REVIEW

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New insights into redox homeostasis as a therapeutic target in B-cell malignancies

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Purpose of review
The goal of this review is to summarize recent advances in our understanding of the regulation of redox homeostasis and the subtype-specific role of antioxidant enzymes in B-cell-derived malignancies. Furthermore, it presents selected prooxidative therapeutic strategies against B-cell neoplasms.

Recent findings
Recent reports have shown that the disturbed redox homeostasis in B-cell malignancies is regulated by cancer-specific signaling pathways and therefore varies between the individual subtypes. For instance, in a subtype of diffuse large B-cell lymphoma with increased oxidative phosphorylation, elevated reactive oxygen species are accompanied by higher levels of thioredoxin and glutathione and inhibition of either of these systems is selectively toxic to this subtype. In addition, growing number of small molecule inhibitors targeting antioxidant enzymes, such as auranofin, SK053, adenanthin, or decreasing glutathione level, such as imexon, buthionine sulfoximine, and L-cysteinase, trigger specific cytotoxic effects against B-cell malignancies. Lastly, attention is drawn to recent reports of effective treatment modalities involving prooxidative agents and interfering with redox homeostasis provided by stromal cells.

Summary
Recent findings reveal important differences in redox homeostasis within the distinct subsets of B-cell-derived malignancies that can be therapeutically exploited to improve existing treatment and to overcome drug resistance.

Keywords
antioxidant enzymes, B cell, leukemia, lymphoma, reactive oxygen species

INTRODUCTION

Constant generation of reactive oxygen species (ROS) is a natural biochemical event in the life of all aerobic organisms. Data accumulated in recent years suggest that cancer cells, including certain subtypes of B-cell malignancies, generate higher levels of ROS mainly because of metabolic dysregulation. As ROS are potentially mutagenic, a tentative strategy was to use antioxidant compounds in hope to prevent carcinogenesis and delay cancer progression. However, this approach has been largely disappointing [1]. The interplay between ROS and cancer was proved far more complex and multifaceted than expected. ROS appeared to be not solely an end waste product of cellular metabolism, but an important oncogenic signaling second messenger and mediator of drug resistance. In line with these findings, tumors in patients with advanced disease and poor prognosis have dysregulated expression of certain ROS-metabolizing enzymes and exhibit prooxidant phenotype [2,3]. Thus, a simple antioxidant treatment of advanced tumors may actually help cancer cells to cope with oxidative stress conditions and progress more rapidly [4,5].

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KEY POINTS

- Certain B-cell malignancies are exposed to higher levels of ROS and have deregulated redox homeostasis but the underlying mechanisms are different in specific subtypes of the disease and depend on cancer-specific signaling pathways.
- Stromal cells aid malignant B cells survival by supporting the redox stress management; therefore, targeting the stromal support is essential to gain clinical efficacy.
- Prooxidative monotherapies involving inhibitors of single antioxidant enzymes show limited clinical efficacy because of the overlapping functions of these enzymes and stromal support.
- Therapies involving various prooxidative agents alone or in combination with classical chemotherapeutics or SMAC mimetics, show remarkable efficacy in various *in vitro* and *in vivo* setups in CLL, Burkitt lymphoma, DLBCL, and B-ALL.

In recent years, the new hope has been placed into using targeted and synergism-based prooxidant therapies to push cancer cells toward death. An increasing evidence suggest that this kind of approach seems especially promising in B-cell malignancies [6,7▪▪].

Malignant B cells have higher ROS levels compared with normal counterparts because of altered metabolism [8,9▪▪] and oncogenic signaling, such as MYC proto-oncogene [10] or BCR-ABL1 fusion protein. [11]. Moderate ROS levels, via oxidative damage to DNA, lead to genomic instability and facilitate tumor progression [12]. In addition, elevated ROS can react with sulfur-containing amino acids side chains, leading to oxidation of sulfhydryl groups and inactivation of cellular phosphatases. This favors B-cell receptor (BCR) and oncogenic kinase signaling, and thereby promotes malignant B-cell survival [13]. However, in contrast to tumor-supporting role of moderate ROS levels, the excessive production of ROS induces damage to cellular biomolecules and organelles and leads to cell death. Thus, eliminating or inhibiting cellular mechanisms that neutralize and maintain 'safe' ROS levels might actually shift the balance of protumorigenic ROS activity toward cancer cell death. Experimental therapeutic approaches, following this concept, have been investigated for at least a decade. However, these studies are only beginning to elucidate specific redox targets, or their combinations, in distinct subtypes of B-cell neoplasms. Malignant cells utilize several, to some extent redundant, antioxidant systems, including low-molecular-weight compounds, for example, glutathione (GSH), and antioxidant enzymes, such as superoxide dismutases (SODs), catalases (CAT), peroxiredoxins (PRDXs), and thioredoxin (TRX) system (Table 1, Fig. 1, reviewed extensively in [22–24]). It is now being recognized that exaggerated antioxidant response, although quite commonly occurring in many types of B-cell cancers, is regulated by cancer-specific signaling pathways and, therefore, is distinct in individual subtypes of the disease. Herein, we summarize the current knowledge on redox homeostasis in malignant B cells and present selected prooxidative strategies to treat B-cell cancers.

REDOX SIGNATURES IN B-CELL MALIGNANCIES

Genes controlling the redox homeostasis are important players in the pathogenesis of multiple types of cancer. Consistent with the hypothesis suggesting the causative role of ROS in cancer initiation, single-nucleotide polymorphisms of certain genes involved in the production and detoxification of ROS significantly increase the risk of diffuse large B-cell lymphoma (DLBCL) development [25,26]. In addition, in patients with established disease, single-nucleotide polymorphisms within the myloperoxidase gene are associated with shorter overall survival and progression-free survival [27]. In accordance with this, DLBCL patients with the worst prognosis exhibit decreased expression of SOD2 and vitamin D-upregulated protein (VDUP1, a protein that inhibits TRX activity), but increased TRX expression [3].

Although these earlier results clearly highlight the pathogenic relevance of redox homeostasis in the biological and clinical characteristics of the disease, these studies do not take into account the inherent molecular heterogeneity of the disease [28,29]. For example, a large subset of DLBCLs is reliant on BCR activity ('BCR'-DLBCLs). Additional subset of tumors that do not exhibit addiction to BCR signaling features transcriptional program associated with increased oxidative phosphorylation ('OxPhos'–DLBCLs) [29]. Energetic metabolism of these DLBCLs is fueled predominantly by palmitate β-oxidation and oxidative phosphorylation of palmitate-derived carbons [30]. Accordingly, 'OxPhos'-DLBCLs overexpress multiple components of mitochondrial electron transport chain (subunits of complex I, II, and V) that increase mitochondrial superoxide production. The mechanism to counteract this burden potentially relies on overexpression of multiple enzymes involved in ROS detoxification, for example, SOD2 and TRX [9▪▪,30]. Efficient clearance of ROS would also ensure that oxidative phosphorylation could remain elevated in 'OxPhos'-DLBCLs. Consistent

Table 1. Major families of antioxidant enzymes in cells

Function	Enzyme	Isoforms and localization	References
Dismutation of superoxide	Superoxide dismutase	SOD1 (c) SOD2 (m) SOD3 (e)	[14]
Reduction of H_2O_2 and/or peroxides	Catalase	CAT (p, c, m)	[15]
	Glutathione peroxidase	GPX1 (c) GPX2 (c) GPX3 (e) GPX4 (c, m) GPX5 (e) GPX6 (e) GPX7 (er) GPX8 (er)	[16]
	Peroxiredoxins	PRDX1 (c) PRDX2 (c) PRDX3 (m) PRDX4 (er) PRDX5 (m, p, c) PRDX6 (c)	[17]
Reduction of disulfide bonds	Thioredoxin	TRX1 (c) TRX2 (m)	[18]
	Glutaredoxin	GRX1 (c) GRX2 (c, m) GRX3 (n) GRX5 (m)	[19]
Re-cycling of oxidized enzymes/substrates	Thioredoxin reductase	TR1 (c) TR2 (m)	[18]
	Glutathione reductase	GR (c, m)	[20]
	Sulphiredoxin (SRX)	SRX (c, m)	[21]

c, cytosol; e, extracellular; er, endoplasmic reticulum; m, mitochondria; n, nucleus; p, peroxisomes.

with this, inhibition of either antioxidant system was selectively toxic to this lymphoma subtype. In addition, cells lacking TRX were uniformly more sensitive to ROS and doxorubicin-induced apoptosis than control cells. Toxicity of TRX inhibition in 'OxPhos-DLBCL' cells was at least partially dependent on reduced proapoptotic activity of Forkhead box protein O1 (FOXO1) [9▪▪]. TRX reduces disulfide bonds between FOXO1 and p300, formed in response to oxidative stress. As acetylation of FOXO1 increases its apoptotic potential, depletion of TRX facilitated p300-mediated acetylation of FOXO1, and favored FOXO1-dependent cell death. Knockdown of FOXO1 in 'OxPhos-DLBCL' cells with silenced TRX expression markedly reduced cell apoptosis in response to ROS, demonstrating that FOXO1 is a redox-sensitive mediator of DLBCL cells' responses to oxidative stress [9▪▪]. These findings underscore the role of TRX in the pathogenesis of DLBCL and are a substantial step toward therapeutic exploitation of the redox environment modulation in DLBCLs.

Recently, a very interesting drug-resistance mechanism involving antioxidant enzymes was also reported for a subset of DLBCL with chronic active BCR signaling, termed 'activated B-cell (ABC)-like DLBCL' [31▪]. In ABC–DLBCLs, doxorubicin, a major component of lymphoma treatment protocols, localizes predominantly to the cytoplasm and triggers cytoplasmic oxidative stress instead of nuclear DNA damage response. Activity of signal transducer and activator of transcription 3 (STAT3) in these DLBCLs led to upregulation of antioxidant enzyme SOD2 and mediated doxorubicin resistance [31▪]. Small molecule STAT3 inhibitor overrode this SOD2-dependent resistance mechanism and restored doxorubicin sensitivity in preclinical models.

The elevated levels of oxidative stress biomarkers were also reported in serum of B-cell chronic lymphocytic leukemia (CLL) patients when compared with healthy study participants. It was accompanied by elevated expression of selected antioxidant enzymes, free thiols, and higher overall antioxidant capacity, presumably as an adaptation to elevated ROS [8]. In addition, mitochondrial biogenesis and increased oxidative phosphorylation were observed in a subset of patients with the highest ROS levels. Interestingly, this subset was sensitive to prooxidative treatment with a benzodiazepine derivative, an inhibitor of ATP synthase composed of subunits F_1 and F_0 [8].

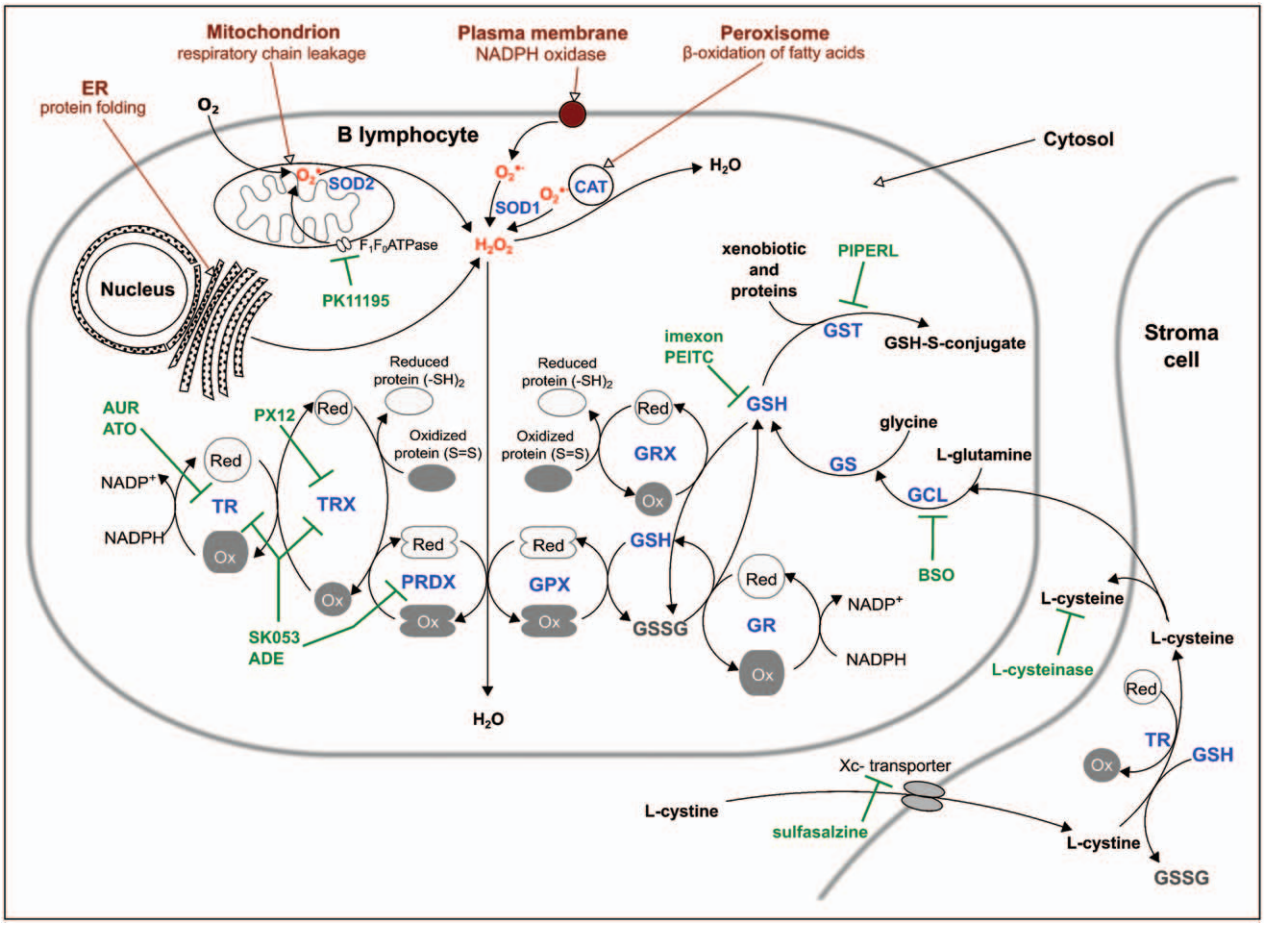

FIGURE 1. Schematic representation of cellular antioxidant pathways and their inhibitors. Major sources of cellular ROS (in red): mitochondrion, peroxisome, ER, NOX. Enzymes of the thioredoxin system: TRX, TR, PRDX. Enzymes and substrates of the glutathione system: GSH, GSSG, GPX, GRX, GR, GST. Enzymes involved in GSH synthesis: GCL, GS. Inhibitors targeting antioxidant pathways (in green). Abbreviations: ADE, adenanthin; ATO, arsenic trioxide; AUR, auranofin; BSO, buthionine sulfoximine, ER, endoplasmic reticulum; GCL, glutamate cysteine ligase; GPX, glutathione peroxidase; GR, glutathione reductase; GRX, glutaredoxins; GS, glutathione synthetase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione-S-transferase; NOX, NADPH oxidase; Ox, oxidized enzyme; PEITC, phenethyl isothiocyanate; PIPERL, piperlongumine; PK11195, inhibitor of F1F0 ATP synthase; PRDX, peroxiredoxin; TR, thioredoxin reductase; TRX, thioredoxin; Xc-transporter, L-cystine-L-glutamate antiporter system; Red, reduced enzyme.

INVOLVEMENT OF REDOX PATHWAYS IN STROMA-MALIGNANT CELL COMMUNICATION

During their differentiation in bone marrow niches and maturation in secondary lymphoid organs, normal B cells rely to a large extent on the support from the surrounding microenvironment. In recent years, a considerable amount of evidence has been gathered regarding the communication between B-cell-derived cancer cells and the microenvironment via BCR-mediated signals, direct cell–cell interactions, and by soluble factors (reviewed in [32,33]). Some studies also highlighted biochemical and metabolic pathways that are crucial for stromal support of malignant cell growth, including the management of cancer-related oxidative stress. The study by Zhang *et al.* [34] reported a potent reduction of oxidative stress in CLL cells by bone marrow-derived mesenchymal stromal cells (BMSC) via the highly specific cystine–glutamate antiporter system, Xc-. Stromal cells utilize this system to import L-cystine and convert it to L-Cys, which is then released into the tumor microenvironment; subsequently, CLL cells uptake the reduced amino acid to facilitate synthesis of GSH (Fig. 1). This observation is in accordance with the previous report that B-cell-derived malignancies can intrinsically display limited capacity for L-Cys uptake and, therefore, are more prone to ROS-induced cell death [35].

The role of microenvironment in alleviation of cellular oxidative stress has been also documented in DLBCL. In patient-derived DLBCL xenografts (a

system that retains microenvironmental components) GSH supply from stromal cells to lymphoma cells has been identified as a major mechanism responsible for adaptation of DLBCL cells to oxidative stress. Importantly, this mechanism was targetable with a small molecule compound, pyrvinium pamoate, which exhibited potent antitumor *in vitro* and *in vivo* activity [36]. The dependence on stromal support has also been reported in certain precursor B-cell acute lymphoblastic leukemia (B-ALL) [37]. Moreover, bone marrow-derived stromal cells (BMSC) induced a multidrug resistant phenotype of B-ALL cells, at least in part by secretion of soluble factors that facilitate adaptation of tumor cells to oxidative stress. In a coculture model, BMSC-conditioned medium triggered upregulation of antioxidant enzymes (SOD2, glutathione peroxidase 1/2) and antiapoptotic protein Myeloid cell leukemia 1 (Mcl-1) in B-ALL cells. Blocking such metabolic remodeling by inhibiting antioxidant production restored drug sensitivity, indicating that metabolic plasticity in leukemic cells is a targetable mechanism of chemoresistance, and potentially disease recurrence [38]. Similarly, a recent study has demonstrated that adipocytes, a prominent component of the bone marrow microenvironment, support survival of daunorubicin-treated B-ALL cells via oxidative stress response [39].

Taken together, these data highlight the crucial role of stromal support in the management of oxidative stress and induction of drug resistance phenotype in precursor and mature B-cell malignancies alike. Nonetheless, it has to be noted that most of these studies involved *in vitro* coculture models; even though 3-D extracellular scaffold systems are quite advanced in recapitulating tumor – microenvironment interactions, the translational value of these findings need to be further confirmed in additional *in vivo* studies.

INHIBITION OF ANTIOXIDANT DEFENSE AND OTHER REACTIVE OXYGEN SPECIES-INDUCING THERAPEUTIC STRATEGIES: PRECLINICAL AND CLINICAL STUDIES

Increased production of ROS in certain tumor cells because of metabolic dysregulation and specific reliance on antioxidant systems opens a way to specific targeting these pathways in tumor cells. In addition, as multiple classical chemotherapeutics (such as doxorubicin or melphalan) have been shown to produce ROS, these strategies might be synergistic with them. In a number of studies, the researchers have tried to utilize pharmacological inhibition of antioxidant enzymes or other ROS-inducing compounds. In the current work, we mainly describe the use of compounds specifically targeting antioxidant defenses or inducing ROS production. Table 2 and Fig. 1 present several examples of such compounds.

Table 2. Examples of oxidative stress-inducing agents utilized against B-cell malignancies

Compound	Antioxidant defense target	Malignancy	Research phase	Reference/clinical trial identifier
Auranofin	TR	CLL cHL	*in vitro*, murine models Clinical Trial Phase 2 *in vitro*, murine models	[6] NCT01419691 [40]
SK053	TRX, TR, PRDX1–4	Burkitt lymphoma	*in vitro*	[7▪▪,41]
Adenanthin	TRX, TR, PRDX1, PRDX2	Burkitt lymphoma	*in vitro*	[42]
ATO	TR	CLL Burkitt lymphoma B-cell lymphoma	*in vitro* *in vitro* *in vitro*	[43] [44] [45]
BSO	GCL, inhibits GSH biosynthesis	DLBCL, mantle-cell lymphoma	*in vitro*	[46]
Imexon	GSH-depletion	B-cell NHL	Clinical Trial Phase 2	[47] NCT01314014
Piperlongumine	GST-π	Burkitt lymphoma	*in vitro*	[48]
Sulfasalazine	Xc- cystine transporter	DLBCL	*in vitro*	[49]
Ellagic acid	Pro-oxidant	CLL	*in vitro*	[50]
Ascorbic acid (vitamin C)	Pro-oxidant	Burkitt lymphoma NHL	*in vitro* Pilot Clinical Trial	[51] NCT00626444

ATO, As_2O_3, arsenic trioxide; BSO, buthionine-sulfoximine; cHL, classical Hodgkin lymphoma; CLL, chronic lymphoblastic leukemia; DLBCL, Diffuse Large B-Cell Lymphoma; GCL, glutamate-cysteine ligase; NHL, non-Hodgkin lymphoma.

TRX and GSH are two major antioxidant systems in cells. Thus, several small molecule inhibitors of various components of these systems have been investigated, so far mostly in preclinical models of B-cell cancers. SK053, which directly binds and inhibits both TRX [52] and dimeric PRDXs, the H_2O_2-scavenging enzymes, triggers ROS-induced extracellular signal–regulated kinases 1/2 (ERK1/2) activation, cell cycle arrest, and apoptosis in Burkitt lymphoma cell line models [7▪▪]. Similar antilymphoma effects were also reported for another inhibitor with similar specificity – adenanthin [42]. Accordingly, auranofin, the inhibitor of thioredoxin reductase, an upstream enzyme of TRX system, has recently demonstrated antitumor activity in preclinical models of CLL [6] and classical Hodgkin lymphoma [40]. Auranofin, a gold complex approved decades ago for the treatment of rheumatoid arthritis, has recently been evaluated in the phase I/II clinical trial in CLL and small lymphocytic lymphoma patients. The final results of the study have not been published yet, however, preliminary conference reports reveal only transient response and limited clinical efficacy of auranofin used as monotherapy [53]. Similarly, inhibitors of GSH biosynthesis, like buthionine sulfoximine, are effective only in combinations with other therapeutic agents (see the paragraph below) [6,46].

Another long known, though still not fully understood phenomenon, is the particular susceptibility of B-cell lymphoma cells to ROS-inducing agents. As published by Chen *et al.* [51], lymphoma cells are much more sensitive to direct exposure to exogenous H_2O_2 as compared to normal B cells. Accordingly, lymphoma cells are among the most sensitive to L-ascorbate ($LD_{50} \sim 0.5$ mmol/l), which generates exogenous H_2O_2 [51]. Despite the high sensitivity *in vitro*, the antilymphoma activity of high dose parenteral L-ascorbate *in vivo* is limited [54]. However, another clinically applicable ROS-inducing compounds are being investigated in clinics. A prominent example is imexon, a cyanoaziridine antineoplastic agent binding intracellular thiols and thus depleting stores of cysteine and GSH, which in consequence increases ROS levels [55]. The results of Phase II study published in 2014 revealed 30% overall response rate and a good correlation of the clinical response with redox parameters, supporting the use of imexon against lymphoma [47].

SYNERGISTIC COMBINATIONS INVOLVING INHIBITION OF ANTIOXIDANT DEFENSES

As highlighted above, numerous small molecule inhibitors targeting redox homeostasis are investigated for the treatment of B-cell cancers, yet, in majority of cases their efficacy in monotherapy is limited. However, increasing amount of research reports on synergistic effects of inhibitors of antioxidant enzymes combined with other drugs. Importantly, in many cases these therapeutic modalities present high degree of selectivity toward malignant cells. In Table 3 we present several examples of such combination therapies.

It is well established that redox status affects vulnerability to apoptosis and that several antioxidant enzymes including TRX1, PRDX1, play antiapoptotic functions [64,65]. Overcoming apoptosis resistance with inhibitors of antioxidant defense system was also reported for B-cell neoplasms. In B-ALL cell lines and primary cells, buthionine sulfoximine, an inhibitor of GSH synthesis, potentiated the efficacy of SMAC mimetics, molecules that antagonize inhibitors of apoptosis. Similar effect was also reported for a combination of SMAC mimetics with auranofin. Interestingly, the therapies were not toxic to nonmalignant leukocytes nor to mesenchymal stromal cells, underscoring tumor selectivity [56,57].

A likely reason for limited efficacy of antioxidant enzymes' inhibitors used as single agents is the occurrence of several antioxidant systems, which, at least to some extent, play redundant functions. Indeed, it was shown that thioredoxin reductase inhibition leads to a compensatory increase in the activity of GSH system [66]. Accordingly, concomitant targeting of both TRX and GSH redox systems resulted in synergistic cytotoxicity in cell line models of CLL [6], DLBCL [46], and Burkitt lymphoma [60]. Similar synergistic effect was also observed in primary CLL [6] and mantle cell lymphoma cells but not in normal B cells [46]. Hence, the use of inhibitors with broader specificity, such as SK053 or adenanthin, which simultaneously inhibit several antioxidant enzymes, may contribute to better efficacy. In support of this hypothesis, the concomitant downregulation of both PRDX1 and PRDX2 contributed to significant attenuation of Burkitt lymphoma cell growth [7▪▪].

Finally, the restriction of GSH biosynthesis, either by thiol depletion or inhibition of stromal support, emerges as a novel strategy to overcome chemotherapy resistance. As mentioned above, stromal cells provide malignant B cells with L-Cys, a substrate for GSH biosynthesis, which may contribute to drug resistance. In accordance, GSH depleting agent β-phenylethyl isothiocyanate enhances cytotoxic effects of fludarabine and oxaliplatine in primary CLL-stroma coculture models, including cells derived from patients with drug-resistant 17p-deletion [34]. Similarly, phenylethyl isothiocyanate

Table 3. Redox-based combination regimens used for the treatment of B-cell malignancies

Mechanism	Combined compounds	Malignancy, model	References
Enhancement of apoptosis-inducing agents	BSO and SMAC mimetics (BV6, LCL161)	B-ALL cell lines: Reh, Nalm, Tanoue, Primary B-ALL blasts	[56,57]
	Auranofin and SMAC mimetic (LCL161)	B-ALL cell lines: Reh, Tanoue	[57]
	Auranofin and doxorubicine, cisplatin, gemcitabine	cHL cell lines: L-1236, HDLM-2	[40]
	PEITC and fludarabine	Primary CLL cells	[34]
	PX-12 and doxorubicin	DLBCL cells lines	[58]
	TRX-downregulation and doxorubicin	DLBCL cell lines	[9▪▪]
Induction of nonapoptotic cell death	BSO and etoposide BSO and SN-38	Bcl-2 overexpressing 697 human B-ALL cell line	[59]
Dual targeting of antioxidant defense pathways	BSO and auranofin	DLBCL cell lines: SUD-HL6, OCI-LY10, MCL cell lines: Rec-1, Granta	[46]
	BSO and ATO	Burkitt lymphoma cell lines: U937, Namalwa	[60]
	Ethacrynic acid and ATO	Burkitt lymphoma cell lines: Raji, Namalwa, Daudi	[61]
	PRDX1 and PRDX2 downregulation	Burkitt lymphoma cell lines: Raji, Namalwa	[7▪▪]
Inhibition of stromal support	PEITC and fludarabine/oxaliplatine	Primary CCL cocultured with MSC - HS5	[34]
	PEITC and SAHA	Primary CCL cocultured with MSC - HS5	[62]
	L-cysteinase and fludarabine	TCL1-Tg:p53$^{-/-}$ mouse model Primary CLL co-cultured with stromal NKTert cells	[63▪▪]
	PEITC and L-asparaginase piperlongumine and L-asparaginase	Primary B-ALL cells and B-ALL cell lines (REH, SUP-B15) cultured in MSC-derived conditioned medium	[38]

As_2O_3, arsenic trioxide; B-ALL, B-cell acute lymphoblastic leukemia; BSO, buthionine-sulfoximine; cHL, classical Hodgkins lymphoma; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; GCL, glutamate-cysteine ligase; MCL, mantle cell lymphoma; MSC, mesenchymal stromal cells; NHL, non-Hodgkin lymphoma; PEITC, phenethyl isothiocyanate; SAHA, Suberoylanilide Hydroxamic Acid.

enhances sensitivity to L-asparaginase of B-ALL cells cultured in a medium containing soluble factors secreted by stromal cells [38]. Another, recently presented strategy to hamper GSH biosynthesis, is to deplete L-Cys via administration of L-cysteinase, an enzyme which degrades L-Cys. L-cysteinase significantly reduces tumor growth as well as increases the efficacy of fludarabine in TCL1-Tg:p53$^{-/-}$mice, a transgenic model of CLL. Importantly, systemic administration of this enzyme is well tolerated, without any signs of toxicity. Moreover, L-cysteinase enhances fludarabine cytotoxicity to primary, patient-derived CLL cells cultured *in vitro* with stroma. The advantage of L-cysteinase over other GSH-depleting agents is that it concomitantly reduces GSH and L-Cys supply, what is manifested with a remarkable *in vivo* efficacy [63▪▪]. It would be interesting to see the efficacy of the combinations of the enzyme with other treatment modalities.

CONCLUSION

Despite a significant progress in our understanding of the role of redox homeostasis in B-cell malignancies in recent years, one must conclude that the purely prooxidant therapeutic approaches against B-cell cancers are still in their early development. Further studies are needed to identify subtype-specific targets and selective inhibitors for such. Importantly, the synergistic effects of prooxidant therapeutics with chemotherapy agents are encouraging and should stimulate further research for

testing their combinations with modern drugs used in clinics.

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Conflicts of interest

There are conflicts of interest.

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