapy, along with acute toxic effects in rapidly dividing normal cells (e.g., hematopoietic tissues and intestinal epithelium). The epidemiological data and reproducing the carcinogenic effects of a drug using animal models afford strong arguments for classifying cytostatic drugs as Group 1 carcinogens [1].

Like other carcinogens, cytostatic drugs may cause genotoxic and/or epigenetic alterations corresponding to the initiation of carcinogenesis. Further selection of proliferating cell clones and other events contributing to tumor emergence occur at the next stage of tumor promotion due to genetic drift and/or exposure to environmental factors. As for antitumor chemotherapy, prevention of drug-related carcinogenesis during tumor treatment (i.e., at the stage of carcinogenesis initiation) is limited, since treatment might be affected. At the end of chemotherapy course, when carcinogenesis

promotion starts, it is possible to selectively inhibit the processes contributing to the final malignant transformation and progression and this should be done, especially for patients in the high-risk groups.

This review focuses on the pathogenesis of secondary neoplasia and the characteristics of high-risk groups for acute therapy-related myeloid leukemia (t-AML) in cancer survivors as an example. It also proposes for the first time to prevent t-AML using natural polyphenols during the latent period of its development.

Therapy-related acute myeloid leukemia (t-AML) is one of the most severe long-term sequelae of anticancer chemotherapy. Over the past 15 years, the number of t-AML cases has increased fourfold in the United States alone. T-AML is more malignant than primary AML since t-AML cells are usually resistant to chemotherapy. Thus, the median overall survival of patients with t-AML is 8-10 months, with the 5-year survival rate not exceeding 10-20% [2].

T-AML most often occurs after anticancer chemotherapy with alkylating agents and topoisomerase II inhibitors, as well as after radiotherapy [3]. Tumor cells resulting from chemotherapy with alkylating agents and radiotherapy are characterized by partial loss of chromosomes 5 or 7 or loss of the entire chromosome 7, with the latency period of their occurrence lasting on average five years [4].

The survival time after the disease onset averages eight months. In patients treated with topoisomerase II inhibitors, the disease manifests itself after 1-2 years [5]. This form of t-AML is more sensitive to therapy than the one induced by alkylating agents. The disease is characterized by an 11q23 translocation involving the *KMT2A* gene. This translocation results in activation of histone-lysine N-methyltransferase 2A (MLL) encoded by *KMT2A*, which is also known as zinc finger protein HRX. Platinum derivatives, antimetabolites, and taxanes are less likely to cause t-AML. Since all of them are used in combination with other drugs, it is difficult to assess the individual carcinogenic contribution of each agent [6, 7].

Additional risk factors are as follows: elderly age and high doses of drugs, especially if pharmacotherapy is followed by transplantation of autologous stem cells that are mobilized using agents such as granulocyte colony-stimulating factor. However, t-AML occurs in only one out of ten patients who have the same type of cured primary tumor and similar clinical course of the disease and were treated using the same protocol. In this regard, a question arises about the reasons for unequal sensitivity of myeloid hematopoietic cells to the carcinogenic effect of cytostatic drugs.

Criteria for predicting predisposition to AML

Prediction based on markers of cytostatic agent metabolism and DNA repair

In 1971, Alfred Knudson proposed a hypothesis known as the “two-hit” theory, which postulates that two successive mutations are required for retinoblastoma to develop in a retinal cell: a mutation inactivating the tumor suppressor gene *Rb* and a mutation converting the mutant *Rb* allele to the homozygous state [8].

Considering what has been known about the carcinogenesis mechanisms back then, it was natural to believe that these mutations emerge due to some features of the metabolism of carcinogenic cytostatic agents and repair of DNA damage caused by them. Numerous attempts to reveal the reasons for predisposition to t-AML have mainly yielded rather ambiguous results. This was because precursors of t-AML cells during the multistage carcinogenesis process are selected based on a series of independent parameters, while the cell properties responsible for cell malignization at one stage can coexist with the oppositely directed processes during another stage. For instance, the high level of secretion of reactive metabolites resulting from activation of a cytostatic agent can be observed simultaneously with high activity of the enzymes responsible for their detoxification or DNA damage repair. For this reason, assessing the risk of t-AML occurrence using a single genetic or epigenetic parameter turned out to be problematic. It was shown to be efficient only in case of mutant high-penetrance genes such as *BRCA1* and *BRCA2*, which are required for initiating homologous recombination, mismatch repair genes *MLH1*, *MSH2*, *MSH6*, and *PMS2* in patients with Lynch syndrome, Li-Fraumeni syndrome, retinoblastoma, and neurofibromatosis. In case of

Fanconi anemia, the risk of t-AML increases in the presence of five inherited homozygous mutations in any of the 16 genes whose abnormal expression is characteristic of this disease. These are mainly the genes *BRCA2 (FANCD1)*, *BRIP1 (FANCJ)*, *PALB2 (FANCN)*, *RAD51C (FANCO)*, and *ERCC4 (FANCQ)* [9, 10].

The genotoxic mechanisms of action of carcinogenic cytostatic agents include malignant transformation of normal cell precursors due to non-fatal damage to DNA structure or function. This results in uncontrolled cell proliferation and genome instability. Genotoxic drugs are conventionally divided into direct-acting agents and the latent forms. The latter ones are converted to active metabolites only after interaction with cellular enzymes. The direct-acting agents are converted to electrophilic molecules in the aqueous phase and are detoxified mainly by conjugating enzymes. The agents used during the initial chemotherapy stage, such as nitrogen mustard, β-propiolactone, N-nitroso-N-alkylureas, ethyleneimines, and a number of their derivatives, exhibit this feature. Other drugs, such as cyclophosphamide, isophosphamide, dacarbazine, doxorubicin, etc., are converted to electrophilic metabolites mainly by cytochrome P450 (CYP) isoforms and, to a lesser extent, by oxidases, hydroxylases, and other enzymes. At the second stage of metabolism, electrophiles are neutralized by acyltransferases, sulfotransferases, glutathione S-transferases (GSTP1; GSTT1), NAD(P)H: quinone oxidoreductase 1 (NQO1), and UDP-glucuronosyltransferase (UGT). In particular, GSTP1 and GSTT1 inactivate the metabolites of doxorubicin, lomustine, chlorambucil, busulfan, cisplatin, cyclophosphamide, etc., while NQO1 converts the resulting quinones into less active hydroquinones.

Thus, the interaction of carcinogenic cytostatic drugs with activating and detoxifying enzymes is the first event in chemical carcinogenesis. The balance between these systems is an important factor influencing both the therapeutic effect of the drug and the individual risk of therapy-related carcinogenesis. Numerous studies aimed at identifying the risk groups using this parameter have been carried out. Patients carrying inactivating polymorphisms were compared to wild-type carriers. It turned out that the findings on the correlation between carrying a polymorphism and the risk of t-AML were ambiguous and difficult to compare, since the studies differed in terms of treatment protocols, patient selection based on the diagnosis, control groups, and other parameters. In their study, K. Takahashi (2019) demonstrated the poor efficiency of searching for the correlation between a single component of therapy-related carcinogenesis and the risk of t-AML [6].

K. Takahashi selected a number of the most similar clinical trials comparing the risk of developing t-AML after the primary tumor is treated with etoposide in patients with wild-type *CYP3A4* and a significantly weaker *CYP3A4A-290G* allele. Both alleles metabolize etoposide to epipodophyllotoxin catechol and then to quinone, which forms DNA adducts. Patients with a weak allele were expected to have a lower risk of t-AML. Two studies confirmed this hypothesis, while four studies revealed no evidence. Etoposide quinone is degraded by NQO1. The C609T variant of NQO1 does not have this ability. Patients heterozygous and especially those homozygous for the mutant NQO1 allele were assumed to have a higher level of active quinone and a higher rate of t-AML complications. Two papers provided a positive result for the hypothesis, while five papers demonstrated negative results. A similar result was also obtained for glutathione S-transferase (GST), which catalyzes the conjugation of electrophilic metabolites with glutathione. Seven studies showed no statistically significant difference in the risk of developing t-AML in individuals carrying a *GSTT1/GSTM1* deletion and wild-type patients (**Table 1**).

Moreover, no relationship was found between the risk of t-AML and the presence of a single nucleotide polymorphism (SNP) in one of the DNA repair genes (*XRCC1*, *XRCC3*, *XPD*, and *RAD51*), as well as the *TP53* and *MDM2* genes (**Table 1**).

The currently available high-throughput technologies and next-generation sequencing (RNA-seq) techniques that have replaced microarrays have made it possible to search for markers of susceptibility to t-AML based not only on the genomic features and disorders but also on changes in the gene expression profile. A large variety of leukemia cell transcriptomes have been revealed, including chimeric RNAs, miRNAs, piRNAs, long non-coding RNAs (lnc-

Table 1. Impact of polymorphism of individual markers on the risk of therapy-related acute myeloid leukemia [6]

Genes	Protein function	Inactivating polymorphism
CYP3A4	Activation of cytostatic drugs	SNP A290G
NQO1	Topoisomerase II inhibitor	SNP C609T
GSTT1, GSTM1	Inactivation of electrophilic metabolites	*GSTT1/GSTM1* deletion
XRCC1	Base and nucleotide excision repair	Arg399Gln
XRCC3	DNA double-strand break repair	Thr241Met
XPD	DNA double-strand break repair	Lys751Gln
RAD51	DNA double-strand break repair	SNP G135C
TP53	Tumor suppressor	Arg72Pro
MDM2	Ubiquitin ligase	SNP T309G

RNAs) and their subtype, circular RNAs. As a result, in addition to a large pool of new findings, searching for primary changes and interpretation of the results have become much more complicated [11].

Prediction based on markers of clonal hematopoiesis

A number of features of t-AML cells indicate that the mechanisms of their occurrence differ from those of primary myeloid leukemia. First, t-AML cells carry much greater number of mutations (especially in *TP53*) compared to the primary forms. However, t-AML cells carry few aging signatures, which are determined by the number of CpG > TpG substitutions in the genome or by telomere length. Second, the set of mutations in the primary tumor that has been treated differs from those in the t-AML cells of the same patient. This fact argues against the existence of the common mechanism of their occurrence. Third, the fact that these patients carry the same mutations in both myeloid and T cells indicates that early hematopoietic precursors become malignant [12]. This is partially because t-AML is often preceded by the occurrence of mutations followed by clonal hematopoiesis of indeterminate potential, and appearance of the clone which is advantageous in cellular competition (i.e., displacement of one cell clone by another one) [13]. Hematological parameters and the features of bone marrow morphology can be normal even if a significant part of the myeloid lineage population is represented by a more actively proliferating mutant clone. Some mutations are found at a low frequency, since they do not influence cell proliferation and violence and they are eliminated by natural selection. Some clones persist throughout a person's life and do not become malignant due to the lack of a sufficient set of driver mutations or epigenetic changes, which can be induced by the genotoxic effect of cytostatic drugs after chemotherapy for primary tumor [14-16].

According to the age-related pattern of clonal hematopoiesis of indeterminate potential, in a normal population its frequency does not exceed 1% until the age of 40 and increases to 10-20% after 70 years. About 2% of people older than 75 years also have other more significant changes presenting as chromosome deletions, insertions and loss of heterozygosity [17-19].

Knight et al. discovered background mutations in t-AML patients with the loss of either chromosome 5 or 7 [20]. These mutations were associated with neither metabolism of cytostatic agents nor DNA repair directly and had therefore escaped the researchers' attention so far. A high correlation between the risk of the disease development ($P < 0.001$) and an SNP of three genes was shown for the patients. The first SNP is an intronic polymorphism (rs1394384) of the gene *ACCN1*, a member of the DEG/ENaC superfamily; its protein product regulates the amiloride-sensitive Na+ channel activated by extracellular protons and associated with sensory functions. The second SNP has the rs1199098 polymorphism; it is in linkage disequilibrium with the gene *IPMK* encoding a multikinase that positively regulates protein kinase B alpha (Akt), which inhibits apoptosis and is therefore considered an onco-

Table 2. Frequency of individual mutations in clones with indeterminate potential [21]

Gene carrying a mutation	Frequency, %	Protein function
DNMT3A	50-60	CpG methylation
TET2	10-15	Regulation of hematopoietic stem cells
ASXL1	8-10	Epigenetic regulation of the genome
SF3B1	2-5	Splicing factor
GNB1	3-4	Integration of signals from receptors to effector proteins
SRSF2	1-2	Splicing factor
GNAS	1-2	Adenylyl cyclase activation

gene. Functions of the third gene with the rs1381392 polymorphism are unknown [21].

Mutations in the *DNMT3A, SRSF2* and *SF3B1, TET2,* and *ASXL1* genes account for up to 80% of all the observed mutations in hematopoietic cells with undetermined potential. Of these, inactivating mutations in *DNMT3A* encoding DNA methyltransferase responsible for CpG methylation occur in more than 25% of AML cases (**Table 2**) [22].

One of the most comprehensive studies conducted to date showed that, among 8,810 patients diagnosed with non-hematological malignancies, clonal hematopoiesis with undetermined potential was found in 25% of individuals prior to treatment, with 4.5% of the cases carrying driver mutations in the *PPM1D* and *TP53* genes. The presence of these mutations correlated with an increased incidence of t-AML and shorter survival in these patients [23].

Cytotoxic therapy, in addition to the induction of mutational and epigenetic changes in the clones, was shown to provide a selective advantage to only those cells with suppressed *TP53* and active expression of adhesion and migration proteins necessary for interaction with the stroma and environment.

In general, the presence of asymptomatic clonal hematopoiesis with undetermined potential combined with known driver mutations increases the risk of the t-AML disease dramatically and can serve as an important prognostic indicator. At the same time, since the presence of any known driver mutation in the clones does not always lead to t-AML, it is obvious that they are just the tip of the iceberg and there are also many other yet unknown components, which are being discovered with the advent of new methods of functional analysis of the genome.

The mutation load in hematopoietic clones can have various origins. These are either congenital abnormalities or mutations arising from genetic drift or exposure to environmental mutagens. It has been previously assumed that mutations or epigenetic changes arise as a result of various factors acting either on hematopoietic stem cells directly or on the precursors of their further differentiation. To date, with the advent of new data on the role of the bone marrow niche in hematopoiesis, it has been suggested that the effect of genotoxic factors, including cytostatic drugs, on the mesenchymal cells of this niche is the primary event.

The role of the bone marrow niche in clonal hematopoiesis and t-AML pathogenesis

Cellular microenvironment is known to play a major role in providing a stem cell with specific functions. It consists of pluripotent mesenchymal cells and their descendants (osteoblasts), endothelium of bone marrow sinusoids, fibroblasts, reticular cells, adipocytes, etc. Hematopoietic stem cells of the periosteal niche proliferate and are restrained from maturation by the chemokine CXCL12 of perisinusoidal stromal cells along with sialoadhesin (CD169) of bone marrow CD169+ macrophages [24]. Mature hematopoietic stem cells of the periosteal niche in the resting phase express the following factors: (1) platelet factor 4 (CXCL4), cytokine TGFβ, and thrombopoietin (TPO) of megakaryocytes; (2) chemokine CXCL12 of reticular cells co-expressing the leptin receptor LepR and the neuron-specific regulatory element of the NES gene (Nes-GFP); (3) chemokine CXCL12 of periarterial cells expressing neural/glial antigen 2 (NG2) and neuron-specific regulatory element of the NES gene (Nes-GFP); (4) stem cell factor SCF and Jag1 (a Notch ligand of endothelial cells of sinusoids). Mature HSCs in the vascular

niche express demyelinated Schwann cells in the resting phase, which activate the latent form of the cytokine TGFβ. Therefore, all phases of hematopoiesis, including replication, proliferation, differentiation, as well as sorting and directed migration of stem cells from the periphery known as homing, involve their direct interaction with HSCs or indirect action through humoral factors, as well as the Notch and WNT signaling pathways [24-26].

Aside from intercellular signaling, normal functioning of the niche is also regulated by neurotransmitters of the sympathetic nervous system, which affect HSC differentiation, as well as the expression of the chemokine SDF-1, a chemoattractant necessary for retaining HSCs in the bone marrow niche, by the microenvironment.

Cytostatic agents remodel the bone marrow niche and affect its homeostasis by inducing the release of inflammatory cytokines such as TNFA, TGFB, and IL-6 as well as generating active oxygen that damages both the mesenchyme and sympathetic neurons. Administration of cytarabine and daunorubicin in mice suppresses regeneration of mesenchymal stem cells of the bone marrow and causes their differentiation into inactive adipocytes and chondrocytes. It further decreases the production of cytokines necessary for hematopoiesis such as SCF, CXCL12, ANGPT1, VCAM1, and IL-7 as well as stimulates apoptosis in hematopoietic stem cells [27, 28].

The first evidence that a change in the stem cell microenvironment in the niche can cause malignant transformation of myeloblasts was obtained in a series of experiments in mice carrying deletions in the genes of either retinoblastoma, retinoic acid receptor-gamma (RAR-γ), transcription factor IκBα or E3 ubiquitin-protein ligase MIB1. In all cases, the mice developed a myeloproliferative disease with characteristic leukocytosis, extramedullary hematopoiesis, and splenomegaly [29-31].

In the same manner, damage by cytostatic agents to the cells innervating the niche causes a series of changes contributing to formation of aberrant clones and malignant transformation of HSCs. In particular, damage to catecholaminergic neurons, the postganglionic fibers of which comprise the bone marrow and innervate the HSC microenvironment, contributes to the onset and aggressive progression of leukemia, while restoration of their effects using a selective β3-adrenoceptor agonist inhibits its progression [32]. Damage to the pool of osteogenic cells by cytostatic agents has an even greater impact. Knockdown of the Dicer1 endonuclease gene in mesenchymal precursors of osteoblasts promotes in acute myeloid leukemia. Meanwhile, the level of the ribosome maturation protein Sbds decreased in the same way as observed in individuals with Shwachman-Bodian-Diamond syndrome, which predisposes to leukemia due to a *SBDS* mutation [33].

Myeloblasts of t-AML patients often exhibit an increased activity of the Notch signaling pathway. To date, activation of this signaling pathway is ascribed to increased WNT/β-catenin signaling in osteoblasts. This triggers the expression of the ligands Jagged-1 and DLL-1 on their cytoplasmic membrane, which activate Notch signaling in hematopoietic cells. Experiments in mice demonstrated that increased activity of the WNT/β-catenin signaling pathway in osteoblasts can occur due to the presence of activating mutations in *CTNNB-1*, which codes for the β-catenin protein. However, in this case, a necessary condition for the development of t-AML is the presence of the functionally active transcription factor FoxO1 in osteoblasts, which acts as a co-activator of β-catenin by facilitating its transfer to the nucleus [34, 35].

Myeloproliferative syndrome and myeloid leukemia can also develop if cytostatic agents cause mutations or knockout of *Dnmt3a* in the mast cells of the HSC microenvironment. This leads to increased secretion of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-13 as well as the development of inflammatory responses that stimulate proliferation and/or suppress apoptosis even in mutant clones [25, 36-39].

The onset of t-AML can begin with deterioration of the Notch/miR-155/κB-Ras1/NF-κb signaling cascade caused by cytostatic agents in endothelial cells of the bone marrow. Notch/RBPJ dysfunction activates the expression of miR-155, which in turn mediates activation of NF-κB and increases the production of pro-inflammatory cytokines and colony-stimulating

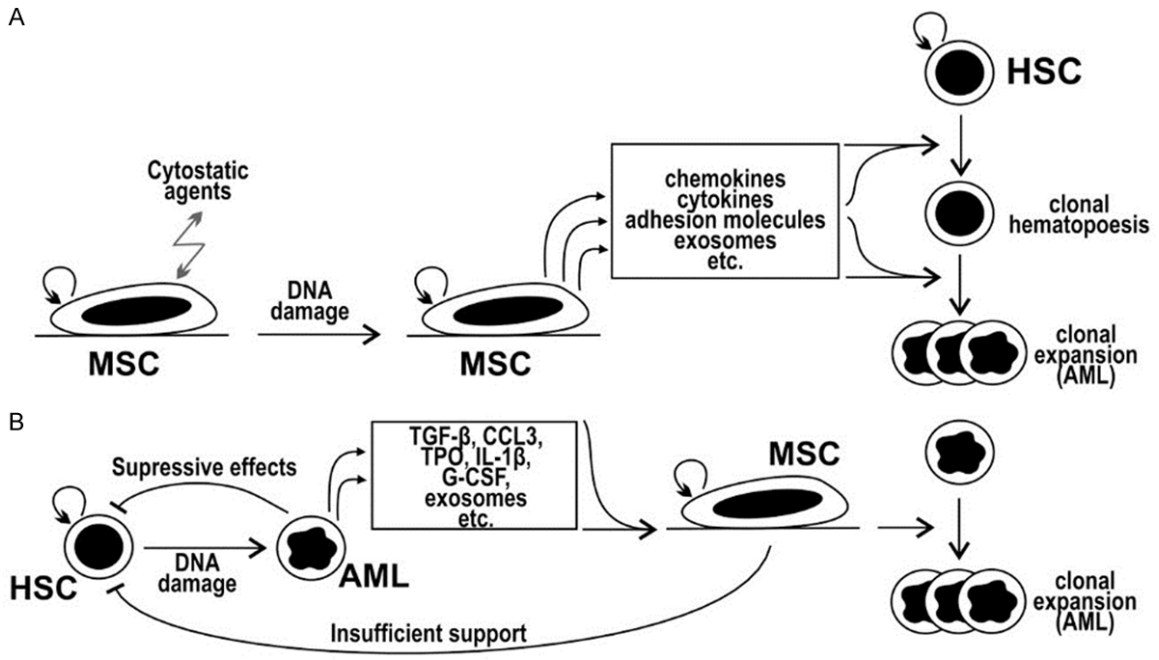

Figure 1. Mechanisms of t-AML pathogenesis. A. T-AML develops as a result of primary damage to the stromal microenvironment of hematopoietic stem cells (HSCs) by cytostatic agents. Functional changes in mesenchymal stromal cells (MSCs) caused by genotoxic stress disrupt their interaction with HSCs thus contributing to the accumulation of genetic and epigenetic changes in HSCs and occurrence of aberrant clones. B. T-AML develops as a result of primary changes in HSCs and progenitor cells of the myeloid lineages. The resulting aberrant clones cause phenotypic and functional changes in the mesenchymal microenvironment of hematopoiesis by turning it into a niche that supports proliferation of malignant cells while disrupting normal hematopoiesis. (Abbreviations: MSCs - mesenchymal stem and progenitor cells; AML - therapy-related acute myeloid leukemia; TGF-β - transforming growth factor β; CCL3 - chemokine (C-C motif) ligand 3; G-CSF - granulocyte colony-stimulating factor; HSPCs - hematopoietic stem and progenitor cells; TPO - thrombopoietin).

factors (G-CSF, SDF-1α, and CXCL16), which activate the proliferation of HSCs and transformed myeloblasts. RBPJ dysfunction in endothelial cells is sufficient for activation of myeloid cell proliferation; however, the contribution of other mesenchymal cells may be essential for the large-scale and rapid progression of a myeloproliferative disease [36, 40-43].

At the same time, cytostatic drugs can induce changes in HSCs themselves. In particular, etoposide, which is activated by myeloperoxidase of myeloid progenitor cells, causes double-strand breaks in the region of the genes *KMT2A*, which encodes histone-lysine N-methyltransferase 2A, and *RUNX1*, which is associated with differentiation of hematopoietic cells [44].

Thus, there are currently two hypotheses regarding the mechanism of t-AML development: primary transformation of progenitor cells and deterioration of the elements of the bone marrow niche that form these cells (**Figure 1**).

Trends in t-AML prevention

When considering the strategies for preventing t-AML and other therapy-related malignancies, one should keep in mind that the attempts to prevent a new disease are accompanied by a risk of primary disease recurrence. Therefore, the measures taken to prevent t-AML should not increase the recurrence risk.

In contrast to spontaneous leukemia, the agents causing t-AML, as well as the main mechanisms of their action, are currently known. Moreover, unlike the spontaneous process, the latent period of the induced process is known to a certain extent. This determines the approaches to preventing the risk of t-AML at the stage of its initiation by cytostatic agents and further progression in the latency period after the end of treatment [45].

Table 3. Inflammation inhibitors proposed for treating myeloproliferative diseases [36]

Drug	Target	Mechanism of action	Phase*
CX-01	TLR	TLR2 and TLR4 inhibitor	1
OPN-305	TLR	Anti-TLR2 antibody	1-2
DUKCPG-001	TLR	TLR9 agonist	2
GNKG168	TLR	TLR9 agonist (oligonucleotide)	1
852A	TLR	TLR7 agonist	2
ARRY614	p38-MAPK	p38 MAPK and Tie2 inhibitor	1
Tocilizumab	IL-6	Anti-IL-6 antibody	1
Siltuximab	IL-6	Anti-IL-6 antibody	2
Etanercept	TNF-α	Anti-TNFR inhibitory antibody	1/2
Bortezomib	NF-κB	Proteasome inhibitor of NF-κB	1 and 2**

*Phase of clinical trial. **Four trial protocols at phases 1 and 2.

Prevention of the initiation of therapy-related carcinogenesis

For now, clonal hematopoiesis with driver mutations in combination with the features of cytostatic drug metabolism and DNA damage repair are considered a comprehensive marker of an increased risk of t-AML. In case of this symptom complex, the best therapy option for treating primary cancer patients would be the one that, with the same effectiveness, has a lower risk of initiating secondary carcinogenesis. This possibility has previously been demonstrated: replacement of the alkylating cytostatic agent melphalan with platinum derivatives in the treatment of ovarian cancer reduced the risk of t-AML fivefold (from 20.8% to 4%) [46].

A similar result was obtained after replacing the MOPP protocol (mechlorethamine, vincristine, procarbazine, and prednisone), which was used during 1971-1984, with an equally effective but more sparing ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) or by using the VMF protocol with cyclophosphamide replacement [47].

Later, a decision was made to limit the use of carcinogenic anthracyclines in the treatment of breast cancer. In particular, for the treatment of HER2-positive breast cancer, the standard regimen containing doxorubicin and cyclophosphamide followed by administration of paclitaxel/docetaxel and trastuzumab has been replaced with an equally effective but less leukemogenic combination of docetaxel, carboplatin, and trastuzumab [48]. While 88% of elderly patients with this disease subtype received anthracycline-based treatment in 2005, only 15% of them received the same treatment in 2011 [49].

The consequences of using colony-stimulating factors for either patient recovery from severe myelosuppression after intensive chemotherapy courses or HSC stimulation prior to bone marrow sampling for its subsequent transplantation are also the topic of active discussion in both Russian and foreign literature. On the one hand, stimulation of hematopoiesis is sometimes necessary for health reasons. On the other hand, it can subsequently increase the risk of AML, since enhanced proliferation of the existing pre-leukemic clones contributes to the higher risk of mutations in them, which are associated with malignant potential [50-53].

Prevention of t-AML promotion and progression in the latency period

Inhibition of inflammation: As shown above, one of the most important factors in the initiation and progression of t-AML is the inflammatory process triggered by cytostatic agents. It is a necessary element of the therapy during treatment, but it can subsequently promote the growth and conversion of the transformed clones to t-AML through stimulation of the cell cycle and inhibition of apoptosis. In this regard, studying the possibility of using inhibitors of pro-inflammatory signaling pathways developed for the treatment of myelodysplasia and primary AML seems promising for prevention of therapy-related malignancies. The positive experimental data obtained provided the basis for clinical trials of inhibitors of toll-like receptors (TLR), interferons (IFN I and II), tumor necrosis factor (TNF-α), interleukins (IL) IL-6 and IL-8, and the transcription factor NF-kB (**Table 3**) [36, 54, 55].

The anti-inflammatory effects of natural polyphenols are described below in the relevant section.

Effects on the bone marrow hematopoietic niche: The question on whether to include the effects on targets in the bone marrow hemato-

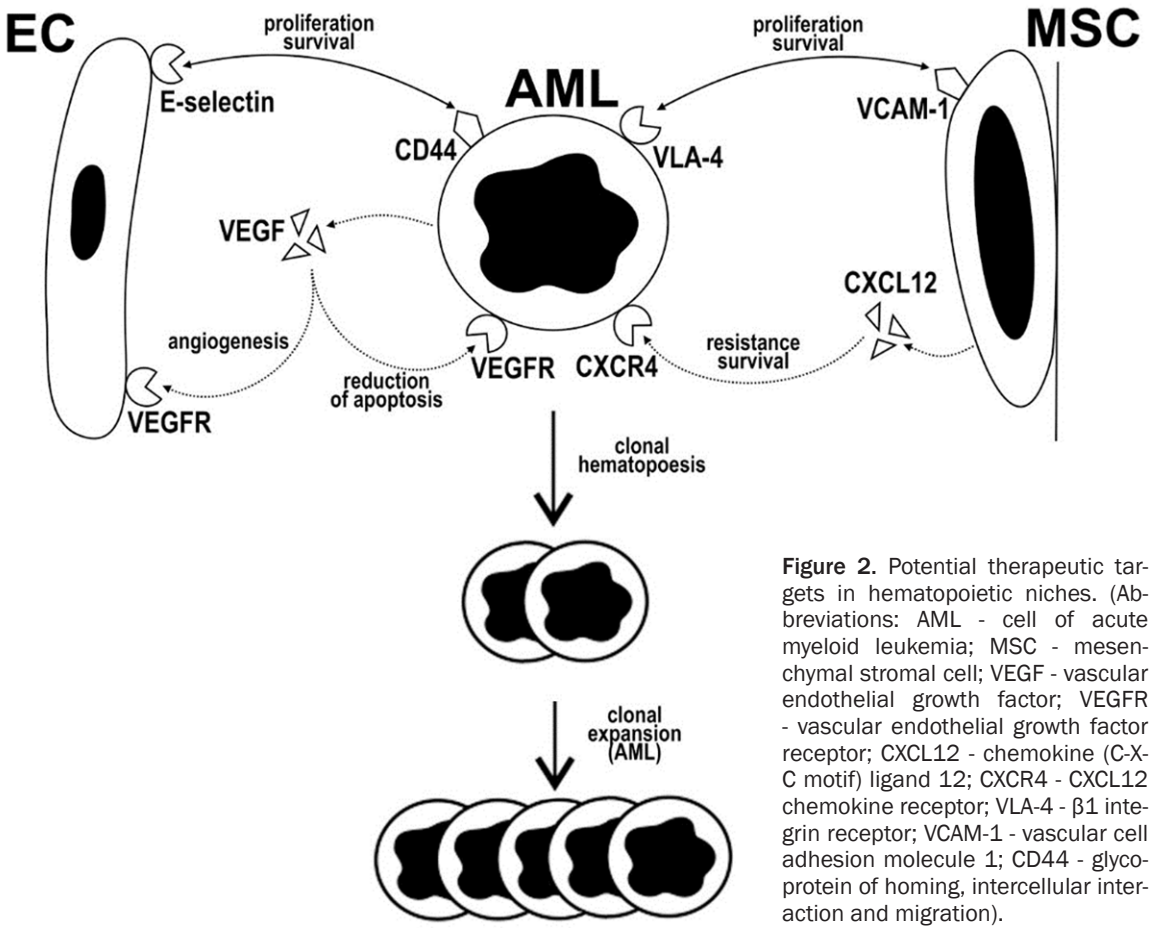

Figure 2. Potential therapeutic targets in hematopoietic niches. (Abbreviations: AML - cell of acute myeloid leukemia; MSC - mesenchymal stromal cell; VEGF - vascular endothelial growth factor; VEGFR - vascular endothelial growth factor receptor; CXCL12 - chemokine (C-X-C motif) ligand 12; CXCR4 - CXCL12 chemokine receptor; VLA-4 - β1 integrin receptor; VCAM-1 - vascular cell adhesion molecule 1; CD44 - glycoprotein of homing, intercellular interaction and migration).

poietic niche in the t-AML latency period in the system for the prevention of therapy-related carcinogenesis has not yet been raised. Studies in this area considered only the treatment of primary AML but not the prevention of its secondary therapy-related variant (**Figure 2**). Furthermore, the results were quite modest, and the side effects turned out to be unacceptable in some cases. In particular, the therapeutic effect of inhibiting angiogenesis in the bone marrow niche using targeted drugs (bevacizumab, semaxinib, sunitinib, etc.) turned out to be very weak, although a positive result had been previously obtained when inhibiting the glycoprotein CD44 in the AML xenograft model using bevacizumab [56-58].

Clinical trials on the use of CXCR4 inhibitors for shifting AML cells from the protected periosteal niche and making them amenable to chemotherapy have been more successful. Plerixafor (AMD3100), ulocuplumab, and BL-8040 in combination with cytostatic drugs increased the frequency rate of complete remissions [59, 60].

Encouraging results were obtained for CX-01, an anticoagulant that binds CXCL12 and blocks the CXCL12/CXCR4 signal [61]. Natalizumab, a monoclonal antibody against VLA-4, a β1 subfamily integrin receptor, was shown to be effective in the AML xenograft model but caused unacceptable demyelization of the CNS cells [62, 63]. The trial of another VLA-4 inhibitor, AS101, turned out to be more successful. It attenuated the drug resistance of tumor cells and thereby enhanced the effect of chemotherapy in experimental conditions [64].

The use of these drugs for prevention of therapy-related tumors in the form of monotherapy does not seem justified due to their limited scope of action, which cannot cover the entire spectrum of genetic and epigenetic changes preceding the t-AML onset. Since transformation can be initiated by cytostatic agents in the

Table 4. Effect of plant polyphenols on epigenetically regulated cellular processes [71, 72]

Polyphenol	Signaling pathways		DNA methyl-transferase	Histone modification enzymes		microRNA	
	Activation	Inhibition	Inhibition	Activation	Inhibition	Activation	Inhibition
Curcumin	-	MAPK/ERK MAPK/JNK WNT mTOR NF-κB Notch Shh/Gli	DNMT1 DNMT3a DNMT3b	-	HDAC1 HDAC3 HDAC4 HDAC8 EZH2	let-7a; miR-9 miR-15; miR16 miR22; miR-181b	miR-21; miR-27a miR-34a; miR-186 miR-208
Resveratrol	MAPK/ERK mTOR Notch	MAPK/ERK MAPK/JNK MAPK/P38 WNT mTOR NF-κB Notch	DNMT1 DNMT3a DNMT3b	-	11 HDACs LSD11 EP300	miR-328	miR17-92; miR-106a-363 miR-106b-25; miR-19 miR-21; miR-25 miR-30a-5p; miR-92a-2
EGCG	MAPK/JNK MAPK/P38	MAPK/ERK WNT mTOR NF-κB Notch Shh/Gli	DNMT1 DNMT3a DNMT3b	SIRT1	HDAC EZH2 HMT	miR-210 miR-7-1 miR-34a miR-99a	miR-25; miR-92 miR-141; miR-200a miR-98-5p; miR-125a-3p miR-92; miR-93 miR-106b
Genistein	-	MAPK/ERK WNT mTOR NF-κB Shh/Gli	DNMT1 DNMT3a DNMT3b MBD2	HAT EZH2 SIRT1	HDAC	let-7b; let-7e miR-200b; miR-200c miR-15a; miR-29a miR-548b; miR-574-3p miR-1256; miR-1296	miR-15b; miR-23b-3p miR-27a; miR-125a miR-125b; miR-145 miR-151; miR-155 miR-208b; miR-211 miR-223; miR-320 miR-376a; miR-411 miR-520g; miR-542-5p miR-1260b

form of a combination of mutations and epigenetic abnormalities, preventative interventions should be polytropic, i.e. they should prevent the formation of the greatest possible number of transcriptional aberrations. Small molecules belonging to plant polyphenols meet the majority of these criteria. Such compounds act mainly through epigenetic mechanisms and exert anticarcinogenic and antitumor activities. They are pleiotropic and non-toxic at physiological doses [65-68].

Anticarcinogenic and antitumor activities of plant polyphenols: Numerous experiments have demonstrated that these substances exhibit an anticarcinogenic and antitumor activity. For instance, they were shown to inhibit the formation and growth of breast cancer in mice and other animals [69, 70].

The main way the most studied polyphenols influence cellular processes is via their epigenetic modification (**Table 4**). The data listed in the table indicate that these substances act on many targets, including various transcription factors, chromatin proteins, DNA methyltransferase, histone acetylases and deacetylases, histone methylases and demethylases, the abnormal functions of which are observed in malignant transformation [71, 72]. Being able to modulate the epigenetic regulatory mechanisms, which are altered in tumor cells, natural polyphenols can affect their proliferation, migration, and death. This can contribute to cell cycle inhibition, tumor cell differentiation or their death via apoptosis, necrosis, autophagy or mitotic catastrophe directly or by changing the tumor microenvironment. In this regard, a combination of chemotherapeutic drugs and natural polyphenols in specific therapeutic strategies, taking into account the effects of both, may become promising. Furthermore, recent studies in the cultures of multidrug-resistant tumor clones have shown that dietary polyphenols can significantly inhibit their growth [73]. These cell lines turned out to be hundreds of times more sensitive to the inhibitory effect of a number of flavonoids than to the antitumor cytostatic agents used in clinical practice (**Table 5**). In addition, most of the studied polyphenols were orders of magnitude less toxic for normal blood cells [73, 74].

Table 5. Effect of flavonoids on leukemic cell lines resistant to cytostatic agents [73]

Parental cell line	Resistant subline	Relative resistance to cytostatic drugs (RR)*	Relative resistance to flavonoids (RR)
HL-60	Anthracycline-resistant HL-60-R	Doxorubicin 328 Epirubicin 72 Daunorubicin 1367 Mitoxantrone 3700 Vincristine 5900	Quercetin 0.50
K562	Doxorubicin-resistant K562/A	Doxorubicin 57	Quercetin 2.6
	Imatinib-resistant K562-R	Imatinib > 50	Apigenin 4
	Imatinib-resistant K562/sti	Imatinib 125	Epigallocatechin gallate 1.1
CCRF-CEM	Multiple resistance to cytostatic agents CEM/ADR500	Doxorubicin 1036 Epirubicin 484 Docetaxel 438 Paclitaxel 200 Vincristine 613	Casticin 1.6
MOLT-4	Daunorubicin-resistant MOLT-4/ADR	Daunorubicin 13.8	Baicalein 1.4 Nobiletin 0.6 Tangeretin 0.5 Wogonin 1.0 Epigallocatechin-3-gallate 1.0 Quercetin 0.9

*RR - relative resistance calculated as the ratio of half-maximum inhibitory concentration (R-IC50) for a resistant subline to that for the parental sensitive line (S-IC50).

Optimization of the use of plant polyphenols: (1) Methods for increasing bioavailability. The success of in vitro experiments does not always predict the effectiveness of the drug in the body, as well as its side effects. In the case of plant polyphenols, this is due to their insufficient bioavailability and numerous metabolic transformations they undergo in the body. In plants, flavonoids exist mainly in the form of glycosides, which are hydrolyzed to aglycones in the intestine and then form complexes mainly with glucuronic acid and enter the bloodstream. Thus, the properties of the intact molecules, including antitumor and anticarcinogenic ones, change significantly [75, 76]. As a result, the concentration of the parent molecules in the blood quickly drops below 10 μM even after eating a meal rich in flavonoids [74, 75].

In order to overcome these disadvantages, modifications are being developed that increase the stability and bioavailability of flavonoids, in particular, by protecting reactive hydroxyls using acetate groups [77].

Conjugation of the flavonoid glycoside phlorizin with fatty acids dramatically increased its ability to inhibit the growth of THP-1 AML cells and two human tumors: MDA-MB-231 breast adenocarcinoma and HepG2 hepatocellular carcinoma. The effect was comparable to that of sorafenib, doxorubicin and daunorubicin. Moreover, the conjugates were not toxic to normal cells [78]. Studies focused on modifying quercetin to enhance its antitumor activity have recently been carried out [79].

Nanotechnology approaches are also being developed to increase the bioavailability of plant polyphenols through their incorporation into lipid-based carrier nanoparticles and targeted delivery to tumor cells [80, 81]. In particular, the combination of quercetin and cisplatin nanoparticles inhibited the growth of a cisplatin-resistant transplanted bladder tumor via the suppression of WNT-16 by quercetin in fibroblasts of the tumor environment, the protein that confers tumor cells with this resistance [82]. These and similar data indicate that, in addition to preventing secondary tumor, plant polyphenols can also enhance the antitumor effect of cytostatic agents [83-85].

(2) Overcoming the side effects of polyphenols. Like any other biologically active compound, plant polyphenols have side effects that can occur both when they are used as monotherapy

and in combination with other drugs. When considering this issue, one should take into account that experimental data on the toxic effect of high doses of pure polyphenol do not always reflect the effect of the natural product. This is because the compound is present at a lower percent and in combination with many other active compounds in its complex nutritional matrix. In addition, when developing the methods for therapeutic and preventative use of polyphenols, one should clearly understand that these compounds can exert exactly the opposite effects depending on the dose, the target system, as well as type of cells and their biological status. On the one hand, they can inhibit the components of the tumor microenvironment that stimulate its growth. On the other hand, they can also suppress the effect of anticancer immunotherapy drugs and facilitate the selection of clones capable of inhibiting antitumor immunity [86].

Moreover, some flavonoids are mutagenic. In particular, this feature of quercetin was demonstrated in the bacterial Ames test and mammalian cell cultures: DNA adducts were found in mammalian cells. The data on the carcinogenicity of quercetin in rodents are ambiguous. High doses of quercetin caused tumors in rats in some experiments, while other studies did not confirm these results [87].

In addition, since quercetin, genistein, and kaempferol are topoisomerase II inhibitors, they can induce *MLL* gene rearrangements in the human cell culture, which are often observed in pediatric leukemia and t-AML after treating primary tumor with etoposide. There is an assumption that dietary flavonoids consumed by mother may concentrate in fetal tissue and cause *MLL* mutations in HSCs. The 11q23 translocation characteristic of the effect of topoisomerase II inhibitors is found in blasts [88-90]. Preincubation of MCF-10A human breast cancer cells with genistein reduced DNA damage caused by two mutagens: the product of lipid peroxidation of 4-hydroxy-trans-2-nonenal (4-HNE) and benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide [91].

Many flavonoids induce the activity of the cytochrome P450 system, since they are ligands and agonists of the aromatic hydrocarbon receptor AhR. Galangin, quercetin, diosmin, and diosmetin induce *CYP1A1* transcription, while flavone and tangeretin induce CYP1A1/2 and CYP2B1/2 (to some extent) proteins. This can significantly affect the concentration of other pharmaceutical drugs used by the patient due to a reduced therapeutic effect or an overdose. Meanwhile, these polyphenols can inhibit the activity of CYP isoforms in certain conditions. Molecular docking studies demonstrated that numerous mechanisms are involved in interaction between flavonoids at various concentrations and the active site of P450, which determines the nature of their impact on catalytic activity [92].

In general, the effectiveness and safety of using polyphenols to prevent secondary carcinogenesis can be described using the Paracelsus quote: “Poison is in everything, and no thing is without poison. The dosage makes it either a poison or a remedy”.

Conclusion

Thus, during last 15 years the number of secondary cancers has raised several folds as a result of the increasing population of cancer survivors and this worsening problem requires solution. t-AML is one of the most frequent secondary cancers, which latency period lasts 5 years and survival time after the disease onset averages 8 months. Two mechanisms of t-AML development have been elucidated: primary transformation of progenitor cells and deterioration of the elements of the bone marrow niche that form these cells. t-AML is induced by alkylating agents or topoisomerase II inhibitors. Platinum derivatives, antimetabolites, and taxanes are less likely to cause t-AML. Carcinogenic effects of genotoxic drugs used in the therapy of primary cancers were expected to depend on the pattern of CYP isoforms and on the levels of detoxification enzymes, however, contradictory results were obtained on the association between metabolism enzyme activity and the risk of t-AML. Inherited genetic mutations in genes of DNA repair, in particular BRCA1, BRCA2, MLH1, MSH2, MSH6, BRIP1, PALB2, RAD51C, and ERCC4 were shown in patients with a number of syndromes that predispose to t-AML. Mutations in the DNMT3A, SRSF2 and SF3B1, TET2, and ASXL1 genes account for up to 80% of all the observed mutations in hematopoietic cells with undetermined potential. PPM1D and TP53 mutations corre-

late with an increased incidence of t-AML and shorter survival in these patients. The strategy for reducing the risk of therapy-related carcinogenesis includes individual selection of a sparing chemotherapy course and further avoidance of contacts with the agents of occupational and environmental mutagenesis. Constant use of inhibitors capable of preventing the progression of potentially malignant clones by inhibiting their proliferation and docking in the bone marrow niche is required after the end of the chemotherapy course, during the latency period of therapy-related carcinogenesis. It seems promising to search for compounds that can normalize the mesenchymal environment of stem cells damaged by cytotoxic agents in the bone marrow niche. Such compounds are found among plant polyphenols comprising the human diet. Preliminary data on their ability to inhibit therapy-related carcinogenesis and proliferation of tumor cells, including those resistant to cytostatic agents, make the rational use of plant polyphenols promising.

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Disclosure of conflict of interest

None.

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