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Berries and leukemia: a systematic review of experimental studies and the *in vitro* effect of ellagic acid on chronic myeloid leukemia cells

Frutas vermelhas e leucemia: uma revisão sistemática de estudos experimentais e o efeito *in vitro* do ácido elágico sobre células de leucemia mieloide crônica

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ABSTRACT
Diet is directly involved in cancer etiology, influencing positively or negatively. The aim of this study was to conduct a systematic review on the effect of berries on leukemia, identifying the main bioactive compounds involved in biochemical and molecular mechanisms through which they act and also to test the *in vitro* effect of ellagic acid, one of the phytochemicals found in berries, on leukemia cells. The work was subdivided into two parts: the first consisted of analysis of abstracts in Pubmed / Medline databases using MeSH (Medical Subject Headings) terms, synonyms, related and free terms, such as: leukemia, berries, anthocyanins, ellagic acid and flavonoids resulting in 274 articles. After abstract analysis, based on the eligibility criteria, this number was reduced to 21 articles. The second part was the *in vitro* investigation of the effect of ellagic acid by the MTT reduction in chronic myeloid leukemia cell lines (CML) resistant or not to chemotherapeutic agents. *In*

vitro, *in vivo* and *ex vivo* studies associated the bioactive compounds present in berries with anticancer effect, acting mainly by induction of metabolic enzymes, modulation of gene expression and cell proliferation, chemotherapic resistance, free radical scavenging and induction of apoptosis. The present work also highlights the induction of collateral sensitivity by ellagic acid, since this compound was able to preferentially act on chemotherapeutic resistant cells. However, it is emphasized that animal models and clinical trials are for establishing the main mechanisms of action and possible dosages of berries intake daily or by dietary supplementation that could contribute to leukemia's treatment.

Key word: leukemia, berries, anthocyanins, ellagic acid and flavonoids.

RESUMO

A dieta está diretamente envolvida na etiologia do câncer, influenciando positivamente ou negativamente. O objetivo deste estudo foi realizar uma revisão sistemática sobre o efeito das frutas vermelhas na leucemia, identificando os principais compostos bioativos envolvidos nos mecanismos bioquímicos e moleculares por meio dos quais atuam e também testar o efeito *in vitro* do ácido elágico, um dos fitoquímicos encontrados em frutas vermelhas, sobre células de leucemia. O trabalho se subdividiu em duas partes: a primeira consistiu em análises de resumos nas bases de dados *Pubmed* / *Medline* utilizando-se termos MeSH (*Medical Subject Headings*), sinônimos, termos relacionados e livres, tais como: leucemia, frutas vermelhas, antocianinas, ácido elágico e flavonoides, resultando em 274 artigos. Após análise dos resumos, com base nos critérios de elegibilidade, esse número foi reduzido para 21 artigos. A segunda parte tratou-se da investigação *in vitro* do efeito do ácido elágico por meio do ensaio de redução de MTT em linhagens celulares de leucemia mielóide crônica (LMC) resistentes ou não a quimioterápicos. Estudos *in vitro*, *in vivo* e *ex vivo* associaram os compostos bioativos presentes nas frutas vermelhas com efeito anticâncer, atuando principalmente pela indução de enzimas metabólicas, modulação da expressão gênica e proliferação celulares, resistência quimioterápica, eliminação de radicais livres e indução de apoptose. O presente trabalho ressalta, ainda a indução de sensibilidade colateral pelo ácido elágico, uma vez que esse composto foi capaz de atuar preferencialmente sobre células resistentes a quimioterápicos. No entanto, destaca-se que modelos animais e ensaios clínicos são cruciais para o estabelecimento dos principais mecanismos de ação e possíveis dosagens diárias de consumo de frutas vermelhas ou suplementação dietética que podem contribuir para o tratamento da leucemia.

Palavras chave: leucemia, frutas vermelhas, antocianinas, ácido elágico e flavonoides.

1 BACKGROUND

Cancer can be defined as a non-communicable disease characterized by uncontrolled cell proliferation. According to the International Agency for Research on Cancer/ World Health Organization (IARC/WHO), cancer is a major cause of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million deaths related to this disease and an expectation of 70% increase in new cancer cases in the coming decades (STRATTON, 2011). Its occurrence depends on multiple factors, among which dietary habits and lifestyle factors play an important role. Its development results from alterations

of cellular DNA which accumulate over time due to the ability of these damaged structures to escape from cellular control mechanisms, such as cell cycle arrest and inhibition of proliferation (FERLAY *et al*., 2012).

Leukemia is a clonal disorder that occurs in a heterogeneous group of haematopoietic progenitor cells which loses their abilities to normally differentiate and respond to normal cellular regulators (ESTEY & DOHNER, 2006). Understanding of chemoresistance mechanisms is an important step in overcoming obstacles to a successfull therapeutic intervention. Many drugs used in cancer therapy have been developed from plants, including plant foods, due to its rich composition on bioactive compounds. Among common foods, berries are known for their nutrients and phytochemicals profile, associated with the reduced risk for various diseases (NILE & PARK, 2014). Berries include blackberries, blueberries, cranberries, strawberries, raspberries, and others such as *jabuticabas (Plinia cauliflora)*.

The benefits of berries can be due to the combined action of micronutrients with the variety of polyphenols already identified (ZHAO, 2007). Polyphenols comprise different classes of compounds, such as those derived from hydroxybenzoic and hydroxycinnamic acids, anthocyanins, proanthocyanins, flavonoids, flavonones, flavonols, flavonones, stilbenes and lignans, all present in different concentrations, in berries. Anthocyanins represent one of major phytochemicals present in most colorful berries and have been suggested as potential chemoprotective agents and a large and growing body of evidence from *in vitro* cell culture studies and *in vivo* animal model tumor systems (STONER & SEERAM, 2011) . Many of these bioactive compounds have shown some protective effect against cancer by induction of metabolic enzymes, gene modulation, chemotherapeutic resistance control, induction of apoptosis, among others, even in leukemia models (SEERAM, 2008). Therefore, the aim of this study is to identify the possible role of berries in chemoprevention and chemoprotection against leukemia through a systematic review of *in vitro, in vivo* and *ex vivo* studies and also to test the *in vitro* effect of ellagic acid, one of the phytochemicals found in berries, on leukemia cells.

2 METHODS

PART 1: SYSTEMATIC REVIEW

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2.1.1 Search strategy and Study Selection

Searches were performed on the electronic databases MEDLINE via PUBMED. A hand-searched was also done in the reference list of relevant publications and those which met the inclusion criteria were included. The initial search date was october 2015 with an update in april 2020, using the search strategy initially defined.

The following keywords, "leukemia" and "berries", and all descriptors were identified by searching the Medical Subject Headings Terms - MeSH Terms - (MeSH). The established terms, synonyms, related and free terms of the search strategy are represented in (**Table, Supplementary material),** and were defined based on the elements of the PICO strategy where P is by definition population and the descriptions are leukemia cells (*in vitro* and *ex vivo* studies), I is by definition interventions and the descriptions are bioactive compounds present in berries, C is by definition control and doesn´t have description and O is by definition outcome and the descriptions are the effects of berries on leukemia. There was no restriction of language or date of publication applied of studies.

To guarantee the quality of the search, the included studies met the following criteria: (1) not being epidemiological and review papers; (2) had used as intervention berries fruits or individual compounds isolated from berries; (3) had being conducted on any kind of leukemia; (4) for the duplicate articles, the newest or most informative one was selected, and (5) articles that did not have enough information in the titles and abstracts were excluded. The search and inclusion criteria analysis were made separately by two authors (Juliana Garcia Borges Fernandes and Ana Luisa Kremer Faller) with no disagreement between them.

2.1.2 Data Extraction

Independently, each author listed the following information from the articles selected: first author's name, publication year, study characteristics, cell type, compounds, doses used and results obtained. Any disagreements were resolved by discussion and the third and fourth researchers (Julia Quarti and Eliane Fialho) were consulted.

PART 2: EXPERIMENTAL

2.1.3 Reagents

All reagents used in this study were of analytical grade. RPMI-1640, β-mercaptoethanol, ellagic Acid, vincristine (VCR), daunorubicin hydrochloride (DNR), dimethylsufoxide (DMSO), and 3,4,5-dimethiazol-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA); penicillin and streptomycin were from Invitrogen (Carlsbad, CA, USA); fetal bovine serum from Cultilab (Campinas, SP, Brazil).

2.1.4 LMC Cell Lines

In this study we used three leukemia cell lines: a chemotherapeutic-sensitive parental cell line, K562 and 2 two MDR cell lines, Lucena-1 and FEPS, that were derived from K562. K562 cells were originated from a patient with blast crisis of chronic myeloid leukemia and are characterized by expressing the chimeric oncogene BCR-ABL on the Philadelphia chromosome (KOEFFLER & GOLDE, 1980).

Lucena-1 and FEPS cells were developed in the Laboratório de Imunologia Tumoral of the Universidade Federal do Rio de Janeiro and were developed by continuous exposure of K562 cells to increasing concentrations of cytotoxic drugs, vincristine sulfate (VCR) for Lucena-1 and daunorubicin hydrochloride (DNR) for FEPS (RUMJANEK *et al*., 2001; DAFLON-YUNES *et al*., 2013). Lucena-1 cells present five times more copies of the MDR1 gene (encoding P-gp) than its K562 parent cell line (RUMJANEK *et al*., 2001). FEPS cells have higher P-gp expression and activity than Lucena-1 and also expresses the multidrug resistance-associated protein (MRP-1) (DAFLON-YUNES *et al*., 2013).

2.1.5 Cell Cultures

K562, Lucena-1 and FEPS cells were maintained in RPMI-1640 medium, pH 7.4, supplemented with 50 mmol/ L β-mercaptoethanol, 25 mmol/ L HEPES, 60 mg/ L penicillin, 100 mg/ L streptomycin and 10% fetal bovine serum. Lucena-1 and FEPS were maintained with 60 nM of VCR and 500 nM of DNR, respectively. To perform the experiments, the drugs were removed for three days from the cells culture. All cells were passaged at a concentration of 2×10^4 cells/ mL every three or four days and kept at 37 °C in a 5% CO_2 humidified environment.

2.1.6 Cytotoxicity assay - MTT

K562, Lucena-1 and FEPS (2×10^4 cells/ mL) were incubated for 72 and 96 hours with ellagic acid (5–25 µM) diluted in 0.25% dimethyl sulfoxide (DMSO). Cell viability was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay. For this, 20 µL of MTT, diluted in Phosphate-Buffered Saline (PBS), were added at a final concentration of 0.5 mg/ mL in each well of a 96-well microplate. The plates were then kept at 37°C, 5% CO_2 for 3 hours. After centrifugation, 200 µL of DMSO were added into the wells in order to dissolve the dark blue crystals formed by the reduction of MTT. The absorbance of the converted dye in living cells was measured at a wavelength of 492 nm. The experiments were performed in triplicate. The IC_{50} values were calculated from dose response curves; the IC_{50} was defined as the concentration of drugs that reduced the number of viable cells to 50% of the control. GraphPad Software 5.0 was used for the IC_{50} calculations.

2.1.7 Statistical Analysis

The results of the experiments were submitted to descriptive statistical analysis, mean and standard error, analysis of variance (ANOVA) followed by Tukey's post-test, using the program GraphPad Prism 5. Results with p values <0.05 were considered statistically significant.

3 RESULTS

PART 1: SYSTEMATIC REVIEW

3.1.1 Literature Search

Two hundred and sixty-three potentially eligible studies were identified initially (263 records in the Pubmed database and 11 by hand-search selection). Of those, most articles (253 records) were excluded after titles and/or abstracts analyses. The reasons for exclusion were studies that used fruits other than berries (230 articles), and that addressed other types of cancer (23 articles). The detailed steps of study selection were shown in

Figure 1.

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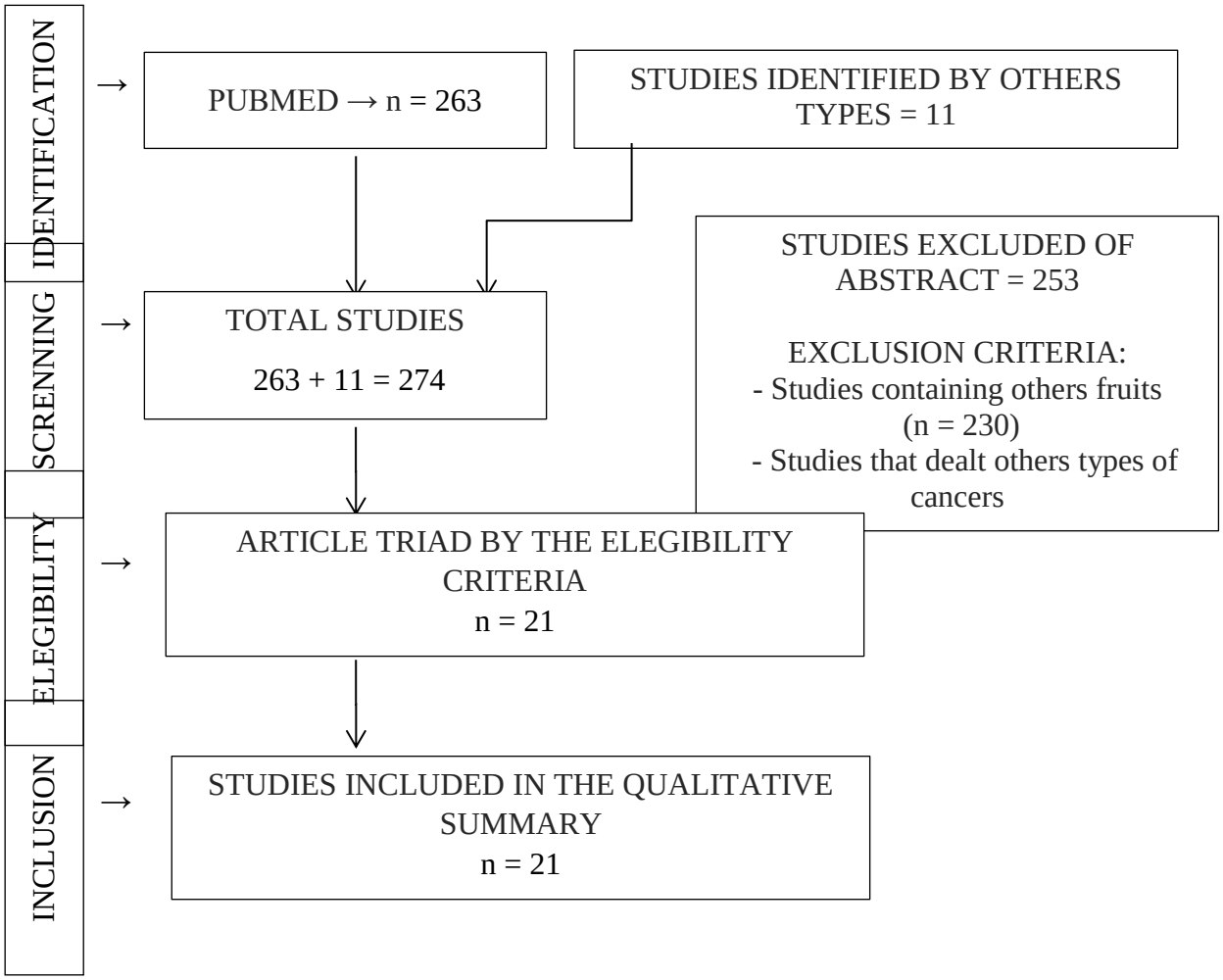

Figure 1: Flow diagram of the search and selection process.

TERMS: (leukemia[mh] OR leukemia[tiab]) AND (redorberries) AND (fruit[mh] OR fruit[tiab] OR anthocyanins[mh] OR anthocyanins[tiab]).

3.1.2 Study Characteristics

The selected studies were published between 2002 and 2018 and were conducted in United States (5), Japan (4), China (2), France (2), Poland (2), Italy (1), Malaysia (1), Republic of Korea (1), Spain (1), The Netherlands (1) and United Kingdom (1). Of the 1 articles selected for this systematic review, 20 were *in vitro* studies and 1 an *ex vivo* study. Among the *in vitro* studies, a wide diversity of cells was used, and both myeloid and lymphoid leukemias which are presented in **Table 1**. Studies included had the use of berries extracts as well as isolated phenolic compounds, such as ellagic acid, resveratrol, quercetin and anthocyanins. The main mechanisms identified were induction of apoptosis and autophagy, modulation of cellular proliferation, antioxidant action and chemoresistance control (**Table 1**).

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Table 1: Systematic Review Articles

AUTHORS	STUDY TYPE	TREATMENT	RESULTS
HAGIWARA *et al.*, 2010	*In vitro* study with Ellagic Acid (EA) and retinoic acid (ATRA) in HL60 acute myeloid leukemia cells.	HL60 cells were treated with 5 to 25 µM of EA for 24 or 96 hours. Besides this, HL60 cells were exposed to 15 - 25 µM of EA in the presence of 0.2 µM or 2 µM of ATRA.	EA inhibited cell growth, induced cell cycle arrest in S-phase and apoptosis associated with caspase 3 activation in HL60 cells in a dose dependent manner. EA also potentiates ATRA induced differentiation in HL60 cells.
DAHLAWI *et al.*, 2013	*In vitro* study with Pomegranate Juice Extracts (PGJE) in lymphocytic leukemia (CCRF - CEM and MOLT3) and myeloid leukemia (HL60 and THP1) cell lines.	CCRF - CEM, MOLT3, HL60, and THP1 cell lines were treated with 6.25%, 12.5% and 25% of PGJE for 24, 48 or 72 hours.	Induction of caspase 3 dependent apoptosis by PGJE in a time and dose dependent manner for all leukemia cell lines. Beside this PGJE promoted S phase arrest at all concentrations.
SHARIF *et al.*, 2010	*In vitro* study with Red Wine Polyphenolic extract (RWPs) in Jurkat cells.	Jurkat cells were treated with 10 - 100 µg/ mL of RWPs for 24 hours.	RWPs inhibited the growth and proliferation of Jurkat cells and induced G0/G1 cell cycle arrest in a concentration-dependent manner. Moreover, RWPs induced caspase 3 dependent apoptosis and promoted ROS accumulation.
GE *et al.*, 2013	*In vitro* study with resveratrol in T-cell acute lymphoblastic leukemia cells (T-ALL, CEM-C7-14, MOLT4, JURKAT and CEM-C7-15).	CEM-C7-14, MOLT4, JURKAT, and CEM-C7-15 cell lines were treated with 200 µM of resveratrol for 24 and 48 hours.	Resveratrol inhibited the growth and induced caspase 3 dependent apoptosis in T-ALL cells in a time and dose dependent manner. It also induced cell cycle arrest in G0/G1 phase by increasing regulation of cyclin-dependent kinase (CDK) inhibitors p21 and p27 and down regulating cyclin A and cyclin D1. Besides this, resveratrol

			induced autophagy in a time dependent manner through inhibiting Akt/ mTOR/ p70S6K/ 4E-BP1 and activating p38-MAPK signaling pathways.
WANG *et al*., 2005	*In vitro* study with Lingonberries anthocyanins in human leukemia HL60 cells.	Human leukemia HL-60 cells were treated with indicated doses of lingonberry (control, 1:160, 1:180, 1:40 and 1:20) extracts for 18 hours.	At dilution of 1:20 of extract of lingoberries was able to induce apoptosis (78% apoptotic cells).
KATSUBE *et al*., 2003	*In vitro* study with berry extracts (low bush blueberry, high bush blueberry, cranberry, raspberry, strawberry, black currant, red currant, blackberry, bilberry and cowberry) and specific anthocyanidins from bilberry (pelargonidin, cyanidin, peonidin, delphinidin and malvidin) in HL60 human promyelocytic leukemia cells.	HL60 cells were treated with 2, 4 or 6 mg/ mL of berry extracts for 24 or 48 hours. This study also determined the growth inhibitory and apoptosis inducing effects of the anthocyanidins (pelargonidin, cyanidin, peonidin, delphinidin and malvidin) at 50, 100 and 200 µM.	Among tested extracts, bilberry was the most effective against HL60 cells by inducing apoptosis. Bilberry extract showed the largest amounts of anthocyanins, wherein 200 µM delphinidin, malvidin or cyanidin were the most capable of inhibiting HL60 cell growth and inducing apoptosis.
SZYMANOWSKA *et al*., 2018	*In vitro* study with raspberry fractions and raspberry juice in J45.01 and HL60 cells.	J45.01 and HL60 cells were treated with crude extracts, anthocyanin-rich fractions and phenolic fractions from raspberry and raspberry juice for 24 hours.	All examined extracts inhibited the viability of J45 cells more effectively than HL60, but the raspberry crude extract showed the greatest cytotoxic effect.

FENG *et al.*, 2007	*In vitro* study with cyanidin-3-rutinoside (C-3-R) extracted and purified from the black raspberry (cultivar Jewel) in human leukemia and lymphoma cell lines HL60 (myeloblastic), MOLT-4 (lymphoblastic), Daudi (lymphoblastic) and CCRF-CEM (lymphoblastic).	HL60, MOLT-4, Daudi and CCRF-CEM were treated with 10-160 µM of C-3-R for 8, 18 and 32 hours.	C-3-R induced caspase-dependent apoptosis in HL60 cells in a time and dose dependent manner. C-3-R also induced apoptosis in others cell lines including MOLT-4, Daudi and CCRF-CEM, but had little toxicity in normal human cells by the MTT assay. Besides this, C-3-R promoted the activation of p38 MAPK and JNK, leading to the ROS accumulation and increasing oxidative stress in HL60 cells.
ASOU *et al.*, 2002	*In vitro* study with resveratrol in myeloid leukemia cell lines (HL60, NB4, U937, THP-1, ML-1 and Kasumi-1) and fresh samples from 17 patients with acute myeloid leukemia.	HL60, NB4, U937, THP-1, ML-1, Kasumi-1 and fresh leukemia cells were treated with 10 - 25 µM of resveratrol for 96 hours. U937 cells were also treated with the combination of $1,25(OH)_2D_3$ (0.1 - 10 nM) and resveratrol (25 µM) or either agent alone for 96 hours. NB4 cells were also treated with the combination of all-*trans*-retinoic acid (10 or 50 nM) and resveratrol (10 µM) or either agent alone for 96 hours.	Resveratrol inhibited the growth and induced death in all cell lines after exposure of 20 µM for 96 hours. In addition, 25 µM of resveratrol for 96 hours promoted typical monocytic differentiation. Besides this, the combination of resveratrol + $1,25(OH)_2D_3$ as well as resveratrol + all-*trans*-retinoic acid had an additive effect on the differentiation of U937 cells and NB4 cells, respectively. Resveratrol (20 µM for 96 hours) also induced differentiation of 8 samples of fresh leukemia cells.
FIMOGNARI *et al.*, 2004	*In vitro* study with cyanidin-3-o-	Jurkat cells treated with 12.5-	In Jurkat cells, 12.5 µg/ mL of Cy-g was sufficient to

	glucopyranoside (Cy-g) in Jurkat and HL60 cell lines.	200 µg/ mL of Cy-g for 24 hours and HL-60 cells treated with Cy-g at the indicated doses for 8 and 30 hours.	increase the number of cells displaying features of apoptosis at 200 µg/ mL. Cy-g-induced apoptosis is associated with significant changes in p53 and bax proteins. When HL-60 cells were exposed to Cy-g for 8 h, recorded a dose-dependent increase in the fraction of apoptotic cells, at 200 mg/ mL: 18% versus 8% in controls. It is noteworthy that HL-60 cells are p53 null. A link might exist between the ability of Cy-g to protect against reactive oxygen species damage and the pro-apoptotic effects observed in this study.
MERTENS-TALCOTT *et al*., 2003	*In vitro* study with ellagic acid and quercetin as single compounds and in combination in human leukemia cells (MOLT4).	MOLT4 cell lines were treated with quercetin (5 or 10 µmol/ L), ellagic acid (5 or 10 µmol/ L) or quercetin + ellagic acid (5 or 10 µmol/ L each) for 12, 24 or 48 hours.	Ellagic acid and quercetin achieve synergistic effects in the reduction of proliferation and viability in MOLT-4 cells. It also promoted cell cycle arrest in G0/G1 and S phases. Besides this, the polyphenols combination induced apoptosis by activation of caspase 3, which was confirmed by an isobolographic analysis for proliferation-reducing effects.
MERTENS-TALCOTT *et al*., 2005	*In vitro* study with ellagic acid, quercetin, and resveratrol as single compounds and in combination in human leukemia cells (MOLT4).	MOLT4 cell lines were treated with 10 µM of ellagic acid, quercetin or resveratrol as single compounds and in combination (10 µM each -	The results showed an additive effect for resveratrol and quercetin combination and for the combination of the three polyphenols, but the combination of ellagic acid with resveratrol exhibited a more than additive interaction. All of the

		ellagic acid + resveratrol; quercetin + resveratrol; ellagic acid + quercetin + resveratrol) for 10, 12, 24 or 48 hours.	double combinations of polyphenols, resulted in a cell cycle arrest in the G0/G1 phase at 12 hours and in an S phase arrest after 24 hours, while the combination of all three polyphenols caused an arrest in the G0/G1 phase after 24 hours, but a synergistic effect of the polyphenols combinations was not observed. Caspase 3 activity assay indicates synergy for either of the combinations (ellagic acid + resveratrol and quercetin + resveratrol), but the combination of quercetin + resveratrol exceeded that of ellagic acid + resveratrol.
SAEDI *et al.*, 2015	*In vitro* study with *Berberis vulgaris* Fruit Crude Extract (BVFCE) in murine myelomonocytic leukemia cell line, WEHI-3.	WEHI-3 cells were treated with BVFCE at 1 to 30 mg/ mL for 24 hours.	BVFCE inhibited WEHI-3 cells proliferation in a dose-dependent manner. All tested concentrations of BVFCE decreased the p53 gene expression of WEHI-3 cells with better results than with chemotherapeutic drug, doxorubicin.
WANG *et al.*, 2012	*In vitro* study with Grape Seed Proanthocyanins (GSPs) in K562 leukemia cells.	K562 cells were exposure to GSP solution at 3.125 to 100 µg/ mL for 72 hours.	At concentrations of 30 µg/ mL, GSPs inhibited K562 cells proliferation and decreased intracelular ROS levels. After treatment with 50 µg/ mL, a typical morphological differentiation was observed and cell cycle arrest in G1 phase at concentrations of 10 µg/ mL and 30 µg/ mL.
GARCIA-ALONSO *et al.*, 2007	*In vitro* study with phenolic-rich juice made from grapes, cherries and berries in human	U937 cells were treated with phenolic-rich juice (10 - 200 µM) for 3 hours.	Pre-incubation of U937 cells with extracts of the phenolic-rich juice partially prevented cell death, abolished the DNA cleavage

	myeloid leukemia cells (U937).		and decreased ROS generation.
WANG *et al*., 2007	*In vitro* study with deerberry fruit extracts in HL60 cells.	HL60 cells were treated with deerberry fruit extracts at 1 to 30 µg/ mL for 1, 18 or 48 hours.	In a dose-dependent manner, deerberry fruit extracts decreased intracellular ROS levels, inhibited cells proliferation and induced apoptosis in HL60 cells.
KATSUZAKI *et al*., 2002	*In vitro* study with anthocyanins isolated from skin of red grape (delphinidin 3-O-beta-D-glucoside, petunidin 3-O-beta-D-glucoside and malvidin 3-O-beta-D-glucoside) in human lymphoid leukemia MOLT4B cells.	MOLT4B cells were treated with anthocyanins isolated from skin of red grape at 0.2 - 1 mM for 72 hours.	Anthocyanins inhibited cell growth in a dose dependent manner. At 1 mM concentration anthocyanins also induced apoptosis and ROS may play an important role in this type of cell death.
SHARIF *et al.*, 2012	*In vitro* study with polyphenol-rich juice from black chokeberry (AMJ) in lymphoblastic leukemia cells (Jurkat, HSB-2, Molt-4 and CCRF-CEM).	Lymphoblastic leukemia cells (Jurkat, HSB-2, Molt-4 and CCRF-CEM) were grown for 24 hours and then they were exposed to AMJ at different concentrations for an additional 24 hours.	AMJ induces in Jurkat cells early and late apoptosis by generating a pro-oxidant signal and triggering mitochondrial membrane potential loss with a subsequent release of cytochrome c. AMJ treatment also induced apoptosis of different human lymphoblastic leukemia cells (HSB-2, Molt-4 and CCRF-CEM), but not in normal lymphocytes.
KWEON *et al*., 2010	*In vitro* study with resveratrol in doxorubicin-resistant acute myeloid leukemia cells (LMA-2/DX30, AML2/DX100 and AML2/DX300).	AML2/DX300 cells were treated with 10 to 100 µM of resveratrol for 24, 48, 72, 96 or 120 hours.	Resveratrol induced cell death in AML2/DX300 cells in a dose and time-dependent manner. Treatment also downregulated the expression of the MRP1 gene and MRP1 protein activity in AML2/DX300 in a dose dependent manner.
SKUPIEN *et al*., 2006	*In vitro* study with berries extracts	HL60, HL60/VINC and	Blueberry extract was the most efficient against HL60

	(blueberry, raspberries and strawberries) in HL60 promyelocytic cell line and its multidrug resistence sublines (HL60/VINC and HL60/DOX).	HL60/DOX cell lines were treated with three berries extracts at concentrations ranging from 0.1 to 2 g/ L for 72 hours.	cells, but presented much lower activity towards resistant cells. In contrast, raspberry and strawberry extract exhibited high cytotoxic activity against both sensitive (HL60) and resistant (HL60/VINC and HL60/DOX) cell types.
WILMS *et al*, 2007	*Ex vivo* study with 168 healthy volunteers (114 females and 54 males) that consumed a blueberry juice with apple.	The volunteers consumed, for 4 weeks, 1 L of blueberry and apple juice (97 mg quercetin and 16 mg ascorbic acid)/ day. Before and after intervention, quiescent peripheral blood lymphocytes were exposed *ex vivo* to oxidative stress and to the food carcinogen benzo[α]pyrene (B[α]P) for 1 and 18 hours, respectively.	The intervention increased plasma antioxidant capacity and protection against oxidative DNA damage.

4 MAIN RESULTS

Cellular apoptosis is an active process of cell death that is important for tumor destruction. Caspases enzymes play an important role in the execution phase. The so-called initiators caspases such as caspases 8 and 9 directly or indirectly activate caspases 3 and 7, responsible for cleaving substrates, including Poly (ADP-ribose) polymerase (PARP), resulting in morphological markers of apoptosis changes (NUNEZ *et al*., 1998).

Myeloid leukemia cells (HL60) incubated with 2 μM of retinoic acid in the presence of ellagic acid (25 μM) for 96 hours inhibited cell growth, induced apoptosis by activation of caspases, and generated a slight accumulation of cells in S phase and reduction in G1 phase (HAGIWARA *et al*., 2010). Acetonitrile fraction of pomegranate juice extracts at concentrations of 6.25%, 12.5% and 25% induced apoptosis in a time and dose dependent

manner in four leukemia cell lines (CCRF-CEM, MOLT3, HL60 and THP1) following 24, 48 and 72 hours incubation by induction of caspase 3 activity (DAHLAWI *et al*., 2013). Similar results with activation of caspase 3 were observed when Jurkat cells were treated with phenolic rich extract (containing catechins, anthocyanins and phenolic acids) obtained from red wine at 100 μg/ mL for 24 hours (SHARIF *et al*., 2010).

Increasing evidences have demonstrated that apoptosis and autophagy may be triggered by common upstream signals and thus affect cancer development and therapy (YANG et al., 2011; WHITE & DIPAOLA, 2009). Autophagy as a type of cellular catabolic degradation response to nutrient starvation or metabolic stress, is considered as a survival mechanism induced in adverse conditions to maintain cell integrity, and it is also involved in a particular mode of cell death called autophagic cell death (MAIURI *et al*., 2007).

Only one study was found on apoptosis and autophagy. Resveratrol inhibited, in a dose and time-dependent manner, the growth of four T-cell acute lymphoblastic leukemia cell lines (T-ALL cells): the glucocorticoid sensitive (CEM-C7-14) and the glucocorticoid resistant (MOLT4, JURKAT, CEM-C7-15) (GE *et al*., 2013). In addition, 200 μM of resveratrol for 48 hours induced apoptosis by decreasing the expression of anti-apoptotic proteins and stimulating pro-apoptotic proteins expression, as well as inducing caspase 3 cleavage, also in a time-dependent manner. Autophagic vacuoles of T-ALL cells were observed under electronic microscopy after resveratrol treatment (GE *et al*., 2013).

Wang *et al*. 2005 found that lingonberry extract induced the apoptosis of human HL-60 leukemia cells in a dose-dependent manner, as indicated by morphology characteristics of apoptosis. Human leukemia HL-60 cells were treated with doses (control, 1:160, 1:180, 1:40 e 1:20) of lingonberry extracts for 18 h. At 1:20 extract concentration was able to induce 78% of cells in apoptosis. This effect may partially be due to its antioxidant properties by perturbing the favorable redox condition in cancer cells and its may be highly effective as a chemopreventive agent that acts by targeting specific oncogenes, such as AP-1 and NF-κB, suppressing cell neoplastic transformation and inducing cancer cell apoptosis.

When evaluating the effect of ten different berries extracts in HL60 leukemia cells, DNA fragmentation was observed, a typical process that indicates apoptosis (KATSUBE *et al*., 2003). Bilberry extract at concentrations of 4 mg/ mL and 6 mg/ mL incubated for 6 hours resulted in the highest decrease of HL60 cells viability, 84% and 88%, respectively. The group also showed that the glycosidic forms of delphinidine and malvinidine, two of the anthocyanins present, were responsible for effects observed in the cell line (KATSUBE

et al., 2003). Crude extract, anthocyanin-rich fractions and phenolic fractions from raspberry and raspberry juice were able to decrease viability of H60 and J45.01 cells, derived from human caucasian promyelocytic leukemia and human acute T cell leukemia, respectively. The J45.01 cell line was more sensitive, the value of EC_{50} was significantly lower (0.35 mg FW/ mL) than that noted for the H60 cell line (0.80 mg FW/ mL) (SZYMANOWSKA *et al*., 2018).

With isolated compounds, Feng *et al*. (2007) demonstrated that 50 μM of cyanidin-3-rutinoside induced apoptosis in approximately 50% of HL60 cells within 18 hours of incubation and almost all cells were apoptotic at concentration of 120 μM. Cyanidin-3-rutinoside also induced apoptosis in other human leukemia/lymphoma cell lines, such as, MOLT4, Daudi and CCRF-CEM cells, but showed little toxicity against normal human cells (FENG *et al*. 2007). In another study, 20 μM of resveratrol inhibited proliferation and induced cell death in six myeloid leukemia cell lines (HL60, NB4, U937, THP-1, ML-1 and Kasumi-1) after exposure for 96 hours. In the same work, 19 bone marrow samples from acute myeloid leukemia patients were obtained from survivors of the Hiroshima atomic bomb. Each sample contained more than 95% leukemic cells, and eight of the nineteen samples reduced NBT (the reduction of NBT within them seems to be correlated with their phagocytic activity) after exposure to the same conditions described above, demonstrating the induction of differentiation of these cells by resveratrol (ASOU *et al*., 2002). Differentiation therapy could be used on leukemia's treatment, since cancer cells can be forced to differentiate and arrest proliferation, thereby controlling their malignant potential (GUTTERIDGE & HALLIWELL, 2000).

One another study with cyanidin-3-0-glycopiranoside (Cy-g) treatment to induce apoptosis in Jurkat and HL-60 cells was conducted. In Jurkat cells, even the lowest concentration tested (12.5 mg/ mL) was sufficient to increase the number of cells displaying features of apoptosis at 200 mg/ mL. When HL-60 cells were exposed to Cy-g for 8h, a dose-dependent increase in the fraction of apoptotic cells were observed. HL-60 cells were, therefore, more resistant to Cy-g-induced apoptosis than Jurkat cells (FIMOGNARI *et al*., 2004). In order to delineate the events leading to apoptosis elicited by Cy-g, the expression of different proteins were analyzed. Jurkat cells were treated for 24 hours with Cy-g 200 mg/ mL and showed a marked increase in p53 and bax protein levels, whereas bcl-2 and c-myc levels were substantially unchanged. The analysis of protein levels in Jurkat cells showed that Cy-g-induced apoptosis is associated with significant changes in p53 and bax proteins,

suggesting that alterations in the levels of these proteins are directly responsible for the death signal delivered by Cy-g. It is noteworthy that HL-60 cells are p53 null. Thus, the induction of apoptosis in this system indicates that Cyg may exert its effects independently of the p53 gene. Cy-g is well known for its antioxidant properties, which have been demonstrated in several cell systems. A link might exist between the ability of Cy-g to protect against reactive oxygen species damage and the pro-apoptotic effects observed in theis study (FIMOGNARI *et al*., 2004).

Isolated compounds and their combinations were used to determine whether these compounds may act in an additive, synergistic or antagonistic manner when inducing apoptosis (MERTENS-TALCOTT *et al*., 2003). To test this hypothesis, MOLT4 cells were incubated with ellagic acid (10 μM), quercetin (10 μM) and the combination of both at the same concentrations for 12, 24 and 48 hours. The association of the two compounds was more potent than the sum of individual effects, suggesting a synergistic mechanism promoting proliferation and viability reduction as well as induction of apoptosis (MERTENS-TALCOTT *et al*., 2003). Similar result was obtained by the same research group when testing ellagic acid (EA) at 68.4 μmol/ L, quercetin (Q) at 12.8 μmol/ L and resveratrol (R) at 54 μmol/ L individually for 24 hours or in combination (EA + Q + R) at a fixed concentration of 10 μmol/ L each for 10 hours (MERTENS-TALCOTT *et al*., 2005).

Another possible chemoprotective mechanism promote by berries is cell cycle arrest, thereby modulating cell proliferation. Saedi *et al*. (2015), demonstrated that the incubation of *Berberis vulgaris* extract (1, 7, and 30 mg/ mL) for 24 hours on WEHI-3 (murine myelomocytic leukemia cells), decreased the expression of p53 gene at all concentrations, with better results than the obtained with doxorubicin, a chemotherapeutic drug (SAEDI *et al*., 2015). This cytoplasmic protein, p53, is known to suppress tumorigenesis by acting on the regulation of G1 phase checkpoints allowing DNA repair or the removal of damaged cells through apoptosis. Mutations in p53 tumor suppressor gene are found in approximately 50% of all human cancers, leading the cell to replicate damaged DNA (HOLLSTEIN *et al*., 2015).

Wang *et al*. (2012) also showed cell cycle arrest on K562 leukemia cells exposed for 72 hours to 30 μg/ mL of a proanthocyanins solution from grape seeds. Grape seeds proanthocyanins reduced proliferation by induction of the cell cycle arrest in the G1 phase, the initial phase of the cycle preceding the S-phase of DNA doublin. Similarly, resveratrol exposure (200 μM for 24 hours) also resulted in induction of cell cycle arrest in G0/G1 phase

and decreased the number of cells in S and G2 phases, indicating cell cycle arrest in four T-cell acute lymphoblastic leukemia cells, both glucocorticoid sensitive (CEM-C7-14) and glucocorticoid resistants (MOLT4, JURKAT, CEM C7-15) types (GE *et al*., 2013). The mechanisms identified for the cell cycle arrest seems to be by the increase in p73 expression (p53 homologous protein) that can block G1 phase and trigger apoptosis when overexpressed (STRANO et al., 2000). In response to DNA damage, p73 is required to trigger the p53-dependent apoptosis mechanism (FLORES et al., 2002).

Oxidative stress may also play a role in many chronic diseases such as cancer. Garcia-Alonso et al. (2007) evaluated the ability of a phenolic-rich juice made from a mixture of grapes, cherries and other berries to protect human myelogenous leukemia cells (U937) from oxidative stress caused by tert-butyl hydroperoxide (tBOOH) (GARCIA-ALONSO *et al*., 2007). The results showed that pre-incubation of cells with the juice extract at 100μM for 3 hours provided protection against cell toxicity, DNA cleavage, and generation of intracellular oxygen reactive species. Deerberry extract also had high free radical scavenging capacity and anti-cancer properties, by inducing apoptosis, in human leukemia HL60 cell lines in a dose-dependent manner (30-120 μg/ mL) (WANG *et al*., 2007). Besides that, the study conducted by Katsuzaki *et al*. (2003) investigated the effects of anthocyanins isolated from skin of red grape (delphinidin 3-O-beta-D-glucoside, petunidin 3-O-beta-D-glucoside and malvidin 3-O-beta-D-glucoside) in human lymphoid leukemia MOLT4B cells. These researchers found that MOLT4B cells treated with anthocyanins showed typical morphological change of apoptosis like apoptotic bodies (1 mM anthocyanins for 72 hours) and fragmentation of genomic DNA after increasing concentrations of these anthocyanins from 0.2 to 1 mM for 72 hours. However, the antioxidant N-acetyl-L-cysteine suppressed the DNA fragmentation caused by anthocyanins, suggesting that reactive oxygen species (ROS) is involved in the induction of apoptosis in this experimental model. Polyphenol-rich black chokeberry (*Aronia melanocarpa)* juice (AMJ) containing predominantly chlorogenic acids, some cyanidin glycosides, and derivatives of quercetin and present a anticancer effect in the acute lymphoblastic leukemia Jurkat cell line, which is deficient for p53. AMJ induces early and late apoptosis by generating a pro-oxidant signal and triggering mitochondrial membrane potential loss with a subsequent release of cytochrome c. AMJ treatment also induced apoptosis of different human lymphoblastic leukemia cells (HSB-2, Molt-4 and CCRF-CEM), but not in normal lymphocytes (SHARIF *et al*., 2012).

Chemoresistance is one of the major obstacles to successful treatment of leukemia. One promising alternative is the identification of chemosensitive agents obtained from foods. This has been shown for some bioactive compounds, such as resveratrol that seems to have the ability to modulate drug-carrying proteins, such as P-glycoprotein (P-gp) and multidrug resistance protein (MRP1) (GUPTA *et al*., 2011). Increased expression of P-gp and MRP1 results in low accumulation of the drug in cells and has been implicated in the development of resistance to a variety of treatments (BARAN et al., 2007). Three cell lines of acute myeloid leukemia resistant to doxorubicin (AML2/DX100, AML2/DX100 and AML2/DX300) were treated with 50 μM of resveratrol for 72 hours. In this study, resveratrol was able to decrease regulation of the MRP1 gene and induce apoptotic death, suggesting that this bioactive compound may facilitate the effectiveness of doxorubicin (KWEON *et al*., 2010). Skupien *et al*. (2006) tested the effect of three different berry extracts (blueberry, raspberries and strawberries) against HL60 and two resistant cell lines (HL60/VINC and HL60/DOX). Among the extracts evaluated, blueberry at 0.240 g/ L for 72 hours was the most efficient against HL60 cells, but showed low activity against resistant cells. In contrast, raspberry (0.130 and 0.772 g/ L) and strawberry (0.133 g/ L and 0.609 g/ L) extracts exhibited high cytotoxic activity against HL60 and resistant cell lines at 72 hours.

The only study conducted in humans indicated a possible chemopreventive effect of blueberry/apple juice intake when evaluating oxidative or genotoxic risk biomarkers. After 4-weeks intervention of 1 L/ day of blueberry and apple juice, equivalent to 16 mg/ L of ascorbic acid and 97 mg/ L of quercetin an increase in plasma concentration of these compounds was observed as well as an anti-genotoxic effect and DNA oxidative damage protection (WILMS *et al*., 2007).

PART 2: EXPERIMENTAL

4.1.1 Main results

One strategy to overcome MDR is to identify compounds which can act selectively on MDR cells, a rare phenomenon known as collateral sensitivity (CS). In our laboratory we have demonstrated this effect with ellagic acid. We performed a cytotoxic assay on leukemia cells such as K562 (sensitive to drugs), Lucena-1 (MDR, expressing P-gp) and FEPS (MDR,

expressing P-gp and MRP1) (KOEFFLER & GOLDE, 1980; RUMJANEK et al., 2001). After 72 hours treatment with 25 µM of ellagic acid, the cytotoxicity was higher on FEPS (28.3 ± 3.3 % of viable cells) followed by Lucena-1 (75.3 ± 5.5% of viable cells) and it was not statistically significant on K562 (**Figure 2A**). The effect of ellagic acid after 96 hours of treatment was even higher on FEPS (26.3 ± 10.3% of viable cells) and Lucena-1 (64.0 ± 7.0% of viable cells) but it was not cytotoxic on K562 cells (**Figure 2B**). Therefore, both in 72 and 96 hours of exposure, ellagic acid showed to have cytotoxic effect on the most resistant cell line, FEPS, with no significant effect on sensitive cell line, K562. The results were confirmed by IC_{50} calculation with higher values for K562 and Lucena-1 cells and lower values for FEPS (**Table 3**). The identification of possible CS promoters could be effective in preventing MDR or making chemotherapy treatment efficient again.

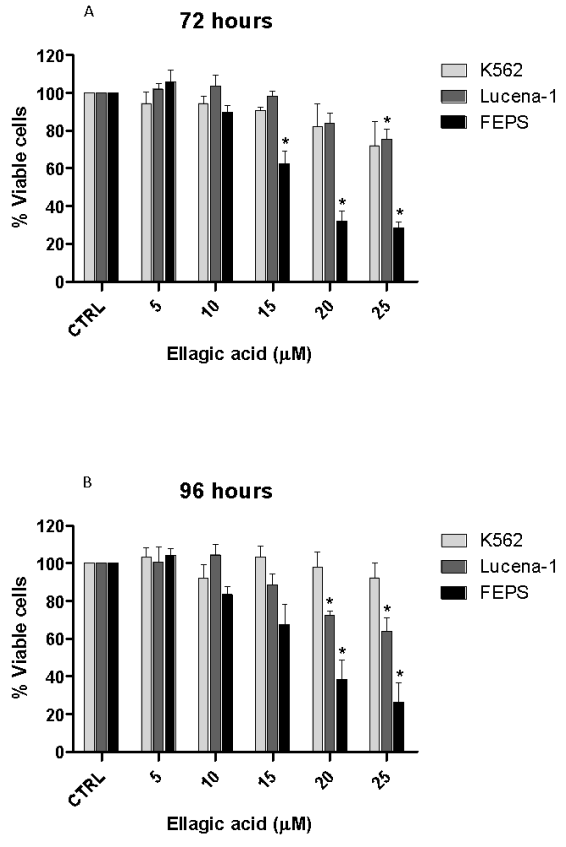

Figure 2: The effects of ellagic acid on the cell viability of K562, Lucena-1 and FEPS cells. Cells were treated with different concentrations of ellagic acid for 72 (A) and 96 hours (B). The cells were plated at the final concentration of 2 x 10^4 cells/ mL. Afterwards, were treated with different concentrations of ellagic acid

dissolved in 0.25% of DMSO or 0.25% of DMSO (CTRL). After treatment, 20 µL of MTT were added, diluted in PBS, at final concentration of 0.5 mg/ mL in each well. The plates were kept in an incubator at 37°C for 3 hours. Posteriorly, the plates were centrifuged at 200 g, for 7 minutes, the supernatant was discarded and the formazan crystals were dissolved in 200 µL of DMSO. The color intensity was measured in a microplate reader, with wave length of 492 nm. The percentage of cell viability was calculated as the ratio of treated cells to control cells. Data represent the mean ± SE of three independent experiments.

Table 3: Cytotoxicity of ellagic acid (IC_{50}) in K562, Lucena-1 and FEPS cell lines. IC_{50} values were calculated from dose response curves.

IC_{50} of ellagic acid (µM)		
Cell line	**72 hours**	**96 hours**
K562	39.9	33.9
Lucena-1	32.3	28.9
FEPS	17.4	17.9

5 CONCLUSIONS

Berries contain a complex mixture of phenolic compounds, such as ellagic acid, resveratrol, quercetin and anthocyanins. *In vitro* studies have shown that these fruits extracts, alone or in combination, as well as their isolated compounds may have a beneficial effect against leukemia. However, both animal models and clinical studies are still limited but crucial to consolidate evidence supporting the beneficial dietary dosage. Although some mechanisms have been identified, such as apoptosis induction, cell cycle arrest and induction of collateral sensitivity, its viability and efficacy in clinical studies are still unknown.

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SUPPLEMENTARY MATERIAL

Table - Conceptual map with the study question: What is the role of red fruits in leukemias?

Terms	DeCS/MeSH*	Synonyms
Leucemia de Células Pilosas	Leucemia de Células Pilosas/Leukemia, Hairy Cell	Reticuloendoteliose Leucêmica, Leukemias Hairy Cell
Leucemia L1210	Leucemia L1210/Leukemia L1210	L1210 Leukemia
Leucemia L5178	Leucemia L5178/Leukemia L5178	Linfoma L5178

Leucemia Linfoide	Leucemia Linfoide/ Leukemia, Lymphoid	Leucemia Linfocítica Leukemias Lymphoid, Lymphocytic Leukemia*, Leukemias Lymphocytic
Leucemia P388	Leucemia P388 /Leukemia P388	Leucemia P388D(1) P388 Leukemia
Leucemia Plasmocitária	Leucemia Plasmocitária	Leucemia Plasmocítica, Leucemia de Plasmócitos
Leucemia Induzida por Radiação*	Leucemia Induzida por Radiação/ Leukemia, Radiation-Induced	Leukemias Radiation-Induced
Leucemia Eritroblástica Aguda	Leucemia Eritroblástica Aguda/ Leukemia, Erythroblastic, Acute	Doença de Di Guglielmo, Mielose Eritrêmica, Eritroleucemia, Leucemia Mieloide Aguda M6 Erythroleukemia
Leucemia Mieloide	Leucemia Mieloide/ Leukemia, Myeloid	Leucemia Granulocítica, Leucemia Mielocítica, Leucemia Mielógena Myeloid Leukemia*, Myelogenous Leukemia*
Leucemia Megacarioblástica Aguda	Leucemia Megacarioblástica Aguda/ Leukemia, Megakaryoblastic, Acute	Leucemia Megacariocítica Aguda, Leucemia Mieloide Aguda M7, Leucemia Megacariocítica Leukemia Myeloid Acute M7, Leukemias Megakaryocytic
Leucemia Monocítica Aguda	Leucemia Monocítica Aguda / Leukemia, Monocytic, Acute	Leucemia Monoblástica Aguda, Leucemia Mieloide Aguda M5 Leucemia Mieloide Tipo Schilling Leukemia Myeloid Acute M5, Monoblastic Leukemias Acute
Linfoma de Burkitt	Linfoma de Burkitt/ BurkittLymphoma	Linfoma Africano, Leucemia de Células de Burkitt, Linfoma-

		Leucemia de Burkitt, Tumor de Burkitt
Leucemia de Mastócitos	Leucemia de Mastócitos/Leukemia, Mast-Cell	Leucemia Mastocitária Leukemias Mast Cell
Leucemia Prolinfocítica Tipo Células B	Leucemia Prolinfocítica Tipo Células B/ Leukemia, Prolymphocytic, B-Cell	Leucemia Prolinfocítica de Células B, Leucemia Prolinfocítica B
Leucemia Linfocítica Granular Grande	Leucemia Linfocítica Granular Grande/ Leukemia, Large Granular Lymphocytic	Leucemia Linfocítica Granular Grande de Células Matadoras Naturais, Leucemia Linfocítica Granular Grande de Células T, Leucemia Linfocítica Granular de Células Grandes Tipo T Leukemia LGL, Leukemia Lymphocytic Large Granular
Leucemia-Linfoma Linfoblástico de Células Precursoras	Leucemia-Linfoma Linfoblástico de Células Precursoras/ Precursor CellLymphoblasticLeukemia-Lymphoma	Leucemia Linfoblástica Aguda de Células T, Leucemia Linfocítica Aguda de Células T, Leucemia Aguda de Células T, Leucemia Linfocítica Aguda Tipo T, T-ALL, Leucemia de Células T Aguda, Linfoma-Leucemia Linfoblástica de Precursor T, Leucemia-Linfoma Linfoblástica de Células T Precursoras, Leucemia-Linfoma Linfoblástico de Células Precursoras-T
Leucemia-Linfoma Linfoblástico de Células T Precursoras	Leucemia-Linfoma Linfoblástico de Células T Precursoras/ Precursor T-CellLymphoblasticLeukemia-Lymphoma	Leucemia Linfoblástica, Leucemia Linfoide Aguda, Leucemia-Linfoma Linfoblástica de Células Precursoras, Linfoma Linfoblástico, Leucemia LinfocíticaAguda LeukemiaLymphoblasticAcute
Leucemia Mieloide Crônica Atípica BCR-ABL Negativa	Leucemia Mieloide Crônica Atípica BCR-ABL Negativa/ Leukemia, Myeloid, Chronic, Atypical, BCR-ABL Negative	Leucemia Mieloide Crônica Atípica, BCR-ABL Negativa, Leucemia Mieloide Crônica Atípica, Leucemia Mieloide Filadélfia-Negativa, Leucemia Mieloide Negativa para Filadélfia, Leucemia Mieloide Filadélfia Negativo Leukemia Myelogenous Ph1-Negative, Leukemia Myeloid Philadelphia Negative
Leucemia Mielomonocítica Juvenil	Leucemia Mielomonocítica Juvenil/ Leukemia, Myelomonocytic, Juvenile	Leucemia Mielógena Crônica Juvenil

Leucemia de Células B	Leucemia de Células B/ Leukemia, B-Cell	Leukemia Lymphocytic Cell B (28031)/Leukemia, B-Cell (15689)
Leucemia Linfocítica Crônica de Células B	Leucemia Linfocítica Crônica de Células B/ Leukemia, Lymphocytic, Chronic, B-Cell	Leucemia Linfocítica Crônica de Células B/ Leukemia Lymphocytic Chronic B-Cell (245)
Leucemia Basofílica Aguda	Leucemia Basofílica Aguda/ Leukemia, Basophilic, Acute	Leukemia Basophilic Acute (196)
Leucemia Eosinofílica Aguda	Leucemia Eosinofílica Aguda/ LeukemiaEosinophilicAcute	Leukemia Eosinophilic Acute(796)
Leucemia-Linfoma de Células T do Adulto	Leucemia-Linfoma de Células T do Adulto/Leukemia-Lymphoma, Adult T-Cell	Leukemia-Lymphoma, Adult T-Cell (796)
Leucemia Aguda Bifenotípica	Leucemia Aguda Bifenotípica/ Leukemia Biphenotypic Acute	Leucemia Bifenotípica aguda/ Leucemia linfocítica aguda de células mistas Leukemia Biphenotypic Acute (553)/ Acute Lymphocytic Leukemia Cells from Mixed (320)
Leucemia Mieloide de Fase Acelerada	Leucemia Mieloide de Fase Acelerada/ Leukemia Myeloid Accelerated Phase	Leucemia Mieloide de Fase Acelerada/ Leucemia Mieloide de Fase Agressiva/ Leucemia Mielógena Crônica de Fase Agressiva/ Leucemia Mieloide Crônica de Fase Acelerada/ Leucemia Mieloide Crônica de Fase Agressiva Leukemia, Myeloid, Accelerated Phase (909)/ Chronic myelogenous leukemia Phase of Aggressive (87)/ Chronic Myeloid Leukemia Accelerated Phase (911)/ Chronic Myeloid Leukemia Phase of Aggressive (114)
Leucemia Mielogênica Crônica BCR-ABL Positiva	Leucemia Mielogênica Crônica BCR-ABL Positiva/	Leucemia Mielogênica Crônica BCR-ABL Positiva/ Leucemia Mielógena Crônica BCR-ABL Positiva/ Leucemia Mieloide Crônica/ Leucemia Crônica Mielocítica Chronic myelogenous leukemia BCR-ABL Positive (539)/ leukemia Myelocytic Chronic (24439)/ Leukemia Myeloid

		Chronic (24163) Leukemia Myelocytic Chronic (24439)/
Leucemia Mieloide de Fase Crônica	Leucemia Mieloide de Fase Crônica/ Leukemia Myeloid Chronic-Phase	Leucemia Mieloide de Fase Crônica/ Leukemia Myeloid Chronic-Phase Leukemia, Myeloid, Chronic-Phase (3607)
Leucemia Mielomonocítica Aguda	Leucemia Mielomonocítica Aguda/ Leukemia Myelomonocytic Acute (3124)	Leucemia Mielomonocítica Aguda/ Leucemia Mieloide Tipo Naegeli Leukemia Myelomonocytic Acute (3124)
Leucemia Mielomonocítica Crônica	Leucemia Mielomonocítica Crônica/Leukemia, Myelomonocytic, Chronic	Leucemia Mielomonocítica Crônica Leukemia, Myelomonocytic, Chronic (2001)
Leucemia Neutrofílica Crônica	Leucemia Neutrofílica Crônica	Leucemia Neutrofílica Crônica Leukemia Neutrophilic Chronic (5)
Leucemia Mieloide Aguda	Leucemia Mieloide Aguda	Leucemia Mieloide Aguda Leukemia Lymphocytic Acute (32899)/ Leukemia Myeloid Acute (51)
Leucemia-Linfoma Linfoblástico de Células Precursoras B	Leucemia-Linfoma Linfoblástico de Células Precursoras B	Leucemia-Linfoma Linfoblástico de Células Precursoras B Precursor B-Cell Lymphoblastic Leukemia-Lymphoma (6)
Leucemia Prolinfocítica de Células T	Leucemia Prolinfocítica de Células T	Leucemia Prolinfocítica de Células T Leukemia Prolymphocytic T-Cell (589)
Leucemia Promielocítica Aguda	Leucemia Promielocítica Aguda	Leucemia Promielocítica Aguda/ Leucemia Mieloide Aguda M3 Leukemia Promyelocytic Acute (6732)/ leukemia myeloid Acute M3 (957)
Leucemia	Leucemia	Leucemia/ Leukemia (179263)/ leukocytosis (7986)

Leucemia de Células T	Leucemia de Células T	Leucemia de Células T / Leukemia, T-Cell(119)/ Leukemia Lymphocytic Cells T (52)/ Lymphocytic (48)
Frutas	Frutas/fruits/t	
Antocianinas	Antocianinas/Anthocyanins	
Ácido elágico	Ácido elágico /Ellagic Acid	
Flavonoides	Flavonoides/Flavonoids	

*** Related terms:** Segunda Neoplasia Primária, Vírus da Leucemia Induzida por Radiação.