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REVIEW

Mitochondria as emerging targets for therapies against T cell acute lymphoblastic leukemia

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Abstract

Acute lymphoblastic leukemia (ALL) comprises a heterogeneous group of hematologic malignancies, arising from diverse genetic alterations in the early lymphocyte development. T-cell subtype of ALL (T-ALL) accounts for about 15% and 25% of ALL in children and adults, respectively. Being less frequent among ALL subtypes, T-ALL represents a high-risk factor for poor prognosis due to its aggressiveness and resistance to common antileukemic drugs. Mitochondria were widely explored recently as a target for anticancer treatment because they are involved in a metabolic reprogramming of a cancer cell and play key roles in reactive oxygen species generation, Ca^{2+} signaling, and cell death induction. Accordingly, a new class of anticancer compounds named mitocans has been developed, which target mitochondria at distinct crucial points to promote their dysfunction and subsequent cell death. The present review analyses the role of mitochondria in malignant reprogramming and emerging therapeutic strategies targeting mitochondria as an "Achilles' heel" in T-ALL, with an emphasis on BH3 mimetics, sequestering pro-survival BCL proteins and voltage-dependent anion channel (VDAC)1-directed drugs, which promote the suppression of aerobic glycolysis, VDAC1 closure, mitochondrial Ca^{2+} overload, stoppage of the oxidative phosphorylation, oxidative stress, and release of proapoptotic factors.

KEYWORDS

apoptosis, calcium, cancer, mitocans, mitochondria, ROS, T cell acute lymphoblastic leukemia

1 | INTRODUCTION

Leukemia is the most common malignancy in children and adolescents worldwide and acute lymphoblastic leukaemia (ALL) is the most common subtype, accounting for 80% of all cases. Pathophysiology of ALL relies on various genetic aberrations, accumulated during lymphocyte maturation, with consequent alterations in the mechanisms controlling cell growth, proliferation, survival, and differentiation. According to the lineage, ALL are divided in two groups: B cell-derived ALL (B-ALL) and T cell-derived ALL (T-ALL). Significant efforts have been made in the management of ALL over the past 50 yr, resulting in the increase of 5-yr survival rates from 10% to approximately 85%.[1] However, considerable number of cases of nonresponsiveness and relapses remains. In this relation, belonging to T-ALL subtype, less frequent, but more aggressive than B-ALL, represents a risk factor for poor prognosis. A high degree of metabolic plasticity of cancer cells as a result of metabolic reprogramming is currently considered to be linked to more aggressive/resistant phenotypes.[2] Understanding of a complex biology and unique metabolic features of T-ALL is required to pinpoint novel therapeutic targets and propose more efficient antileukemic drugs.

Mitochondrial contribution to oncogenic transformation was proposed to include several mechanisms. First, mitochondria determine the bioenergetic profile of a cancer cell through an interplay and flexible switch between aerobic glycolysis and mitochondrial oxidative phosphorilation (OXPHOS), insuring therefore a rapid anabolism. Next, tumor cells possess a characteristic profile of proteins from the Bcl-2 family that interact specifically one with another and with the voltage-dependent anion channel (VDAC) in the outer mitochondrial

Abbreviations: 2-DG, 2-Deoxyglucose; 3-BP, 3-Bromopyruvate; ALL, Acute lymphoblastic leukemia; AMPK, AMP-activated kinase; ANT, Adenine nucleotide transporter; B-ALL, B cell-derived acute lymphoblastic leukemia; BH, Bcl-2 homology domain; Ca^{2+}, Calcium; CLL, Chronic lymphocytic leukemia; Cn, Calcineurin; DN, Double negative; DP, Double positive; ETC, Electron transport chain; ETP, Early T-cell precursor; HK, Hexokinase; IMM, Inner mitochondrial membrane; MCU, Mitochondrial calcium uniporter; MOMP, Mitochondrial outer membrane permeabilization; mPTP, Mitochondrial permeability transition pore; mtDNA, Mitochondrial DNA; OMM, Outer mitochondrial membrane; OXPHOS, Oxidative phosphorilation; PBMC, Peripheral blood mononuclear cells; PD, Pyruvate dehydrogenase; ROS, Reactive oxygen species; T-ALL, T-cell-derived acute lymphoblastic leukemia; TCA, Tricarboxylic acid; UCP2, Uncoupling protein 2; VDAC, Voltage-dependent anion channel; $\Delta\Psi m$, Mitochondrial membrane potential

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membrane (OMM). These interactions insure a high metabolic rate and an antiapoptotic status of tumor cell. Mitochondria also produce reactive oxygen species (ROS), required for the activation of some oncogenic signaling pathways. Finally, ATP and ROS production as well as survival/apoptosis balance are under the control of mitochondrial Ca^{2+} uptake. For further information on the roles of mitochondria in tumorigenesis, an interested reader may consult some excellent reviews.[3–8]

Nowadays, a new class of small membrane-permeable anticancer compounds, targeting and destabilizing mitochondria, termed "mitocans," has been introduced.[9–12] Diverse mitocans act at different points, affecting syntheses of ATP and of mitochondrial DNA (mtDNA), a balance between glycolysis and OXPHOS, ROS production, and scavenging, as well as pro- and antiapoptotic factors expression.[13] Respective processes are altered in cancerous cells, so that mitocans alone or in a combination with conventional anticancer drugs could selectively eliminate malignant cells.

In the present review, we summarize the available data regarding metabolic features and operation of mitochondria in T-ALL as compared to healthy T lymphocytes and discuss the possible use of mitocans for the T-ALL treatment.

2 | MITOCHONDRIA AND VDAC IN MALIGNANT REPROGRAMMING

It is well known that proliferating cells in general, and tumors in particular, preferentially use glycolysis to produce ATP, even under aerobic condition, the phenomenon known as "aerobic glycolysis" or "Warburg effect."[14–16] Since aerobic glycolysis is less efficient for the ATP production than OXPHOS, proliferating cells enhance the glucose uptake and maintain high glycolytic rates for a faster ATP generation. Abundant glycolytic intermediates are accumulated and shunted into de novo synthesis of nucleotides, fatty acids and nonessential amino acids. Simultaneous operation of the tricarboxylic acid (TCA) cycle, localized in the mitochondrial matrix, also provides various intermediates for de novo macromolecular synthesis and insure anabolic metabolism of rapidly proliferating cells. To overcome limited pyruvate availability under aerobic glycolysis conditions, cancer cells use glutamine to replenish the TCA cycle and to drive mitochondrial metabolism. Accordingly, glutamine anaplerosis is one of essential features of the tumoral reprogramming.[17] The coordination between aerobic glycolysis, which takes place in the cytosol, and mitochondrial OXPHOS represents a central challenge in the anabolic cancer metabolism. VDAC, which is localized in the checkpoint between mitochondria and cytosol, plays a crucial role in this coordination. It may be considered as a master key for metabolic and signaling processes in tumor cells.[18,19]

VDAC acts as a principal two-way gatekeeper that mediates metabolic and ionic exchange between mitochondrion and cytosol. It transports multiple metabolites (pyruvate, malate, succinate, NADH, glutamate, ATP, and ADP) and small ions (e.g., Na^+, Ca^{2+}, Cl^-). As for many other porins, VDAC adopts a β-barrel configuration. A β-barrel pore is relatively flexible, so in addition to a fully open state VDAC may adopt multiple lower conductance states, with a dominant one having 40–50% of the maximal conductance. Upon a transition to this substate the pore diameter is decreased from 3 nm to 1.8 nm, so that VDAC becomes impermeable for large metabolites. Simultaneously, positively charged voltage sensor moves outward and converts VDAC into a cation-selective channel with a higher permeability for Ca^{2+} as compared to the fully open state.[18] Interaction of proapoptotic truncated Bid (tBid) with VDAC induces the channel closure to a dominant conductance substate, what may lead to reduced metabolic exchange, enhanced Ca^{2+} uptake, and mitochondrial collapse.[20]

In cancer cells, VDAC may be stabilized in the fully open state via binding of the hexokinase (HK) to its cytosolic edge (Fig. 1). It prevents mitochondrial Ca^{2+} overload and maintains free Ca^{2+} level optimal for metabolic reactions. Both HK I and HK II isoforms demonstrate a high ability for mitochondrial binding, but it is HK II that is overexpressed in most nonbrain tumors. HK catalyzes the first step in glycolysis, glucose phosphorylation, and binding of HK to VDAC regulates the OXPHOS/glycolysis ratio. Specifically, HK gains a direct access to the intramitochondrial ATP, exported via VDAC, resulting in the increased glycolysis rate. VDAC was shown to contribute also to the apoptosis checkpoint by interaction with anti- and proapoptotic proteins of Bcl-2 family. Importantly, VDAC-HK binding prevents further binding of proapoptotic proteins and a formation of permeability transition pore, mPTP[4,21,22] (see Fig. 1).

Recent studies have demonstrated also an extensive integration of VDAC into the intracellular Ca^{2+} signaling and homoeostasis, not only due to its Ca^{2+}-transporting function, but also via the interaction with multiple proteins/components of the cellular Ca^{2+} toolkit.[23] Several proapoptotic factors induce cytosolic Ca^{2+} elevation. This Ca^{2+} increase was proved to cause the VDAC overexpression (via yet unknown mechanism) and oligomerization, which, in turn, mediates cytochrome *c* release and apoptosis.[24] Importantly, VDAC is overexpressed in many cancers.[25] Specifically, VDAC1 was proposed as a prognostic biomarker for B-ALL, as its expression was increased after prednisolone treatment in GC-sensitive cell lines (Sup-B15, RS4;11 and 697), but not in GC-resistant ones (REH).[26]

3 | GLYCOLYSIS AND RESPIRATION BALANCE EACH OTHER IN T-ALL

The T lineage commitment as well as the further T cell maturation are determined by Notch signaling in thymus.[27] However, constitutive activation of Notch1 is considered as an important trigger of T cell leukemogenesis.[28] Indeed, in most clinical samples and cell lines derived from T-ALL patients a permanent up-regulation of this pathway was found.[29,30] Activating Notch1 mutations augment the self-renewal capacity of the leukemia-initiating cells and ensure their susceptibility to accumulate further genetic abnormalities. Notch signaling activates mTOR/Akt pathway, accompanied by Glut1 up-regulation and high level of glucose uptake in maturating thymocytes.[31] But oncogenic hyperactive Notch was shown to be responsible not only for enhanced aerobic glycolysis but also for retained capacity to switch to OXPHOS.[32] This feature may be related to direct Notch1 target, c-Myc.[29,33] In turn, c-Myc directly regulates

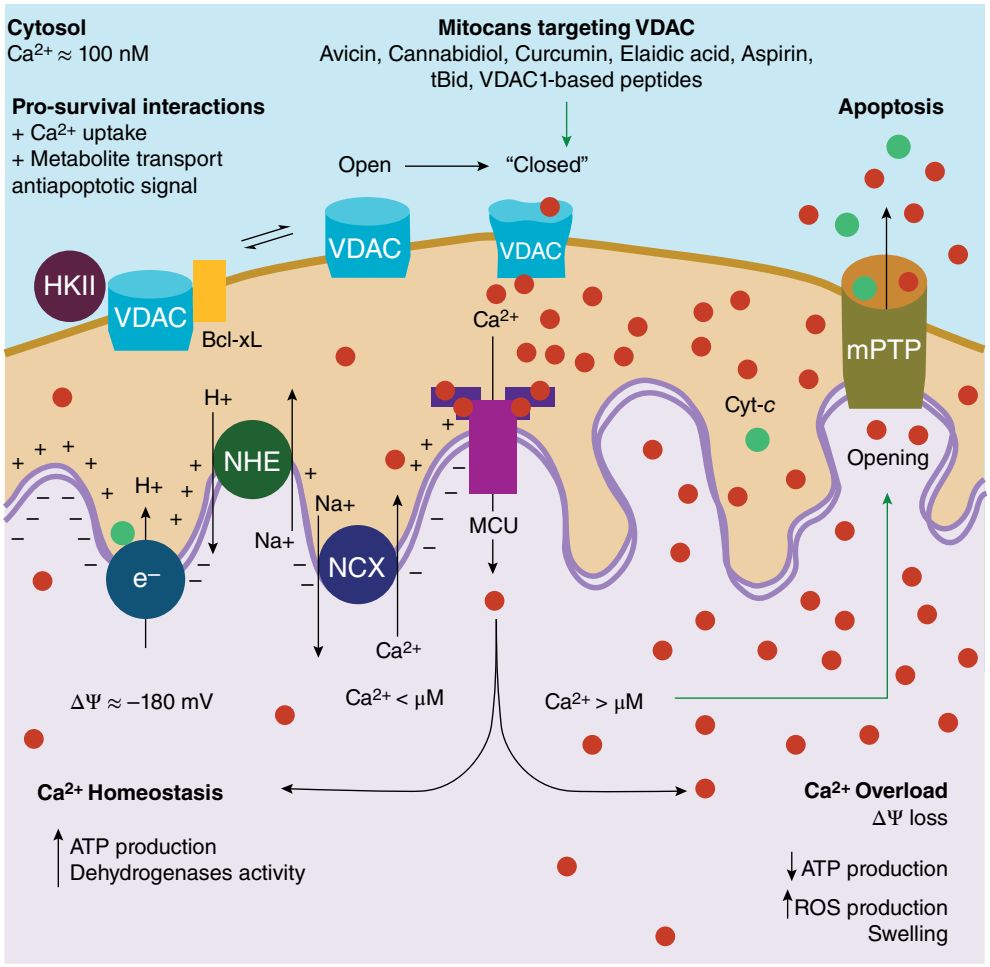

FIGURE 1 **Mitocans targeting VDAC1 cause mitochondrial Ca^{2+} overload and apoptosis.** In homeostatic state (left), due to interaction with HK and Bcl-2 pro-survival proteins, VDAC1 is stabilized in its open state characterized by low permeability for Ca^{2+} but is suitable for active metabolite exchange between mitochondria and cytosol. Operation of the electron (e^-) transport chain in the inner mitochondrial membrane polarizes it, making the matrix side much more negative ($\Delta\Psi$ approx. −180 mV). This implies a large matrix-directed driving force for Ca^{2+}. At resting conditions, Ca^{2+} level is maintained at a submicromolar range by a balance between a strongly limited influx through mitochondrial Ca^{2+} uniporter MCU and active Ca^{2+} extrusion by Na^+/H^+ and Ca^{2+}/Na^+ antiporters.[126,127] Mitocans targeting VDAC1 and VDAC1-related complexes cause change of VDAC1 conformation to the "closed" state highly permeable for Ca^{2+} (right). Consequently, Ca^{2+} level in the intermembrane space rises to the micromolar range, activating MCU of the inner membrane, and causing Ca^{2+} overload in the mitochondrial matrix. The latter provokes the formation of the mitochondrial transition pore (mPTP) through both mitochondrial membranes, resulting in the $\Delta\Psi$ collapse, stoppage of the oxidative phosphorylation, oxidative stress, and the release of soluble compounds including the excess of Ca^{2+} and proapoptotic proteins (cytochrome *c*)

the genes associated with the metabolism of both glucose (e.g., glucose transporter GLUT1, HKII, phosphofructokinase, enolase) and glutamine, allowing a concurrent conversion of glucose to lactate and glutamate oxidation via the TCA cycle (reviewed in Miller et al.[34]). Importantly, c-Myc seems to be responsible for the mitochondrial biogenesis, because over 400 genes, related to mitochondrial metabolism, are identified as c-Myc targets.[7,34,35]

Sometimes activated healthy T cells were suggested as a model for a cancer metabolism, due to their dependence on the aerobic glycolysis and a high proliferation rate.[36] Then the question arises, to which extent the metabolic profile of activated T lymphocytes is similar to or differs from those for T-ALL? There is recent experimental evidence that long-lived T-ALL cells have adapted their metabolic strategies to balance glycolysis and mitochondrial oxidative capacities in a very efficient manner, rather than simply rely on the glycolysis.[37] Using Notch1-dependent T-ALL murine model as well as primary T-ALL clinical samples, these authors have demonstrated that the aerobic glycolysis in T-ALL was less active than in proliferating T cells. Metabolomic analysis demonstrated that T-ALL cells had elevated levels of metabolites, associated with both aerobic glycolysis and TCA cycle, when compared to naïve T cells. The underlying mechanism in Notch1-dependent T-ALL seems to involve a dual effect of the pro-oncogenic Notch1: it promotes the glycolysis, but simultaneously up-regulates the 5′-AMP-activated kinase (AMPK). Subsequently, AMPK is responsible for the restraining of aerobic glycolysis through the mTORC1 inhibition and promotion of the mitochondrial complex I activity. The authors suggested that combination of moderate aerobic glycolysis along with alternative mitochondrial metabolism may favor T-ALL cells to survive and maintain an enhanced proliferative capacity over the long periods.

4 | Bcl-2 PROTEINS PATTERNS IN T-ALL

The balance between survival and cell death plays an important role in T cell biology. Developing thymocytes, which express nonfunctional or autoreactive TCR, are deleted by apoptosis. Expanded clones of effector T cells are also eliminated by apoptosis during the terminal phase of immune response. All antiapoptotic BH1-4 proteins, Bcl-2, Bcl-xL, Mcl-1, and Bcl2A1 (known also as Bfl-1 or A1), are involved in the T lymphocytes development and survival.[38–40]

Primary B- and T-ALL cells overexpress antiapoptotic Bcl-2 proteins as a strategy to suppress apoptosis and promote survival.[41–44] To evade cell death and therapy, ALL may also decrease the expression of proapoptotic proteins (e.g., Bax), what may lead to a more complicated clinical scenario upon chemotherapy.[45,46]

4.1 | BH3 profiling predicts mitochondrial apoptotic priming in leukemic cells

Compared to cancers developed from longeval cell types, hematopoietic malignancies are considered to be "primed" to apoptosis, given a relatively low "buffering" capacity of the antiapoptotic Bcl-2 pool.[47] This buffering is related to the binding of activator BH3-only Bid and Bim to pro-survival Bcl-2 proteins; addition of de-repressor BH3-only proteins will tend to unleash the activators and, eventually, induce the cell death via the induction of mitochondrial outer membrane permeabilization (MOMP).[48] The specificity of buffering depends on the functional repertoire of pro- and antiapoptotic Bcl-2 proteins, which shows a great variability for leukemias.[49,50] To address this issue, then Letai group has proposed a diagnostic method termed "BH3-profiling."[47,51,52] In brief, peptides mimicking diverse BH3-only effector proteins are added in separate to slightly permeabilized leukemic cells, and MOMP is evaluated as a percentage of cytochrome *c* release from mitochondria in each case. This test also shows how close respective leukemic cells are to the apoptosis threshold. BH3 profiling has been applied to ALL primary cells and ALL cell lines.[53–55] By a comparison of the obtained BH3 profile with known binding chart for BH3-only peptides and antiapoptotic Bcl-2 proteins, an accurate prediction may be made on the relative sensitivity to different anti-Bcl-2 drugs. Alternatively, a simplified scheme, using the mimetics for promiscuous BH3-only members may be used, to evaluate at least the degree of priming, hence predicting the relative chemoresistance.[39]

4.2 | The pattern of Bcl-2 pro-survival proteins is changed during T cell maturation

Reciprocal dependence on Bcl-2/Bcl-xL was observed during the double negative (DN) and double positive (DP) phases of T cell maturation. Namely, early DN cells were dependent on the Bcl-2, whereas more mature immature single positive (ISP) and DP thymocytes were Bcl-xL dependent. Thymocytes, which survived both positive and negative selection and became mature CD4+ or CD8+ single positive T cells, are dependent on the Bcl-2 again.[39] Bcl-xL was reported to exhibit a lower affinity to the proapoptotic Bak and Bax proteins as compared to the Bcl-2.[56] Thus, Bcl-2 seems to be more efficient as an antiapoptotic factor. These data are in accordance with requirements of high flexibility of apoptosis regulation during positive and negative selection in the DP thymocyte population.[57]

4.3 | The pattern of Bcl-2 pro-survival proteins in T-ALL is related to correspondent phase of T-cell maturation

To evaluate Bcl-2 and Bcl-xL dependence in T-ALL, BH3 profiling was performed in T-ALL cell lines and primary clinical samples.[39] The LOUCY cell line distinguished by early T-cell precursor (ETP) phenotype, arising from the early DN immature phase, express high Bcl-2, but low Bcl-xL and Mcl-1 levels, with a high Bcl-2/Bcl-xL ratio (Fig. 2). Similar pattern was observed in the most ETP-ALL samples. In contrast, leukemic cells, derived from primary clinical samples of typical (not ETP) T-ALL, demonstrated high Bcl-xL and low Bcl-2 expression, similar to DP thymocytes. Independent studies also reported relatively low Bcl-2 level in newly diagnosed childhood T-ALL when compared to B-ALL.[49,50] Interestingly, cell lines of typical T-ALL, derived from the DP phenotype, express both Bcl-xL and Bcl-2, although the Bcl-xL level is slightly higher. Both, cell lines and patient-derived cells of typical T-ALL express a high level of Mcl-1, which is in contrast to ETP-ALL patient samples and cell lines. The marked variability in expressing patterns of Bcl-2 proteins is of a great clinical relevance: it may be used for diagnostic and risk prediction, and also should be considered for treatments with specific BH3 mimetics. In particular, these findings are important for the resistant ETP subtype, for which novel therapeutic strategies and drug targets are needed.[39]

4.4 | Bcl-2 pro-survival proteins are involved in metabolic pathways in T-ALL

Remarkably, mTOR was shown to form a complex with Bcl-xL and VDAC1 in leukemic T cells, where Bcl-xL serves as a substrate for the mTOR. This complex appears to function as a cellular switch between glycolytic and respiratory metabolism.[58]

In cancer cells and activated lymphocytes, long-lasting glycolytic metabolism seems to stabilize also another antiapoptotic protein, Mcl-1, via the inhibitory phosphorylation of the glycogen synthase kinase 3α and 3β (GSK-3α/β), which otherwise promotes the Mcl-1 degradation.[59,60]

4.5 | BCL2A1 expression is enhanced in T-ALL

During thymocyte maturation, the DN3/DN4 transition phase is characterized by metabolic and proliferative burst. It was observed that the pre-TCR–mediated survival depends on the antiapoptotic multimeric BH1-4 protein A1 rather than on the prototypic antiapoptotic molecules Bcl-2 or Bcl-xL.[38] Notably, in contrast to all other Bcl-2 pro-survival proteins, A1 does not possess conserved calcineurin (Cn) interaction sites within the BH4 region. Thus, A1 is not able to sequester Cn and, as a result, A1 does not handicap Cn-mediated gene expression and cell proliferation. Remarkably, A1 up-regulation was suggested to be important factor in the leukemic

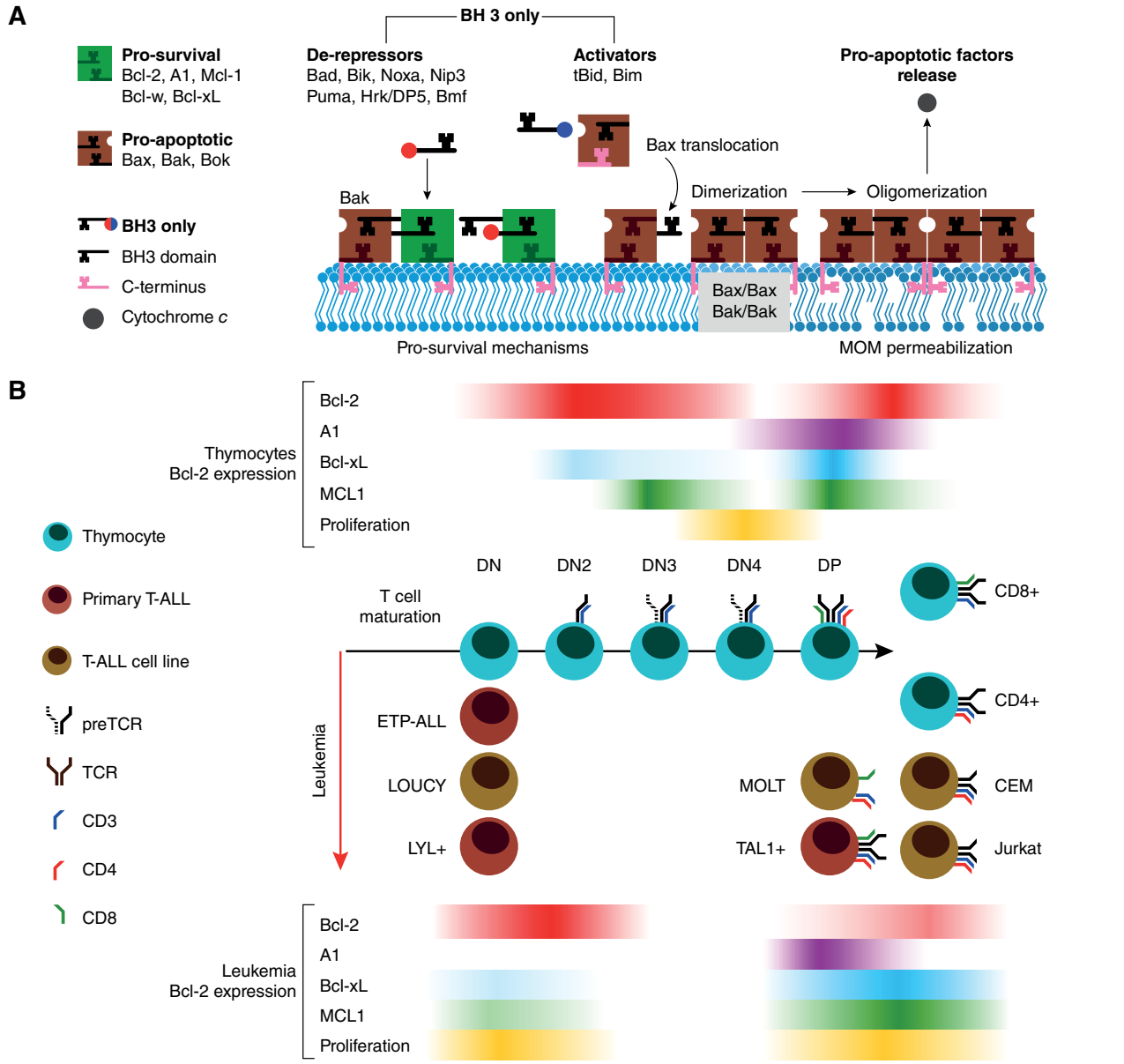

FIGURE 2 **Bcl-2 family proteins' expression profile and apoptosis resistance.** (A) Proapoptotic Bcl-2 proteins, Bak and Bax, by oligomerization form large pores in the outer mitochondrial membrane, which mediate a release of the proapoptotic factors (like cytochrome *c*) from the intermembrane space. Pro-survival BH3 proteins impede the oligomerization by a formation of nonfunctional heterodimers with Bak and Bax or by sequestering activator BH3 domain-only proteins. Another group of BH3 domain-only proteins could "buffer" pro-survival Bcl-2 proteins, thus restringing their activity. BH3 domain is crucial for molecular interactions between all types of Bcl-2 proteins.[128] Each malignancy case may represent a specific profile of the Bcl-2 expression and a balance between proapoptotic and pro-survival activities. Leukemia appears to be highly "primed" cancer type, due to its closeness to the apoptosis threshold. (B) Expression patterns of pro-survival members of Bcl-2 proteins family during the thymocyte maturation (upper panel) and in T-ALL (lower panel)

transformation of pre-TCR cells.[38] In T-ALL, an enhanced A1 expression level was related to more aggressive highly proliferative cases resistant to chemotherapy,[61] and was predominantly associated with the advanced or metastatic stages of the disease.[62]

5 | TARGETING MITOCHONDRIA IN THERAPY OF T-ALL

In relation to above described features of T-ALL, this section summarizes the advances of the usage of mitocans, small molecules targeting mitochondrial functions, as potential drugs for antileukemic therapy, with focus at T-ALL (see also Table 1 for reference).

5.1 | Strategies targeting hexokinase

HK is a classical target for anticancer therapy. Among widely used drugs are a competitive HK inhibitor, glucose analog 2-deoxyglucose (2-DG) and a halogenated analog of pyruvic acid 3-bromopyruvate (3-BP). The anticancer therapeutic efficiency of 2-DG by itself was reported to be poor, but it was able to increase the chemosensitivity of cancer cells.[13] Indeed, both compounds strongly sensitized human

TABLE 1 Mitocans tested in T-ALL models

Mitocan	Targeted structure/ process	Experimental system	Effects	Preclinical stage/FDA approval	References
2-DG	HKII inhibition	T-ALL cell lines: Jurkat, MOLT-4	Cytotoxicity, sensitization to glucocorticoids	NT	63
		Primary ALL cells	Cytotoxicity	NT	
3-BP	HK dissociation	T-ALL cell lines: Jurkat, MOLT-4	Cytotoxicity	NT	63
Methyl-jasmonate		MOLT-4	↓ ATP levels, cell death	NT	129
Avicin	VDAC closure	Jurkat / Mitoplast	↓ ATP levels ↓ Oxygen consumption	NT	70,71
VDAC-based peptides	VDAC interaction	Jurkat, MOLT-4	Cytotoxicity, HK detachment, cytochrome *c* release	NT	69
[a]ABT-737	Bcl-2 family	CCRF-CEM, Molt-3, Molt-4, COG-LL-317	Cytotoxicity, sensitization to chemotherapy, cytochrome *c* release	NT	91
		CEM-c1	Apoptosis		53
ABT-199 / Venetoclax		Primary ALL cells	Cytotoxicity		54
		ALL		Phase 1	[b]NCT03319901
		ALL		Phase 1	[b]NCT03236857
		Relapsed ALL		Phase 1	[b]NCT03181126
[c]ABT-263 / Navitoclax		Primary ALL cells	Cytotoxicity		54
		T-ALL xenograft	↓ Leukemia progression ↑ Survival Sensitization to chemotherapy ↓Tumor size		130
		ALL		Phase 1	[a]NCT03181126
		Relapsed ALL		Phase 2	[a]NCT03504644
Ara-C	Mitochondrial ETC/ROS levels	Jurkat, MOLT-4	Apoptosis ↑ROS production	NT	97
Arsenic trioxide		CEM-C7, CEM-C1, MOLT-4, Jurkat	↑ROS production, cytotoxicity, sensitization to chemotherapy	NT	99
Adaphostin		Jurkat	Caspase activation, apoptosis, ↑ROS production, antiproliferative	NT	131
VE analogs		Jurkat, MOLT-4	Apoptosis $\Delta\Psi m$ loss	NT	107
Resveratrol		Jurkat	Apoptosis	NT	111
Tigecycline		CCRF-CEM, DND-41, MOLT-4	Antiproliferative, apoptosis, sensitization to chemotherapy	NT	112
Menadione		Jurkat	$\Delta\Psi m$ loss, ↑ROS production, caspase activation	NT	117

NT: not tested.
[a]This compound possesses poor aqueous solubility and it is not orally bioavailable.
[b]Clinical trial identifier code (clinicaltrials.gov).
[c]This compound induces a rapid but reversible thrombocytopenia.

T-ALL-derived glucocorticoid (GC)-resistant cell lines Jurkat and Molt4 to prednisolone treatment.[63] Poor sensitivity of human T-ALL to HK inhibitors may be explained by a high plasticity of their metabolism and a possible switch to OXPHOS as a rescue strategy under the conditions of glycolysis inhibition. On the other hand, the glycolytic metabolism seems to be important to escape the GC-related cell death.

The main limitations of 2-DG clinical use are related to metabolic perturbations and occurred when the drug was administered systemically. In particular, glycopenia was generated in the central nervous system by the nonmetabolizable glucose analogue with consequent activation of the alternative metabolic pathways such as lipolysis and glycogenolysis.[64] In dose-dependent manner, 2-DG caused polyphagia, body temperature decrease, and altered water intake in rats[64] and humans.[65] In high doses (2000 mg/kg) 2-DG caused a fall in the blood pressure and respiratory frequency decrease in mice.[66] Phase I clinical trials showed that 45 mg/kg were well tolerated, however, already 60 mg/kg dose was cardiotoxic, promoting QT interval prolongation.[67]

5.2 | Strategies targeting VDAC1-related complexes

The interaction of VDAC1 channel with HK and pro-survival Bcl-2 family proteins ensures an efficient anabolic metabolism and prevents

the apoptosis of tumor cells. VDAC1 domains, responsible for these interactions, were identified and peptides with amino acid sequences identical to those domains were designed.[68] Resulting cell-penetrating VDAC-1 based peptides were shown to trap HK I/II, Bcl-2, and Bcl-xL, preventing corresponding interactions and causing cell death in tumors. The mechanism was shown to involve the mitochondria-bound HK detachment, a significant decrease of cellular ATP levels, cytosolic Ca^{2+} rise, VDAC1 oligomerization, and cytochrome *c* release. Tumors of different histogenesis demonstrated a differential sensitivity to these peptides.[69] In studies using N-Ter-Antp and Antp-LP4 peptides, 41 cancerogenic cell lines were divided into three groups, according to their sensitivity: highly sensitive (EC_{50} = 0.9-4.5μM), sensitive (EC_{50} = 6.5-10μM), and less sensitive (EC_{50} > 18μM) tumors. Notably, GC-resistant Molt-4 and Jurkat T-ALL cell lines showed an extremely high sensitivity to these peptides. Importantly, PBMC derived from healthy volunteers were significantly less sensitive to the treatment.[68] These authors also announced the development of optimized VDAC1-related peptides with improved tumor selectivity, due to the inserted transferrin receptor internalization sequence (Tf). It was reported that VDAC-based Tf peptides did not cause cell death in noncancerous cells; they were tested on some cancer cell lines, but not yet on T-ALL.[69] Accordingly VDAC1-based peptides may be considered as a valid perspective for T-ALL treatment.

Using xenograft mouse models of human glioblastoma, breast and lung adenocarcinoma, it was demonstrated that optimized R-Tf-D-LP4 peptides alter the expression levels of important metabolic enzymes and transporters, such as glucose transporter (Glut-1), HK-I, glyceraldehyde dehydrogenase (GAPDH), VDAC1, and citrate synthase in cancer cells.[69] Taking into the account the importance of these proteins for cell metabolism in general, a potentially harmful effect of R-Tf-D-LP4 on healthy cells and tissues may not be excluded at this moment. Thus, to propose VDAC-based peptides for clinical trials, their possible toxic effects should be first studied in more detail on animal models.

In addition, a variety of small molecules exert their proapoptotic activity by their interaction with VDAC.[70] Of particular interest are avicin,[71] cannabidiol,[72] curcumin, elaidic acid, and aspirin[73–75] which all were proved to provoke the VDAC closure. A simplest explanation of their proapoptotic action, then, may be the block of metabolic exchange between mitochondria and cytosol. Additional, nonexclusive explanation is based on the Ca^{2+}-permeable nature of the VDAC "closed state."[76] Thus, locking VDAC in the "closed" state would facilitate the mitochondrial Ca^{2+} uptake and overload, leading to the formation of the mPTP and induction of apoptosis. Aspirin and curcumin were shown to induce apoptosis in some leukemic cell lines,[70] but these or similar VDAC-interacting small compounds have not been tested yet for any T-ALL model.

5.3 | Bcl-2 targeting compounds

The first synthetic BH3 mimetic compound, ABT-737, was shown to bind Bcl-2, Bcl-xL, and Bcl-w, but displayed a low affinity and weak inhibitory effect against Mcl-1 and A1.[48,77] As mentioned before, the latter are often overexpressed in hematopoietic malignancies, in particular in T-ALL. The effectiveness of ABT-737 as a single agent was tested on ALL cell lines, where CEM line derived from T-ALL was significantly more resistant to treatment than B-ALL cell lines.[53] In this regard, studies with use of different hematopoietic malignances evidenced that the up-regulation of A1 may cause ABT-737 resistance. So, resistance to ABT-737 in chronic lymphocytic leukemia (CLL) was related to concurrent up-regulation of Bcl-xL and A1 in culture conditions mimicking lymph node microenvironment.[78] In another work, B lymphomas initially sensitive to ABT-737 after long-term exposure to treatment became resistant to this drug due to MCL-1 and A1 up-regulation.[79]

Because of the low solubility of ABT-737, for clinical applications two derivatives were synthesized: ABT-263 (navitoclax) and ABT-199 (venetoclax), with activity against Bcl-2, Bcl-xL, and Bcl-w or selective for Bcl-2, respectively. Both compounds have shown high efficiency to suppress ALL in vitro, especially, primary ALL cell lines.[54] Regretfully, ABT-263 has a pronounced side effect: it provokes thrombocytopenia due to BCl-xL dependence of platelets.[80] So, up to now, ABT-199 is the only BH3 mimetic to be approved by FDA for CLL clinical treatments.[81] Bcl-2 is dominated over Bcl-xL in T-ALL derived from ETP (ETP-ALL), with an opposite situation at the more late maturation stages (DP, Fig. 2B). Consequently, preclinical studies demonstrated that ABT-199 treatment was very efficient in ETP-ALL.[39,82] In general, in contrast to CLL, which solely depends on the Bcl-2, an efficient treatment of typical (non-ETP) T-ALL seems to require a concurrent inhibition of Bcl-xL, Mcl-1, and A1. An exception is the case of MLL-rearranged ALL, which mainly relies on the Bcl-2 overexpression and is efficiently suppressed by ABT-199.[55,83] An interesting example of the BH3 mimetics is represented by α-tocopheryl succinate, which not only targets Bcl-2 and Bcl-xL, but also has a second site for its proapoptotic activity, namely, ubiquinone binding site in the ETC.[10,84]

Direct and specific inhibitors against Mcl-1 are so far lacking. The progress in the search of small compounds, selective against Mcl-1, is summarized in recently published reports.[81,85] Mcl-1 plays a central role in chemoresistance to anti-tubulin agents as vincristine. Mcl-1 stabilization in T-ALL was shown to be dependent on the defect in Fbw7, which is the substrate binding component of the ubiquitin ligase complex. Once phosphorylated by a specific glycogen synthase kinase-3, GSK-3 (AKT pathway), Mcl-1 is polyubiquitylated by the ligase complex, thus, marked for a further rapid proteosomal degradation, underlying a short life of Mcl-1 in healthy tissues.[86,87] Sorafemib, a multi-kinase inhibitor, in vitro suppressed T-ALL, resistant to Bcl-2 antagonist by inhibiting MAPK and/or activating GSK-3.[86] Thus, promising therapeutic strategies may be targeted to up-stream elements, regulating the Bcl-2 family proteins expression/recycling, in particular, to the kinases machinery.[81] Mcl-1 is up-regulated via PI3K/AKT/mTOR pathway. Thus, a combined inhibition of the anti-apoptotic Bcl-2 proteins and PI3K/AKT/mTOR, for example, by known catalytic mTOR inhibitors, acts synergistically for the induction of apoptosis in diverse cancers.[88] Dual inhibition of the PI3K/mTOR by a small synthetic compound P-103 suppressed T-ALL,[89,90] but we are not aware of the combined treatment with inhibitors of PI3K/AKT/mTOR and BH3 mimetics on ALL models. Use of BH3 mimetics combined with other anticancer agents increases B- and T-ALL sensibility to vincristine, dexamethasone, and L-asparaginase

in vitro and in vivo.[91] Finally, therapeutic approaches leading to the overexpression of proapoptotic Bcl-2 proteins may be equally promising. It was shown, for instance, that dexamethasone up-regulates Bim in ALL, whereas Bim is inactivated by extracellular signal-regulated kinase, ERK. Thus, joint treatment with dexamethasone and ERK inhibitors promoted apoptosis in a synergistic manner in ALL cell lines and primary cultures.[92]

5.4 | Antioxidant suppression or ROS induction. Mitocans targeting electron transfer chain

It is widely accepted that cancer cells possess a higher intrinsic level of ROS, and leukemic cells are not the exception.[93–95] Mitochondrial electron-transfer chain appears to be a primary source for ROS in leukemic cells. Increased ROS production in primary ALL cell cultures is only partly compensated by a concomitantly increased activity of the majority of antioxidant system components.[94] Elevated ROS levels in cancer cells favor ROS signaling, cell proliferation, genetic instability, mutations acquisition, and drug resistance.[96] However, excessive ROS levels lead to cell death. An attractive idea, thus, is a pro-oxidant treatment, either via induction of extra-ROS production or by inhibition of the antioxidants, to cross the threshold for the execution of cell death scenario. Given intrinsically higher ROS levels and exhaustion of the antioxidant system, such a treatment could be selective for leukemic cells vs. noncancer ones. Both approaches, either the induction of ROS by cytabarine,[97] arsenic trioxide[98–100] and adaphostin,[101] or glutathione depletion[102] and SOD suppression[103] by phenethyl isothiocyanate or 2-methoxyestradiol, respectively, were able to induce cell death in diverse B- and T-ALL cell lines or sensitize them to chemotherapy.

Aforementioned pro-oxidant treatments are not necessarily targeting ROS of mitochondrial origin or respective evidence is lacking. In the case of adaphostin, a synthetic dihydroquinone derivative, it was demonstrated that its ROS-inducing activity decreased by more than 75% in a mutant rho° cells, lacking ETC complexes, as compared to a parental MOLT-4 T-ALL cell line; a more detailed analysis revealed that adaphostin did not affect complexes I and II activity, but the cytochrome *c* reduction by complex III.[104] As_2O_3 apparently has multiple targets, but, among them, it has been shown to provoke a leak of electrons from the mitochondrial ETC.[105] There are additional anticancer drugs, whose targets within the ETC are more certain. Metformin is one of the promising anti-ALL drugs at the stage of clinical trials.[106] It has several different modes of action, but one of the principal ones is due to its inhibitory action on the complex I of mitochondria.[13] Complexes I and II transport electrons from the TCA to complex III. Interruption of the ETC at this level causes an increased leak of electrons to oxygen. Consequently, vitamin E analogues, epitomized with succinate (alpha-tocopheryl, α-TOS, and its derivatives), which inhibit complex I and/or II, provoke oxidative stress and apoptosis in different cancers, including a T-ALL model, Jurkat cells.[13,107–109]

Specificity and efficiency of mitocans, targeted to ETC, may be enhanced by chemical modification, favoring their intramitochondrial accumulation. A common way is their linkage to a membrane permeable cation, triphenylphosphonium (TPP^+), which tends to localize mitocans at the interface between mitochondrial matrix and inner mitochondrial membrane (IMM), thus, improving their contact to target-ETC complexes and the overall anticancer efficiency.[13] For example, the mitochondrial-targeted mitoVES is up to 50 times more efficient apoptosis inducer in Jurkat cells as compared to the original compound.[110] Mitochondrial-targeted resveratrol, a polyphenol compound of plant origin, primarily inhibits complexes I and III and acts as a pro-oxidant, hence, provoking death of Jurkat cells.[111] Last but not the least: mitochondrial respiration in general may be attacked by a restriction of the mitochondrial ETC proteins biosynthesis. Thus, tigecycline, a broad-spectrum antibiotic, which reduces biogenesis of mitochondria by causing energy crisis and enhanced ROS production, was shown to be a strong and selective (vs. healthy counterpart cells) suppressor of leukemic cells, in particular, ALL.[112] Recently, Wang and coworkers demonstrated a very peculiar tactics of T-ALL cells, which were able to preferentially export their mitochondria to stromal cells via nanotubes, thus, preventing the excessive mitoROS production in source cells in a response to chemotherapy.[113] In general, a protective role of stromal cells is a very essential factor for chemoresistance and needs to be considered and targeted in anticancer treatments trials.

5.5 | Other mitochondrial associated strategies

As already mentioned, the OXPHOS in cancer cells may be suppressed by the interference with the mtDNA translation, which precludes the biosynthesis of the ETC complexes. Similar effect may be achieved at the mtDNA transcriptional stage. Vitamin K3 (menadione) called clinical attention as it exhibits anticancer effects. Menadione was proved to be a selective DNA polymerase γ inhibitor.[114] This mitochondria-specific polymerase is essential for the mtDNA replication and repair.[115] When exposed to menadione, T-ALL cells exhibited mitochondrial dysfunction, $\Delta\Psi$m loss, nuclear fragmentation, and increased ROS production.[116,117] On the other hand, high doses of menadione may be toxic for healthy cells due to DNA damage.[118] It was reported to cause lesions in the kidney, liver, lungs, and heart of rats at doses as high as 100 mg/kg.[119]

Uncoupling of the respiration from the OXPHOS or, in other words, the ETC operation from the ATP synthesis by mitochondria, via dissipation of the $\Delta\Psi$, may have a dual effect. Whereas some artificial uncouplers sensitize ALL to chemotherapy,[120,121] the up-regulation of the intrinsic uncoupling protein 2, UCP2, observed in leukemias, has an antiapoptotic effect. This increase of the UCP2 activity is rapidly induced by a microenvironment (mesenchymal cells). It promotes a metabolic switch (Warburg effect) and down-regulates mitoROS production, thus, underlying the chemoresistance.[122] Thus, a suppression or modification, for example, via induced glutathionylation,[123] of the UCP2 may be employed for the anticancer therapy.

Mitocans targeting TCA (e.g., dichloroacetate, inhibitor of pyruvate dehydrogenase (PD) kinase, which is a negative regulator of PD and restricts the metabolic flux to TCA) were successfully applied for myeloid leukemia treatments,[13] but we are not aware of their testing against ALL.

Compounds, targeting mitochondrial ANT, could suppress cancer cells in two ways: by the inhibition of the ADP/ATP exchange across

the IMM and by the induction of the mPTP formation (hence, apoptosis). Among such anticancer drugs, a thiol cross-linking reagent 4-(N-(S-glutathionylacetyl)amino)phenylarsenoxide, which targets two cysteine residues in the ANT, or nonhydrolysable adenine-containing metabolite clodronate, a competitive ANT inhibitor, may be given as examples,[6] but their potential against ALL is not yet revealed.

Finally, inhibition of the autophagy (mitophagy), which aggravates the mitochondrial damage and potentiating mitoROS production and mitochondria-mediated apoptosis, sensitizes ALL to anticancer chemotherapy.[124,125]

6 | CONCLUDING REMARKS

Mitochondria are profoundly involved into the neoplastic reprogramming in T-ALL. Respective alterations include high metabolic plasticity and flexible switch between OXPHOS to aerobic glycolysis, an increased intracellular ROS level, and characteristic pattern of Bcl-2 family proteins. For the latter, leukemias represent an extreme case, coming close to the apoptosis "threshold," which makes them an attractive target for anticancer therapy based on the BH3 mimetics and/or manipulation with signaling cascades, regulating the expression of distinct pro-survival and proapoptotic Bcl-2 proteins. In case of the CLL the progress is the most advanced as BH3 mimetic venetoplax already achieved a clinical approval. It is to be expected that intense BH3-profiling of primary ALL in combination with the respective drug development will lead to successful therapies in the near future. In particular, targeting Mcl-1 and A1 needs to be resolved, to yield efficient pharmacologic tools against ALL. Induction of the oxidative stress by compounds, targeting the mitochondrial ETC in ALL should be another winning strategy. Special attention should be given to "double agents," acting as BH3-mimetics and on the quinone-binding sites of the ETC, α-TOS, and its derivatives, chemically modified to promote their mitochondrial accumulation. VDAC1-related therapeutic strategies should also be explored in their application to T-ALL treatments. VDAC1-based peptides are of special interest, because they demonstrated high efficiency against T-ALL GC-resistant cell lines Jurkat and Molt-4.[69] It would be important to test these peptides as well as their optimized modification against clinical samples to be recommended for clinical trials. Another promising group of VDAC-interacting drugs are small molecules of diverse chemical nature, which dock within the channel pore, stabilizing the VDAC highly Ca^{2+}-permeable "closed" state, thus promoting Ca^{2+} overload, mPTP formation, and apoptosis induction. In all cases, of a good choice would be a synergistic combination of differently targeted mitocans and/or mitocans with other anti-cancer drugs (e.g., glucocorticoids), thus, increasing the sensitivity to the latter.

AUTHORSHIP

All the authors equally contributed to this work.

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DISCLOSURES

The authors declare no conflicts of interest.

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