HHS Public Access

Author manuscript

*Trends Cancer*. Author manuscript; available in PMC 2021 November 01.

Published in final edited form as:

*Trends Cancer*. 2020 November ; 6(11): 960–973. doi:10.1016/j.trecan.2020.05.012.

# The Unfolded Protein Response in Leukemia: from Basic Understanding to Therapeutic Opportunities

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## Abstract

Understanding genetic and epigenetic changes that underlie abnormal proliferation of hematopoietic stem and progenitor cells is critical for development of new approaches to monitor and treat leukemia. The unfolded protein response (UPR) is a conserved adaptive signaling pathway that governs protein folding, secretion and energy production and serves to maintain protein homeostasis in various cellular compartments. Deregulated UPR signaling, which often occurs in hematopoietic stem cells and leukemias, defines the degree of cellular toxicity and perturbs protein homeostasis, at the same time offering a novel therapeutic target. Here we review current knowledge related to altered UPR signaling in leukemias and highlight possible strategies for exploiting the UPR as treatment for this disease.

### Keywords

Myeloid leukemia; lymphoid leukemia; hematological malignancy; unfolded protein response (UPR); hematopoietic stem cell (HSC); ER stress

## The UPR in Hematopoietic Stem Cell Biology

**Hematopoietic stem cells (HSCs)** (see Glossary) give rise to numerous progeny over their life span and maintain hematopoiesis under normal or stress conditions [1, 2]. Under steady-state conditions, HSCs remain dormant and rely in part on low protein synthesis rates to sustain **self-renewal** capacity and protect HSC integrity [3, 4]. However, proliferating HSCs show increased protein synthesis to allow expansion of the stem cell pool [5]. Due to poor protein folding capacity, HSCs tend to accumulate more unfolded/misfolded proteins, which in turn promote endoplasmic reticulum (ER) stress. Re-establishment of ER homeostasis in response to this stress requires activation of the unfolded protein response (UPR). UPR components sense protein misfolding in the ER and initiate a cellular response that both transcriptionally and non-transcriptionally aims to alleviate cellular stress, or triggers apoptosis if stress cannot be resolved (Figure 1) [6, 7]. The UPR governs cellular protein homeostasis (**proteostasis)** by modulating protein translation, degradation, and transcriptionally regulates genes functioning in protein folding, protein quality control, and **ER associated degradation (ERAD)** programs [8], which collectively regulate cellular

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responses to ER stress. Crosstalk among these pathways, including mitogen-activated protein kinases (MAPK) signaling and redox signaling, determines cell's ability to cope with consequences of impaired ER function, resulting in **autophagy,** cell death or survival programs [9] (Figure 1). Therefore, the UPR is proposed to sustain a healthy homeostatic balance, in part through clearing individual cells after ER stress.

The interplay between pro-survival and pro-apoptotic signaling of the UPR (Box 1) is crucial for maintaining organismal homeostasis, under both steady state and stress conditions, especially in HSCs to prevent propagation of damaged HSCs and, ultimately, leukemogenesis [10]. Indeed, under steady-state and mild ER stress conditions (such as **hypoxia** and increased metabolic demand), the inositol-requiring enzyme 1 alpha (IRE1α) and the activating transcription factor 6 (ATF6) arms of the UPR reportedly promote HSC survival and stemness potential [11–13]. For example, Estradiol (E2), the most active type of estrogen, specifically upregulates the IRE1α-sXBP1 axis in HSCs by directly activating IRE1α transcription. That activity increases HSC capacity to reconstitute the hematopoietic system upon transplantation in lethally irradiated mice and accelerates hematopoietic regeneration [11]. Consistently, IRE1α pathway activation has been shown to promote HSC survival and stemness potential under ER stress conditions (such as accumulation of misfolded protein and oxidative stress), and HSCs with high IRE1α activity exhibit increased reconstitution potential *in vivo* compared to HSCs with low IRE1α activity [13, 14]. In addition, activating transcription factor 4 (ATF4)-mediated responses in HSCs subjected to low stress conditions, such as amino acid deprivation, exhibit cyto-protection and facilitate HSC persistence [15]. However, under severe ER stress conditions, prolonged activation of the protein kinase R-like endoplasmic reticulum kinase (PERK)-ATF4 axis induces expression of pro-apoptotic factors such as C/EBP homologous protein (CHOP), a transcription factor that controls genes mediating apoptosis [8, 9, 16–22]. To this end, gene expression analysis has revealed enrichment of UPR components in a mixed population of HSC and **progenitor** cells (HSPCs) as compared to downstream progenitors, including components of the PERK-ATF4-CHOP axis [7]. Relative to effects seen in progenitor populations, elevated PERK signaling in HSPCs confers greater sensitivity to ER stress-induced apoptosis, such as following treatment with the ER-inducing drug tunicamycin (Tm) [7]. Consequently, overexpression of the ER co-chaperone ERDJ4 [7], or treatment with chemical **chaperones** [23], protects HSPCs from proteotoxic stress and increases their reconstitution capacity in xenograft assays by blocking upregulation of stress-related genes, such as CHOP. These results suggest that the PERK pathway plays an important role in maintaining HSC integrity, by eliminating damaged stem cells.

Current analysis suggests that distinct arms of the UPR serve dedicated functions in HSCs: sustained PERK activation is associated with programmed cell death, and the IRE1α-sXBP1 arm is mainly linked to survival and regeneration. Additional studies are needed to define how both arms interact to sustain HSC proliferation and self-renewal status. In this review, we summarize how this conserved response to stress is hijacked by leukemic cells to enable their survival during leukemogenesis and leukemia progression as well as to promote resistance to therapy. We summarize current evidence for elevated UPR signaling in chronic

and acute leukemias, providing a rationale for targeting the UPR in a heterogeneous group of these malignancies.

## UPR Function in Leukemogenesis

Leukemias emerge from accumulation of genetic alterations in HSCs or lineage-restricted progeny [24, 25]. Leukemic cells often hijack stem cell programs to fuel leukemogenesis and progression, and myeloid leukemias are, like normal hematopoiesis, maintained by **leukemic stem cells (LSCs)** [2, 25] (Box 2). Given the importance of the UPR in regulating normal HSC survival and self-renewal, activation of these processes in LSCs is expected to confer a clonal advantage enabling LSCs to survive the increased metabolic demands associated with increased proliferation (Figure 2). Indeed, the UPR, and in particular the IRE1α-XBP1 axis have been demonstrated to promote survival of HSCs during early transformation steps in experimental systems [13]. In conditional knock-in mice bearing the $NRAS^{G12D}$-mutated allele in HSCs, constitutive NRAS signaling transforms HSCs into pre-leukemic stem cells (pre-LSCs), leading to a broad spectrum of myeloid neoplasms. In this model, IRE1α-sXBP1 signaling is activated, protecting pre-LSCs from ER-stress-induced apoptosis and enhancing both their ability to compete for nutrients with other cells and their self-renewal capacity [13]. Thus, like normal HSCs, pre-LSCs utilize the UPR to adapt to harsh conditions of the bone marrow niche that trigger ER stress and to cope with increased metabolic demands that underlie oncogene activation and proliferation [13]. Interestingly, expression of other driver mutations, such as the internal tandem duplication in FMS-like tyrosine kinase 3 (FLT3-ITD), one of the most frequent mutations in acute myeloid leukemia (AML) and usually associated with poor outcome [26], does not confer a survival advantage following treatment with ER stress inducers [13], suggesting that different driver mutations utilize distinct mechanisms to protect pre-LSCs from stress. One example for alternate stress adaption is the decrease in protein synthesis rates by downregulating ribosome biogenesis observed in RUNX1-mutated pre-LSCs [27]. RUNX1 is a transcription factor mutated in *de novo* and therapy-related leukemias [28]. Low biosynthetic activity in RUNX1-mutant pre-LSCs is accompanied by reduced UPR and p53 signaling and consequently resistance to genotoxic and ER stress. This stress resistance provides a selective advantage to pre-LSC and allows them to expand in bone marrow and outcompete normal HSPCs [27]. These studies highlight the importance of maintaining ER homeostasis during HSC transformation to leukemia.

The first study addressing UPR function in leukemogenesis was performed in the leukemic cell line NB4, which harbors the t(15;17) (q24.1;q21.2) translocation, a cytogenetic hallmark of acute promyelocytic leukemia (APL). This translocation enables expression of the oncogenic **fusion protein** promyelocytic leukemia-retinoic acid receptor alpha (PML-RARα). In normal cells, wild-type RARα forms heterodimers with a member of the soluble nuclear receptor co-repressor 1 (N-CoR) family of transcriptional co-repressors [29]. Oncogenic PML-RARα binds to N-CoR protein in the cytosol immediately after translation inducing an abnormal N-CoR conformation that triggers UPR-mediated clearance of abnormal N-CoR by the ERAD system [30]. This leads to decrease levels of soluble N-CoR protein in the nucleus and may underlie uncontrolled proliferation seen in APL. UPR cues

may thus impact N-CoR localization and expression levels and define the extent of transcriptional repression on specific promoters affecting myeloid differentiation.

Taken together, aberrant expression of diverse oncogenic drivers in leukemia results in the activation of UPR components, highlighting the importance of UPR signaling in the etiology of this disease.

## The Role of the UPR in Leukemia Cell Survival

Normal and leukemic hematopoietic cells are exposed to harsh environmental conditions in the bone marrow such as hypoxia, elevated **reactive oxygen species (ROS)** levels, and nutrient deprivation, which results in increased cellular metabolism and protein synthesis, culminating in ER stress. Leukemic cells accommodate ER stress by activating the UPR, allowing them to escape cell death and continue proliferation. While somatic mutations in UPR components were not identified so far in leukemia patients, activated UPR signaling clearly contributes to leukemia progression and survival, as discussed below. Correspondingly, activation of the IRE1α-XBP1 branch of the UPR, reflected in increased sXBP1, BiP and calreticulin expression, was found in ~18% of AML patients [31, 32].

The transcription factor c-Jun was recently found to be overexpressed in multiple AML subtypes and required for AML cell survival and progression *in vivo* [33]. Upon ER stress, c-Jun induces transcription of UPR target genes (such as XBP1 and ATF4) by direct binding to their promotors, allowing leukemic cells to resolve ER stress through a cytoprotective UPR. Correspondingly, c-Jun inhibition blocks UPR activation and the ability of AML cells to cope with ER stress. Of note, in different AML subtypes [inv(16), t(8;21) and 11q23] there is a positive correlation between c-Jun expression and that of numerous UPR genes, including ATF3, ATF4, CHOP, HSPA5 and PPP1R15B, confirming c-Jun's importance in the UPR and the etiology of this cancer type. c-Jun is also implicated in chronic myeloid leukemia (CML) stem cell survival [34] and has been shown to be a downstream component of signaling by the heparin-binding growth factor, pleiotrophin (PTN), which is necessary for CML pathogenesis and initiation. PTN promotes CML stem cell growth, survival, and resistance to tyrosine kinase inhibitors via induction of c-Jun and the UPR [34] (Figure 2).

Notably, the ER is the cells major calcium store and calcium is a key player in regulating early and late stages cell death [35, 36]. Calcium release from the ER leads to rapid uptake and accumulation in mitochondria, promoting the **mitochondrial apoptotic pathway**, characterized by mitochondrial swelling, perturbation of the outer membrane, and release of apoptosis-inducing factors into the cytosol [35, 37]. This calcium-dependent ER-initiated apoptotic pathway is inactivated in BCR-ABL-expressing cells, representing a pro-survival mechanism and potential opportunity for therapeutic targeting [38]. BCR-ABL overexpression in immortalized murine 32D cells reportedly decreases calcium release from ER stores and correspondingly reduces capacitative calcium entry (CCE) [38]. Conversely, treatment with imatinib, which blocks BCR-ABL tyrosine kinase activity, reverses CCE and restores calcium release from ER stores. Accordingly, cells expressing high BCR-ABL levels are resistant to classical inhibitors of the mitochondrial apoptotic pathway [38].

Adaptive UPR signaling and tight control of secretory mechanisms is of major importance in B cells, which secrete immunoglobulins. Indeed, promoters of genes encoding several UPR components, including ERN-1 (IRE1), HSPA5 (BiP), PRDM1 (BLIMP-1) and XBP-1, are hypomethylated in pre-B acute lymphoblastic leukemia (ALL) cells relative to either normal pre-B cells or mature B-cell lymphoma [39]. This epigenetic state results in increased expression of these genes in the pre-B ALL, compared with mature B cells or non-Hodgkin lymphomas. Among these genes, Hspa5 is required for survival and malignant transformation of pre-B cells. Hspa5 deletion induces cell cycle arrest in pre-B cells *in vitro* and decreases leukemia burden *in vivo*, resulting in prolonged survival of ALL transplant recipient mice [39]. The importance of elevated UPR activity to ALL etiology is also reflected in analysis of pre-B-ALL, in which elevated XBP1 expression at diagnosis coincides with poor patient outcome. Indeed, *Xbp1* deletion in a mouse model of BCR-ABL-transformed pre-B ALL increased expression of the UPR components *Hsp90b1*, *Del3* and *Atf6* and reduced expression of genes functioning in the secretory pathway and in the Golgi apparatus (such as *Sirpa* and *B3gnt5*). *Xbp1*-deficient mice also show downregulation of immunoglobulin (Ig) light chain assembly genes as well as genes functioning in B cell antigen expression [39]. Similar to its role in B-ALL, XBP1 has also an important function in chronic lymphoblastic leukemia (CLL) etiology. XBP1 deletion in CLL cells (in the Eμ-TCL1 transgenic mouse model) is sufficient to attenuate leukemia development and prolong animal survival [40]. This phenotype is consistent with observed decreases in phosphorylation of Syk and Btk, key B-cell receptor (BCR) signaling components required for CLL cell survival. The authors of this study also noted marked decreases in synthesis of secretory μ heavy chains and decreased IgM secretion in *Xbp1*-deleted CLL model mice [40]. Notably, stimulation of CLL cells with sIgM *in vitro* using anti IgM antibodies increased expression of UPR components, an effect blocked by BTK inhibitors. Lastly, immunohistochemistry and gene expression analysis has demonstrated relatively high levels of UPR components in lymph nodes of CLL patients [41]. Overall, these studies suggest that IRE1α inhibitors may offer a novel therapeutic modality for ALL and CLL.

Hyperactivation of the Myc oncogene promotes robust protein synthesis leading to ER stress induction and UPR activation [42, 43] (Figure 2). Recent findings confirm that Myc regulates UPR activation in T-ALL cells via increasing transcription of the ubiquitin fusion degradation 1 (UFD1) gene. UFD1 is a component of the ERAD complex and facilitates ubiquitin-dependent degradation of misfolded/unfolded proteins. UFD1 inhibition in human T-ALL cells exacerbates ER stress, decreases cell growth, and induces apoptosis, in part by activating PERK-mediated proapoptotic signaling [44]. These findings point to Myc/UFD1 signaling as a key driver of the ER stress response in T-ALL and suggest that this pathway could be exploited therapeutically [44]. Accordingly, PERK activity was recently shown to promote dissemination of leukemic cells into peripheral blood and lymph nodes and thus may play an important role in progression of Myc-driven leukemias [45].

Interactions between leukemic cells and the BM microenvironment mediated by extracellular vesicles (EVs) promote leukemia cell survival [46]. Such transmission of UPR and ER stress factors by leukemic cells via EVs has been implicated in remodeling of the bone marrow niche in AML, which alters composition and function of the BM microenvironment [47]. The ability of EVs to transmit ER stress *in vivo* from AML

xenografts to bone marrow stroma upregulates expression of core UPR components and promotes subsequent differentiation of mesenchymal stem cells, a process requiring cell-cell transfer of Bone Morphogenic Protein 2 (BMP2) by AML-EVs [47]. Notably, transmissible ER stress was previeously indentified as a source of chemoresistance in solid tumors [48].

Taken together, these studies confirm the importance of UPR activation for leukemic cell survival and highlight possible therapeutic strategies for exploiting the UPR as a novel target to ameliorate leukemia patient outcomes.

## The Role of the UPR in Resistance to Therapy

Chemotherapy and targeted therapies are critical modes of cancer treatment. However, their efficacy is compromised by tumor cells' ability to develop intrinsic and acquired resistance [49]. Among resistance mechanisms are blocking drug uptake, alteration of the drug target, induction of drug-detoxification mechanisms, repair of drug-induced damage, and activation of anti-apoptotic and pro-survival pathways [49, 50]. LSCs play important roles in leukemia relapse and drug resistance, hampering complete cure of the disease [51]. Clinical evidence and *in vitro* studies have linked UPR activation to drug resistance in several cancer types [52, 53]. For example, CML treatment improved significantly following development of ABL tyrosine kinase inhibitors (TKIs), such as imatinib, dasatinib, nilotinib, bosutinib, and ponatinib. However, resistance to TKI or cancer recurrence after TKI discontinuation remains an obstacle to cure. Interestingly, PERK-eIF2α phosphorylation positively correlates with resistance to these inhibitors in CML [54]. Ectopic expression of BCR-ABL in 32D myeloid cells or its expression in CML lines (such as K562 and BV173) coincides with elevated levels of ER stress and concomitant activation of the PERK-eIF2α pathway [54]. Along these lines, increased PERK and eIF2α protein expression and phosphorylation is seen in $CD34^+$ cells from peripheral blood of CML patients resistant to imatinib or other TKIs. Correspondingly, expression of dominant negative forms of either PERK or eIF2α sensitizes CML cells to imatinib-mediated cell death. Chemoresistance can also be attributed in part to autophagy [55], a protective mechanism essential for CML cell survival, leukemogenesis and imatinib resistance [55–57]. Indeed, therapy combining imatinib with the autophagy inhibitor hydroxychloroquine decreases CML resistance to imatinib [58].

The importance of UPR signaling to CML therapy-resistance is also supported by reports on ATF6 pathway overactivation in CML cells, which has been attributed to high expression of protein disulfide isomerase A5 (PDIA5) [12]. Those authors showed that PDIA5 was necessary for ATF6 activation upon ER stress by catalyzing rearrangement of ATF6 disulfide bonds under stress conditions in a manner promoting ATF6 export from the ER and activation of its target genes. Genetic and pharmacological inhibition of the PDIA5/ATF6 axis restored imatinib sensitivity in imatinib-resistant K562 leukemia cells [12]. These results are in line with independent studies showing the important role of eIF2α phosphorylation and ATF6 activity in protecting cancer cells from chemotherapeutic drugs [59–61].

A recent study also highlights PERK/NRF2 and autophagy pathways as functioning in resistance to histone methyltransferase G9a inhibition in AML LSCs [62]. G9a regulates

transcription of multiple genes primarily by catalyzing dimethylation of histone H3 lysine 9 (H3K9me2) [63]. These activities consequently induce changes in cellular redox homeostasis, decreasing ROS production [64, 65]. Pharmacological and genetic targeting of G9a inhibits AML cell proliferation and reduces LSC frequency in a mouse model and in human AML cell lines [66, 67]. G9a inhibition by either BIX-01294 or siRNA activates the pro-survival PERK/NRF2 pathway and suppresses ROS generation. Inhibition of PERK/NRF2 or autophagy increases ROS generation and enhances G9a-induced apoptosis of LSCs [62]. This data suggests that treatment with inhibitors of either PERK/NRF2 or autophagy may overcome resistance to G9a inhibition and eliminate LSCs and may serve as treatment for AML.

Overall, leukemic cells hijack UPR signaling as a mean to promote drug resistance. Therapeutic approaches that target UPR sensors and their downstream effectors may be useful to overcome drug resistance phenotypes.

## Targeting the UPR in Leukemias

Targeting the UPR and ER stress is a promising approach in novel anti-cancer therapies (reviewed in [68]). Nonetheless, as noted above, a plethora of cellular functions are controlled by these signaling cascades and could be targeted in various ways. Below is a summary of interventions or drugs either in development or proposed to target UPR-associated signaling pathways relevant to leukemia (Table 1).

### Targeting UPR Sensors (IRE1α and PERK)

To date, drug development targeting the UPR as leukemia treatment has mainly focused on inhibiting pro-survival signaling mediated by IRE1α-XBP1 or PERK-ATF4 signals (Figure 3). Two catalytic domains within IRE1α, the RNase domain and the ATP binding site of the kinase domain, have been exploited in developing inhibitors, and pioneering studies in multiple myeloma (MM) proved their anti-cancer activity [69, 70]. These strategies have also led to development of several compounds, such as STF-083010, MKC-3646, B-I09, and hydroxyl-aryl-aldehydes (HAA), that inhibit the catalytic core of the IRE1α RNase domain [69, 70], blocking its ability to cleave XBP1 mRNA and consequently reducing levels of sXBP1. MKC-3646 in fact has a potent anti-proliferative effect against MM [70] and cytotoxic activity against AML cell lines and primary cells derived from AML patients [71]. Pharmacological inhibition of IRE1α using STF-083010 also promotes apoptosis of primary B-ALL cells and prolongs survival of B-ALL bearing mice *in vivo* [39]. Notably, inhibition of the IRE1α RNase domain predominantly blocks XBP1 splicing, without affecting RIDD, suggesting RNase and RIDD domains constitute distinct targets [72]. Pharmacological inhibition of IRE1 using B-I09 also has anti-tumor effects in CLL cells and in tumor-bearing Eμ-TCL1 mice [40]. Inhibition of the IRE-1/XBP-1 pathway decreases phosphorylation of BTK, and synergizes with ibrutinib in suppressing human CLL cell growth *in vitro* [40], providing a rationale to assess this combination against chronic B cell leukemia. More recently, IRE1 kinase inhibitors have been developed and evaluated in mouse models of MM [73, 74]. This class of inhibitors stabilize IRE1 in the inactive state and prevent activation of

the RNase domain [74]. However, the efficacy and specificity of these inhibitors in leukemia require further studies.

GSK2606414 and GSK2656157, originally designed as selective ATP-competitive inhibitors, are among the most widely studied PERK inhibitors and have high potency and pharmacokinetic properties suitable for use *in vivo* [75, 76]. Both inhibitors have anti-proliferative activity in multiple cancer models *in vivo* including MM [77]. Moreover, combining PERK inhibition with retinoic acid (RA) or arsenic trioxide (ATO) treatment is reportedly effective against APL [78]. Correspondingly, induction of differentiation (by RA) of APL cell lines or primary human APL cells sensitizes them to ER stress. Indeed, combining ATO with Tm has a synergistic cytotoxic effect in both RA-sensitive and RA-resistant APL cell lines. Such sensitivity can be attributed to the PERK pathway, as inhibition of PERK by GSK2606414 enhances toxicity of Tm and ATO [78].

## Exaggerating ER Stress to Trigger UPR-mediated Apoptosis

Therapeutic induction of ER stress emerges as a promising treatment for FLT3-ITD positive leukemia. FLT3-ITD is particularly interesting in the context of the UPR as it is a misfolded protein mostly retained in the ER due to impaired glycosylation [79]. Therefore, drugs that impact FLT3-ITD glycosylation reportedly have anti-proliferative effects on FLT3-ITD-positive AML cells (Figure 3) [80]. Among them, Tm inhibits FLT3-ITD glycosylation resulting in ER-stress-induced apoptosis and synergizes with FLT3-ITD kinase inhibitors [81]. Fluvastatin, a clinically approved inhibitor of mevalonate synthesis, also inhibits FLT3 signaling by inhibiting FLT3-ITD glycosylation and thus prolongs survival of mice with FLT3-ITD leukemia [82]. Lastly, low levels of drugs that generate either ER (Tm) or oxidative (arsenic trioxide) stress combined with retinoic acid was reported to kill AML cells characterized by MLL fusion proteins or the FLT3-ITD mutation [83]. Similar findings have been reported in T-ALL and B-ALL settings, as treatment of ALL cells with 2-deoxy-D-glucose (2-DG), Tm, or metformin leads to UPR induction and ER stress/UPR-mediated apoptosis [84, 85]. Noteworthy, the effects of Tm on a large plethora of cellular components, resulting in global cellular changes, should be considered when evaluating this drug for therapy.

Targeting the ERAD machinery to disrupt proteostasis is another approach to exploit ER stress for therapeutic purposes. The concept is mainly studied in secretory cells such as plasma cells, which -as antibody-producing cells- are strongly dependent on a well-developed secretory system and are pruned to potential protein overload. Here, proteasomal degradation represents the main pathway for ERAD. This led to development of the proteasome inhibitor bortezomib which blocks the 20S proteasome with great clinical success in MM and mantle cell lymphoma [86–89]. Bortezomib is now being studied in combination with other standard chemotherapy drugs for the treatment of relapsed or refractory ALL, newly diagnosed pediatric patients with T-cell ALL and AML [90–92]. Another approach is targeting the AAA-ATPase p97, a key ERAD component [93] (Figure 3). p97 (also known as Valosin-containing protein) is an abundant and conserved ATPase that functions in diverse activities, including protein quality control, chromatin remodeling, autophagy, and DNA repair [93, 94]. Recent efforts to develop small molecule p97 inhibitors

have identified several ATP-competitive and allosteric inhibitors, including Eeyarestatin I (EerI) [95–98]. p97 inhibition induces ER stress and cell death in solid tumors [98] and in MM [29], with promising activity and tolerability in *in vivo* models. Eer1 treatment of hematological cancer cells elicits an integrated stress response program in the ER to activate the UPR transcription factors ATF3 and ATF4, which together activate expression of the BH3-only protein NOXA and induce cell death [99]. Thus, ERAD inhibitors represent a novel class of anticancer drugs targeting the UPR. However, p97 inhibitors should be distinguished from the general proteasome inhibitors as many of the observed effects of p97 inhibition differ from those triggered by proteasome inhibition. This could be explained by the fact that p97 inhibitors activity may not be limited to the ER, as p97 is implicated in control of misfolded proteins at other subcellular locations, including polysomes or nuclear transcriptional complexes, and may also inhibit co-translational protein transport across the ER membrane by inactivating the Sec61complex [93, 100–102].

The neural precursor cell expressed, developmentally down-regulated (NEDD8) conjugation pathway, implicated in the regulation of cullin-RING ligases (CRLs) dependent protein ubiquitination, represents another regulatory component which impacts the UPR with concomitant effects on cell death programs in ALL [103]. CRLs are an integral part of the multi-protein E3 ubiquitin ligase complex SCF. Selective inhibition of NEDD8 with the small molecule Pevonedistat attenuates CRL activity and induces cell death in AML, CLL, and ALL. How CRLs control UPR signaling remains unclear, however, this could be explained by accumulation of proteins which were not efficiently ubiquitinated or degraded due to reduced CRL activity. Since the SCF complex is also implicated in the regulation of ATF4 ubiquitination and degradation [104], limiting SCF activity via NEDD8 inhibitors may limit leukemia progression. Along these lines, NEDD8 inhibitors sensitize ALL and CML cells to ABL kinase inhibitors [105].

Another proposed leukemia treatment involves targeting chaperones that function in maintenance of ER homeostasis, among them, BiP. The peptidomimetic drug 78 (BMTP-78) targets BiP and induces apoptosis of several leukemia and lymphoma lines, as well as primary AML cells derived from patients (Figure 3). However, BMTP-78 has exhibited unacceptable toxicity *in vivo*, potentially attributable to effects on the tumor microenvironment [106]. Epigallocatechin gallate (EGCG), a natural compound from green tea [107], also targets the BiP ATP-binding domain, promoting a conformational change that inactivates the protein and attenuates its anti-apoptotic function. Interestingly, EGCG treatment reportedly induces apoptosis of different B-lineage ALL lines and sensitizes B-ALL cells to eradication by vincristine, a chemotherapy drug commonly used against B-ALL [108]. Although the anti-tumor potential of EGCG has been extensively studied [109, 110], its use as a natural compound component of combination therapies remains untested.

## Concluding Remarks

Studies summarized here reflect emerging appreciation for the importance of the UPR in leukemia. Indeed, in response to extrinsic and intrinsic cues, activation of the UPR network represents an important step in oncogenic transformation and influences several activities relevant to leukemic cells including development, progression, and chemoresistance.

Different UPR arms serve distinct purposes: the PERK pathway may function in autophagy and apoptosis, while the ATF6 and IRE1 arms likely govern cell survival. A balance between UPR arms requires further studies to define changes occurring during transformation, which may reveal mechanisms targetable at early stages of leukemia development (see Outstanding Questions). Moreover, genetic models enabling analysis of UPR components in hematopoietic malignancies, while available, have not been used to assess the impact of targeting either specific UPR arms or their combination. Although targeting ER stress/UPR signaling has great potential as an intervention in leukemias, further studies are warranted to answer unresolved issues. Therefore, further mapping of regulatory components that fine tune UPR sensors should enable one to achieve and exploit a more refined assessment of select UPR signaling in each of the different leukemias.

## Acknowledgments

We thank Ani Deshpande and Daniela Senft for their valuable comments. Support by NCI grants R35 CA197465 and P01 CA128814 (ZR) is gratefully acknowledged.

## Glossary

**Autophagy**
Is an evolutionary conserved homeostatic process that involves degradation and recycling of cytosolic components by the lysosomes

**Chaperones**
Is a ubiquitous family of proteins that functions in protein folding and proteostasis by contributing to the correct folding of polypeptides or their assembly into oligomeric structures

**ER-associated degradation (ERAD)**
Constitutes a cellular pathway which targets misfolded proteins of the ER for ubiquitination and subsequent degradation by the ubiquitin proteasome system

**Fusion-protein**
Is a protein product of two or more genes that originally coded for separate proteins and were fused by chromosomal rearrangements, resulting in protein product for both genes (or part of them)

**Hematopoietic stem cells (HSCs)**
Are cells characterized by their unique ability to self-renew and replenish all blood cell types in the body

**Hypoxia**
Is a state or a condition marked by low oxygen levels (<3%)

**Leukemic stem cells (LSCs)**
Are a subpopulation of leukemia cells that possess stem cell properties distinct from the bulk leukemia cells, including self-renewal and drug resistance

**Proteostasis (Protein homeostasis)**

Reflects complex pathways that controls the biogenesis, folding, trafficking and degradation of proteins in cells

**Progenitors**

Are descendants of stem cells, which are more constrained in their differentiation potential and capacity for self-renewal

**Reactive oxygen species (ROS)**

Are unstable and highly reactive chemicals containing oxygen which can cause cellular damage and stress

**Self-renewal**

Presents a process by which stem cells divide to make more stem cells, perpetuating the stem cell pool throughout life, while maintaining their undifferentiated state

**Mitochondrial apoptotic pathway**

Is regulated by pro-and anti-apoptotic members of the Bcl-2 protein family and characterized by mitochondrial membrane permeabilization and subsequent release of cytochrome c into the cytoplasm to activate caspases

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## Outstanding Questions

- What signals activate the UPR in different leukemia subtypes?
- How does the UPR impact tumor metabolism and interaction with the tumor microenvironment?
- Can modulators of downstream UPR components reverse the course of leukemia development ?
- Would fine-tuning UPR signaling redirect the course of leukemia development or counter treatment resistance?
- Can we develop genetic models to study the role of UPR signaling in leukemias?
- Would precision medicine approaches using advanced computer modeling and analysis tools allow us to define UPR-related treatment modalities? (we did not speak about it in the review at all – should mention?)
- Could transcriptomic and proteomic data from leukemia patients allow us to map new paths underlying UPR dysregulation, in order to monitor and treat the disease?

## Highlights

- Activated UPR contributes to leukemia development and progression
- Intrinsic and extrinsic cues govern/modulate activity of UPR components in leukemia
- Specific UPR components may serve as markers of leukemia development and therapy response
- Targeting the UPR may overcome leukemia resistance to therapy

### Box 1: The UPR in Cell Survival and Death Programs

The mammalian UPR has evolved into a dynamic and flexible network of signaling events that responds to various inputs over a wide range of basal metabolic states. During ER stress conditions, activation of the UPR reduces unfolded protein load through several pro-survival mechanisms. However, when ER stress is not mitigated and homeostasis is not restored, the UPR triggers apoptosis [9]. Two distinct UPR-related responses, the adaptive response and/or apoptotic response, are observed over time in cells undergoing ER stress. In the adaptive phase, PERK inhibits general protein translation through eIF2α phosphorylation [111], and IRE1α activation leads to selective degradation of mRNAs encoding for certain ER-located proteins through regulated IRE1-dependent decay (RIDD) [20]. Autophagy is also activated by ER stress to eliminate accumulation of protein aggregates and possibly damaged ER (a process termed ER-phagy) through the lysosomal pathway [112]. Together, these mechanisms reduce the influx of proteins into the ER to allow adaptive and repair mechanisms that re-establish ER homeostasis. Simultaneously, a massive gene-expression response is initiated through at least three distinct UPR transcription factors: ATF4, sXBP1 and ATF6. These transcription factors promote adaptive responses that aim to restore ER function and maintain cell survival including amino acid transport and synthesis, redox signaling, protein folding and ERAD [113]. Unresolved ER stress results in apoptosis which depends on the core mitochondrial apoptosis pathway regulated by the B cell lymphoma 2 (BCL-2) protein family [114]. When activated at the transcriptional or post-translational level, BCL-2 homology 3 (BH3)-only proteins regulate the activation of BAX and/or BH antagonist or killer (BAK) to trigger apoptosis. Sustained PERK/ATF4 activation induces expression of pro-apoptotic factors such as CHOP, which downregulates the anti-apoptotic protein BCL-2 and induces the expression of some BH3-only genes mediating apoptosis [18, 21]. Under certain conditions, IRE1α can also induce cell death by activating the pro-apoptotic IRE1-TRAF2-JNK pathway, with subsequent downstream engagement of the BCL-2 family members, concomitant with the activation of RIDD, resulting in the degradation of mRNAs encoding key mediators of protein folding [72, 115]. Additional complementary mechanisms are proposed to induce cell death under chronic ER stress, including activation of the BH3-only protein BH3-interacting domain death agonist (BID) by caspase 2, as well as ER calcium release, which may sensitize mitochondria to activate apoptosis [116]. Therefore, it is essential to understand how UPR sensors shift their signaling output to determine divergent cell fate decisions.

### Box 2: Classification of Leukemias

Leukemia is a blood cancer caused by the rapid production of abnormal white blood cells and is the most common cancer in children and adolescents. Clinically and pathologically, leukemia is subdivided into four major subtypes: ALL (acute lymphoblastic leukemia), CLL (chronic lymphoblastic leukemia), AML (acute myeloid leukemia), and CML (chronic myeloid leukemia). [117]. Classification is based in part on the type of blood cell affected and includes both lymphoblastic and myeloid leukemias. In the former, malignant changes occur in the type of marrow cell that becomes lymphocytes, which function in the immune system. With myeloid leukemia, malignancy occurs in cells that usually mature into red blood cells, other types of white cells, and platelets [118, 119]. Classification is also based on whether leukemias take an acute or chronic form based on rate of progression. In acute leukemias, abnormal blood cells are blasts and usually remain poorly differentiated and largely non-functional, leading eventually to defective hematopoiesis. Acute leukemias often rapidly progress and require immediate treatment. In chronic leukemias, some blast cells may remain and may mature and function normally [118, 119]. Thus, progression of chronic leukemias is usually slower and may not require treatment as aggressive as that used against acute leukemias. Overall, etiology, clinical features, treatment, and survival of leukemia cells differ significantly, requiring type-specific analysis.

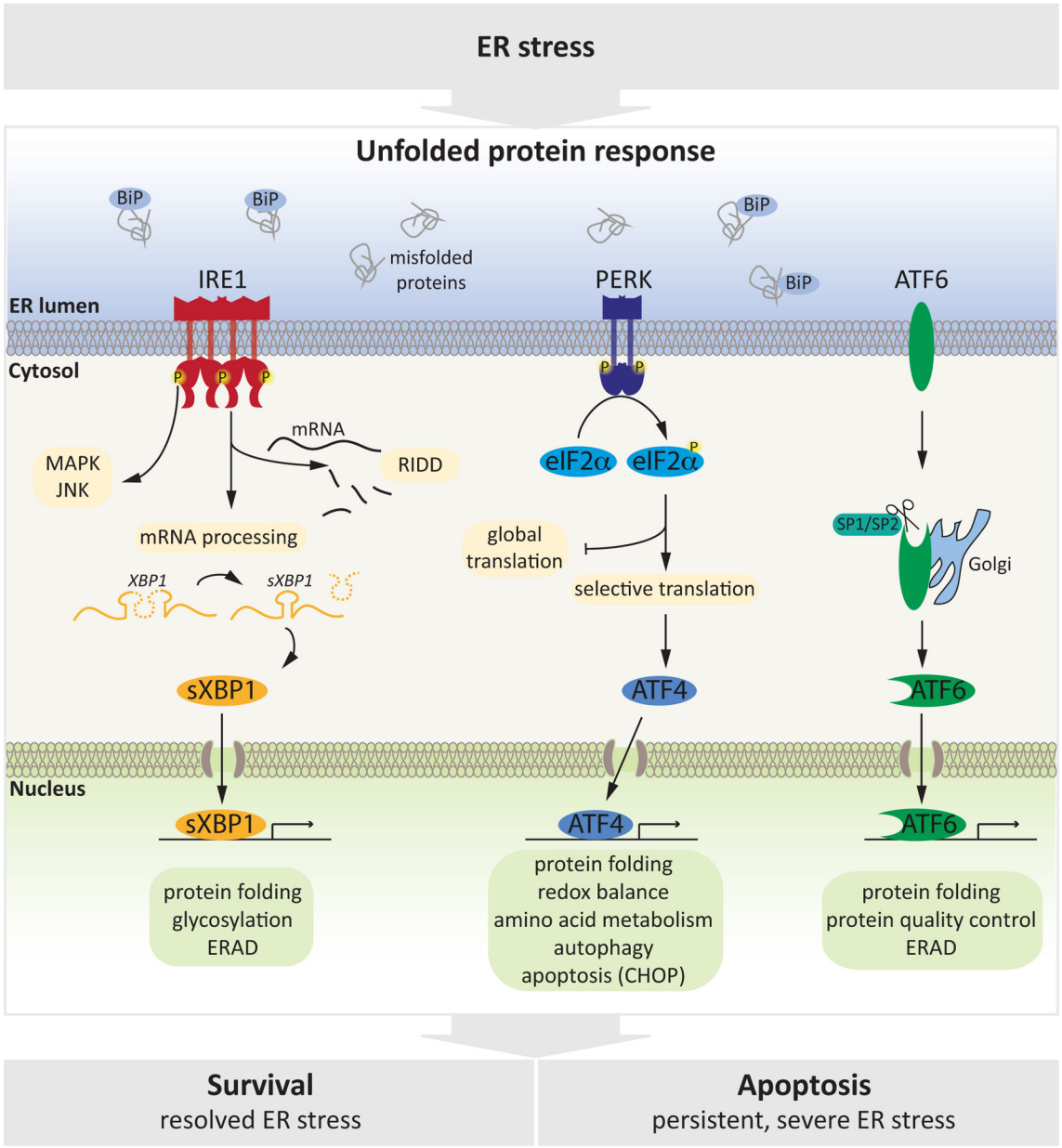


**Figure 1: Molecular Mechanisms of the UPR**

ER stress, triggered by accumulation of un- or mis-folded proteins in the ER lumen is sensed by three transmembrane proteins (IRE1, PERK, ATF6) that serve as ER stress sensors and cooperatively coordinate the UPR. IRE1α and PERK are type I ER transmembrane proteins and are activated by oligo/dimerization and autophosphorylation. IRE1α has a cytoplasmic kinase and endoribonuclease domain and once activated, it catalyzes excision of a 26-nucleotide intron from the mRNA encoding X-box binding protein-1 (XBP-1) which is translated to form the transcription factor spliced XBP1 (sXBP1), and also degrades a subset of ER-associated RNAs through a process called IRE1 dependent decay (RIDD). Through its kinase domain, IRE1 activates further stress-related pathways to modulate response to stress. PERK is composed of an ER luminal stress sensor and a cytosolic protein kinase

domain. Activated PERK attenuates global protein synthesis by phosphorylating serine 51 of the initiation factor eukaryotic translation initiator factor 2α (eIF2α), globally decreasing initiation of mRNA translation while enabling selective translation of stress-related mRNAs including activating transcription factor 4 (ATF4). Finally, ATF6 is a transmembrane protein whose cytosolic domain has transcriptional activity. Following ER stress, ATF6 is transported to the Golgi apparatus where it is processed by site 1 protease (S1P) and S2P, releasing a transcriptionally active ATF6 protein which then enters the nucleus. All three arms of the UPR mediate a transcriptional response (by activating sXBP1, ATF4 or ATF6) to increase ER capacity to resolve stress, by upregulating target genes implicated in protein folding, quality control and ERAD. Upon severe or prolonged stress, the UPR orchestrates a pro-apoptotic response that eliminates damaged cells.

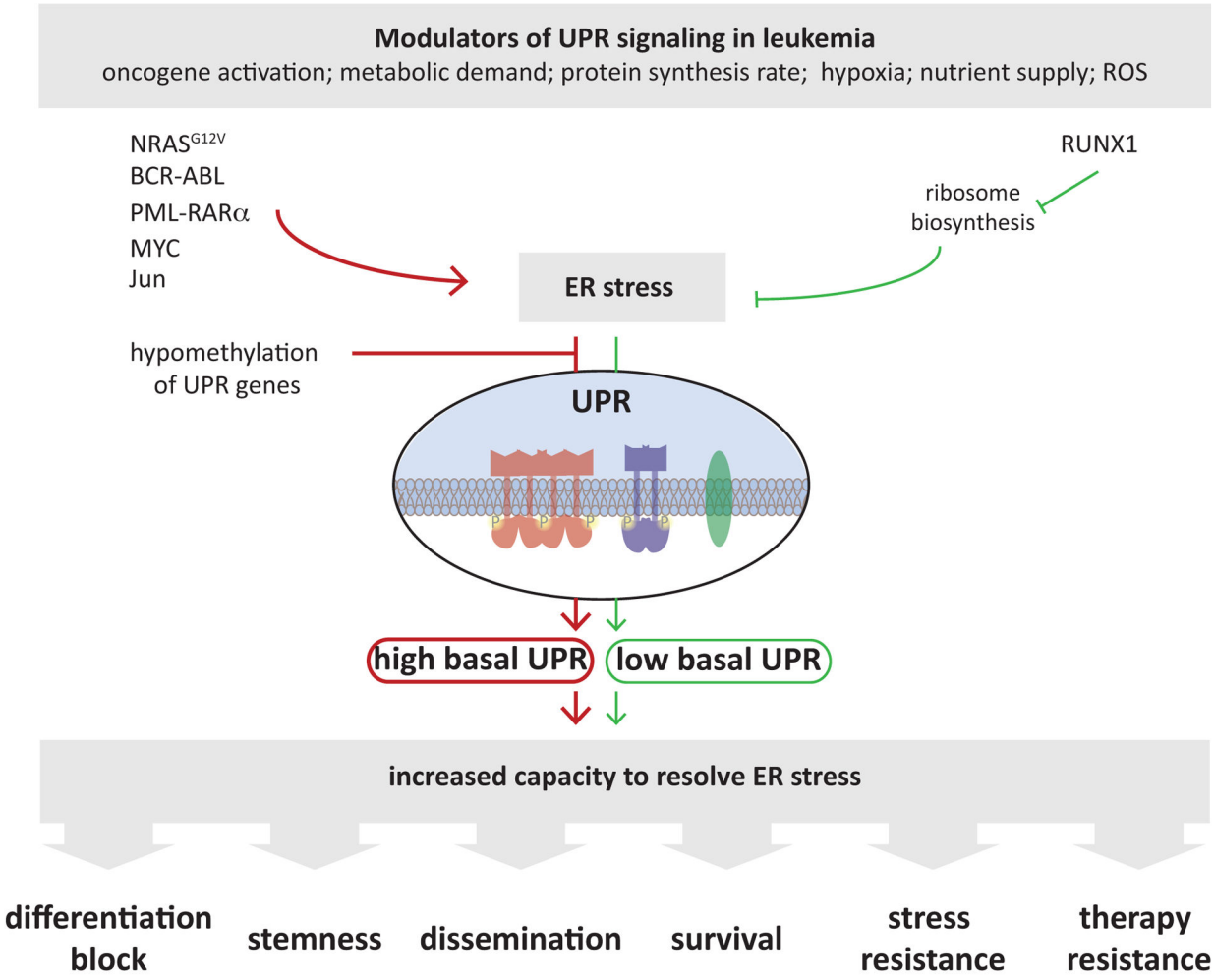


**Figure 2: UPR Signaling in Leukemia Initiation, Progression and Therapy Response**

Leukemic and pre-leukemic cells are exposed to harsh environmental conditions (hypoxia, ROS) that increase protein misfolding and trigger ER stress. Genetic alterations commonly observed in leukemia, such as BCR-ABL, PML-RARα or MYC mutations as well as other oncogenic signaling events, such as increased c-Jun levels, contribute to elevated UPR signaling in leukemias. UPR components can also be upregulated, putatively independent of ER stress in leukemia, e.g. by hypomethylation of UPR genes. All these events (red arrows) culminate to promote UPR signaling and leukemia cells are often characterized by high basal UPR signaling to increases the capacity of cells to cope with stress and restore ER homeostasis. The adaptive signaling events orchestrated by the UPR promotes leukemogenesis and progression by altering diverse cellular processes, from affecting cellular differentiation, as occurs in AML, to increased cell survival and therapy resistance in multiple disease types.

Exemplary alternate routes to maintain ER homeostasis are displayed (green arrows), where RUNX1 mutations decrease ribosome biogenesis, resulting in reduced ER protein load, thereby increasing the capacity of the ER to cope with stressful insults, which also endows RUNX1-mutated cells with increased survival and therapy resistance. Targeting the UPR

thus could prevent leukemia progression and overcome resistance to standard therapeutic regimens.

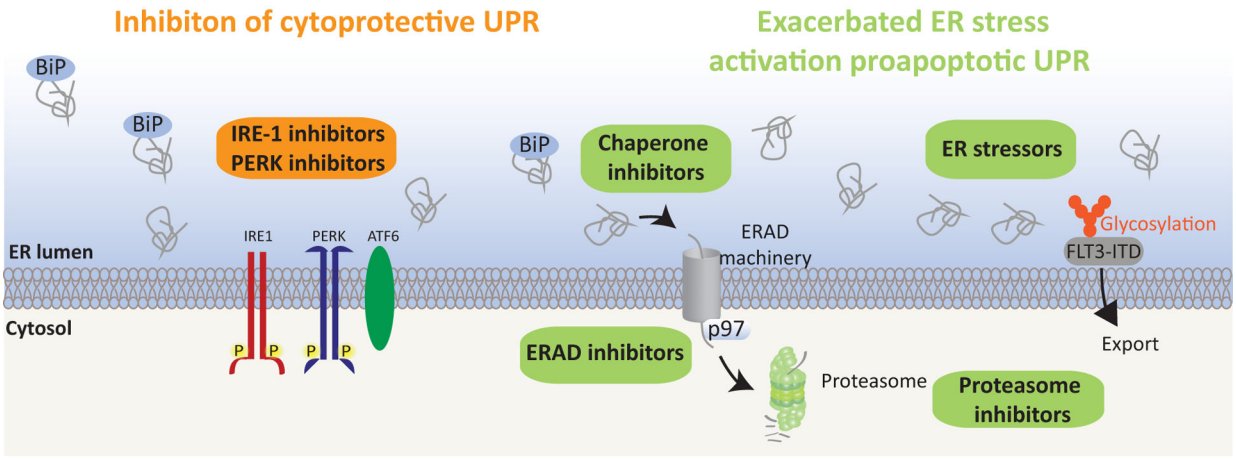


**Figure 3: Therapeutic Targeting of the UPR in Leukemia**

Given the dual ability of the UPR to either promote cell survival or induce death, two differing strategies could be used to target the ER stress response in leukemia. The first (left) includes inhibiting the UPR cytoprotective arms associated with the IRE1/XBP1or PERK axes. The goal of the second approach (right) would be to exacerbate ER stress to promote pro-apoptotic signaling. The rationale is that cancer cells display higher basal ER stress than do normal cells and will thus display increased susceptibility to ER stress-inducing drugs. Among these types of drugs tested preclinically against leukemia are chaperone inhibitors, as indicated, which prevent sequestration of un-/mis-folded proteins in the ER, thereby increasing ER stress. Moreover, ERAD inhibitors, which prevent retro-translocation of un-/misfolded proteins from the ER to the cytosol for proteasomal degradation, may interfere with the ER's ability to resolve stress. Finally, treatment with drugs which prevents protein glycosylation and thus protein export from the ER (right), promotes protein retention in the ER, thereby inducing stress. FLT3-ITD positive AML may be especially vulnerable to such treatment, as in addition to inducing ER stress, these drugs prevent FLT3-ITD glycosylation and export from the ER, thereby decreasing FLT3-ITD oncogenic signaling [81]. Candidate UPR inhibitors are listed in Table 1.

**Table 1:**

List of compounds used to exploit the UPR for leukemia therapy

| Therapeutic drug | Classification/Mechanism | Disease | Development stage | Reference |
|---|---|---|---|---|
| MKC-3946 | IRE1α inhibitor | AML | Preclinical studies | 26934650 |
| STF-083010 | IRE1α inhibitor | ALL, CLL | Preclinical studies | 24821775, 22692508 |
| B-I09 | IRE1α inhibitor | CLL | Preclinical studies | 24812669 |
| A106 | IRE1α inhibitor | ALL, CLL | Preclinical studies | 24821775, 22692508 |
| GSK2606414, GSK2656157 | PERK inhibitors | APL | Preclinical studies | 28776567 |
| Eeyarestatin I (EerI) | ERAD inhibitor | ALL, CLL | Preclinical studies | 19164757 |
| Epigallocatechin gallate (EGCG) | BiP inhibitor | ALL | Preclinical studies | 21517817 |
| Pep42 | BiP inhibitor | ALL | Preclinical studies | 21517817 |
| BMTP-78 | BiP inhibitor | AML | Preclinical studies | 29205207 |
| Bortezomib | Proteasome inhibitor | AML | Phase I-III clinical trials | NCT01861314, NCT04173585, NCT01371981 |
| | | ALL | Phase III clinical trials | NCT02112916 |