Drug Resistance Updates 64 (2022) 100853

Contents lists available at ScienceDirect

Drug Resistance Updates

journal homepage: www.elsevier.com/locate/drup

Understanding ER homeostasis and the UPR to enhance treatment efficacy of acute myeloid leukemia

Martyna Śniegocka [1], Francesca Liccardo [1], Francesco Fazi [*], Silvia Masciarelli [*]

Department of Anatomical, Histological, Forensic & Orthopedic Sciences, Section of Histology & Medical Embryology, Sapienza University of Rome, Rome, Italy

ARTICLE INFO

Keywords:
Acute myeloid leukemia
ER stress
UPR
Oxidative stress
IRS
Proteostasis
Bone marrow niche
FLT3-ITD
Molecular target therapy

ABSTRACT

Protein biogenesis, maturation and degradation are tightly regulated processes that are governed by a complex network of signaling pathways. The endoplasmic reticulum (ER) is responsible for biosynthesis and maturation of secretory proteins. Circumstances that alter cellular protein homeostasis, determine accumulation of misfolded and unfolded proteins in the ER, a condition defined as ER stress. In case of stress, the ER activates an adaptive response called unfolded protein response (UPR), a series of pathways of major relevance for cancer biology. The UPR plays a preeminent role in adaptation of tumor cells to the harsh conditions that they experience, due to high rates of proliferation, metabolic abnormalities and hostile environment scarce in oxygen and nutrients. Furthermore, the UPR is among the main adaptive cell stress responses contributing to the development of resistance to drugs and chemotherapy. Clinical management of Acute Myeloid Leukemia (AML) has improved significantly in the last decade, thanks to development of molecular targeted therapies. However, the emergence of treatment-resistant clones renders the rate of AML cure dismal. Moreover, different cell populations that constitute the bone marrow niche recently emerged as a main determinant leading to drug resistance. Herein we summarize the most relevant literature regarding the role played by the UPR in expansion of AML and ability to develop drug resistance and we discuss different possible modalities to overturn this adaptive response against leukemia. To this aim, we also describe the interconnection of the UPR with other cellular stress responses regulating protein homeostasis. Finally, we review the newest findings about the crosstalk between AML cells and cells of the bone marrow niche, under physiological conditions and in response to therapies, discussing in particular the importance of the niche in supporting survival of AML cells by favoring protein homeostasis.

1. Background

Leukemias are caused by a block in differentiation of hematopoietic precursors which are classified, on the basis of the lineage affected, into lymphoid or myeloid leukemias. They can be either acute (characterized by extended proliferation of immature, non-functional white blood cells) or chronic (characterized by expansion of differentiated cells) (De Kouchkovsky and Abdul-Hay, 2016; Saultz and Garzon, 2016; Jabbour and Kantarjian, 2018; Jimenez et al., 2020). Here we focus on Acute Myeloid Leukemia (AML). AML accounts for around 80% of all leukemias in adults over 60 years old. It is a very heterogeneous pathology caused by chromosomal aberrations and point mutations. AML can be classified into three prognostic risk groups: favorable, intermediate and adverse. Some chromosomal aberrations and point mutations are associated with a better prognosis, like chromosomal translocations t(8;21)(q22;q22), t(16;16)(p13.1;q22), chromosomal inversion inv(16)(p13.1q22) or mutations in nucleophosmin (NPM1). In contrast, other aberrations result in poor prognosis, like the translocation t(6,9)(p23;q34.1) or the internal tandem duplication (ITD) of the tyrosine kinase receptor FLT3 (FLT3-ITD) at high frequency (Pelcovits and Niroula,

List of abbreviations: ER, Endoplasmic Reticulum; UPR, Unfolded Protein Response; AML, Acute Myeloid Leukemia; ROS, Reactive Oxygen Species; OXPHOS, oxidative phosphorylation; FLT3-ITD, internal tandem duplication (ITD) of the tyrosine kinase receptor FLT3; OS, overall survival; BMM, bone marrow microenvironment; MSC, mesenchymal stromal cell; BMSC, bone marrow stromal cell; ERAD, endoplasmic reticulum associated protein degradation; BiP, Binding immunoglobulin Protein; IRE1, inositol-requiring enzyme; ATF6, Activating Transcription Factor 6; PERK, PKR-like ER kinase; PKR, Protein Kinase R; GCN, General Control Non-derepressible; HRI, Heme Regulated Inhibitor; IRS, Integrated Stress Response; eIF2α, Eukaryotic Translation Initiation Factor 2A.

* Corresponding authors.
E-mail addresses: francesco.fazi@uniroma1.it (F. Fazi), silvia.masciarelli@uniroma1.it (S. Masciarelli).
[1] Co-first authors.

https://doi.org/10.1016/j.drup.2022.100853

Available online 8 July 2022

2020; Newell and Cook, 2021). For decades therapy of AML remained constant, based on repeated cycles of intensive chemotherapy with anthracycline and cytarabine. The majority of patients respond with remission after the first cycles of treatment but, unfortunately, most of them relapse because of the frequent emergence of therapy resistant clones. In general, the 5 years overall survival (OS) rate under 60 years is 40–50%, whereas for patients older than 60 years it dramatically drops to 15–20% (Döhner et al., 2017). However, recent therapies are profoundly evolving, with many molecular target drugs approved for clinical trials and clinical practice (Perl, 2017). Nonetheless, these promising therapies, used alone or in combination with standard chemotherapy, very often result in relapses, due to two main factors: clonal selection that promotes the emergence of therapy resistant clones and protection supplied to AML cells by the bone marrow microenvironment (BMM) (van Gils et al., 2021a; McMahon et al., 2019a; Forte et al., 2020). Hence, it is essential to better understand the biology of AML cells and their crosstalk with the BMM in order to tailor more effective therapies.

Endoplasmic reticulum (ER) was first observed in chicken fibroblast-like cells in 1945 (Porter et al., 1945). ER is the largest organelle of most eukaryotic cells formed by a complex network of membrane- enclosed, elongated tubules and flattened disks spreading from the nuclear membrane into a great area of the cytoplasm (Almanza et al., 2019). The ER is responsible for multiple functions. It is the main intracellular site of calcium storage, regulating calcium homeostasis and intracellular signaling pathways (Papp et al., 2020). It is also the major site of lipid biosynthesis for membrane production, lipid droplets formation and energy storage in form of fat accumulation (Almanza et al., 2019). The other main activity of the ER is synthesis, folding, maturation and post-translational modification of secreted and transmembrane proteins, that account for over a third of all cellular proteins. Hence, the ER is part of the network responsible for preservation of proteostasis. The latter is a crasis for “protein homeostasis”, indicating the balance of the complex network of cellular processes that regulate biosynthesis, maturation and degradation of proteins. There are many perturbations which can disrupt ER homeostasis, both intrinsic (like increased protein folding demands, expression of mutated proteins that cannot reach proper maturation, variations in Ca^{++} concentration) and extrinsic (like nutrient deprivation, hypoxia, accumulation of reactive oxygen species (ROS), fluctuations in cytosolic Ca^{++}, hindrance of intracellular protein degradation systems). These cause ER malfunctioning and end up in accumulation of misfolded proteins, a condition defined as ER stress. The ER developed several tight quality control checkpoints to correct or eliminate misfolded proteins, since incorrect secreted proteins would deliver “wrong messages” to other cells. In the event of stress, the ER triggers the Unfolded Protein Response (UPR) a network of pathways aimed to re-establishing homeostasis (Hetz and Papa, 2018). However, if this fails, the response switches from pro-survival “adaptive UPR” to pro-apoptotic “terminal UPR” (Urra et al., 2013; Magagnin et al., 2006; Niewerth et al., 2015; Long et al., 2020).

Disruption of ER stress response and errors in UPR signaling are involved in many physiological and pathological states. As discussed in more details below, the UPR participates to hematopoietic stem cell preservation. Moreover, constitutive activation of some pathways of the UPR is part of the physiology of dendritic, B and T cells and deregulation of the UPR contributes to pathogenesis of infectious diseases (Choi and Song, 2020; Janssens et al., 2014). Misfolding and aggregation of proteins and activation of the UPR underlie many neurodegenerative diseases such as Alzheimer’s, Parkinson’s or Amyloid Lateral Sclerosis (ALS), even though the exact relationships still remain unclear (Ghemrawi and Khair, 2020). ER stress also plays an important role in immunity. Many studies report the relevance of ER stress response dysfunctions in metabolic diseases such as diabetes or obesity (Madhusudhan et al., 2015; Bhattarai et al., 2020). Importantly, different types of cancers reveal crucial impact of ER stress and UPR (Oakes, 2020; Chen and Cubillos-Ruiz, 2021). UPR role in cancer was mainly studied in solid tumors which are characterized by insufficient vascularization, hypoxia and nutrient deprivation leading to high levels of ER stress (Chen and Cubillos-Ruiz, 2021; Urra et al., 2016). Hematological cancers, including leukemias, are characterized by very different features, but cancer cells resident in the bone marrow experience a similarly stressful environment and an important role of the UPR in leukemia development and resistance to therapy is becoming increasingly clear.

In this context, we discuss ER stress and the UPR as possible therapeutic targets for AML treatment, also in relation with the BMM. Recent comprehensive reviews highlight the growing interest in the UPR role in different types of leukemia development and treatment (Khateb and Ronai, 2020a; Martelli et al., 2020; Féral et al., 2021). Here, we focus on AML. After a brief description of the UPR and of its involvement in normal hematopoietic stem cell and in AML biology, we provide an overview about various drugs that are effective in targeting AML and which, directly or indirectly, alter proteostasis, in the perspective of manipulating the UPR to enhance their efficacy and overcome resistance. Finally, we conclude discussing the most recent findings relative to the crosstalk between AML cells and cells of the bone marrow, that involve the proteostasis network.

2. ER stress and the UPR in proteostasis control

Most of the proteins destined to reside in cell membranes or to be secreted are produced by ribosomes bound to the ER membrane and are co-translationally translocated into the ER lumen. Here they are post-translationally processed to acquire the correct 3D conformation and undergo modifications that favor their stability and activity in the extracellular environment, like formation of disulfide bonds and glycosylation. The ER lumen is characterized by an optimal ionic and redox potential to favor these processes, that are guided and catalyzed by a complex folding machinery composed of protein chaperones, glycosylating enzymes and oxidoreductases. Protein chaperones help folding by hydrolyzing ATP thus preventing aggregation of misfolded proteins. The latter tend to aggregate because they exhibit hydrophobic stretches that are normally buried within the folded proteins. To avoid toxic aggregation, ER chaperones, among which the main is BiP (also called GRP78), bind and thus hide hydrophobic residues. Normally, a fraction of proteins cannot reach the final conformation and, after undergoing various rounds of folding attempts, is degraded by a mechanism defined as ER associated degradation (ERAD). Misfolded proteins are retro-translocated into the cytosol via an ER membrane complex, with the aid of the cytosolic ATPase p97, then ubiquitylated and degraded by the proteasome (Christianson and Carvalho, 2022). When the number of client proteins exceeds the protein folding capacity of the ER, misfolded proteins accumulate in the lumen, causing ER stress and activating the UPR. In metazoans, UPR signaling originates from three ER resident transmembrane proteins, IRE1α, ATF6 and PERK. Although the activation mechanism is not completely elucidated, it certainly involves BiP that is normally bound to the luminal domain of the three UPR sensors, maintaining them inactive. When misfolded proteins accumulate in the ER lumen, BiP is titrated away from the sensors and these start their signaling (Kopp et al., 2019).

Release of IRE1α by BiP allows its homo-oligomerization which triggers the kinase activity of the cytosolic domain, leading to trans-autophosphorylation. Once phosphorylated, IRE1α becomes an active endonuclease and executes non-canonical splicing of the mRNA encoding for the transcription factor XBP1, removing 26 internal nucleotides. The spliced form of the messenger encodes for spliced XBP1 (sXPB1) that migrates into the nucleus and drives transcription of genes involved in protein folding, secretion, ERAD, protein translocation into the ER and lipid biosynthesis. Thus, in general, sXBP1 increases the folding capacity of the ER (Acosta-Alvear et al., 2007). However, strong or prolonged ER stress exceeds an oligomerization threshold of IRE1α, that broadens its endonuclease activity to many RNAs, including messenger, ribosomal and non-coding RNAs, located in the proximity of

the ER membrane, that are thus degraded (le Thomas et al., 2021). This activity, defined Regulated IRE1-Dependent Decay (RIDD), modulates important processes like inflammation and especially apoptosis (Maurel et al., 2014; Gómora-García et al., 2021). XBP1 splicing and RIDD are differentially switched on in time and depending on stress intensity. Alternative activity of IRE1α determines cell fate upon ER stress (Ghosh et al., 2014; Han et al., 2009).

ATF6 is a transmembrane protein the cytosolic domain of which is a bZIP transcription factor. When released by BiP, ATF6 can translocate to the Golgi apparatus where two resident proteases (S1P and S2P) cleave it, allowing the transcriptionally active domain (ATF6f) to migrate into the nucleus. ATF6f drives transcription of genes involved in protein folding and ERAD, promotes Ca^{++} homeostasis by upregulating the expression of the ER Ca^{++} pump SERCA and can also heterodimerize with sXBP1 regulating a coordinate transcriptional program (Shoulders et al., 2013).

PERK, similarly to IRE1α, homo-oligomerizes when freed by BiP and undergoes trans-autophosphorylation thus becoming activated. PERK cytosolic domain is a kinase that phosphorylates the α subunit of the translation initiation factor eIF2, inhibiting general protein translation (Harding et al., 1999). This mechanism is an emergency break to quickly reduce the load of client proteins entering into the ER lumen and to allow adaptation of the ER to increased requirements, through the transcriptional programs prompted by ATF6f and sXBP1. While inhibiting translation of most mRNAs, P-eIF2α promotes translation of some that present a peculiar 5′ untranslated region, among which the mRNA encoding the transcription factor ATF4. The latter upregulates transcription of genes involved in protein folding and promotes the antioxidant response and autophagy, thus increasing cell defenses against proteotoxic stress (Harding et al., 2003). Nonetheless, it also upregulates the expression of the transcription factor CHOP (also known as GADD153), which is involved in induction of apoptosis upon excessive ER stress loads (Marciniak et al., 2004). Indeed, CHOP inhibits the expression of the antiapoptotic gene bcl-2 and increases that of BH3-only pro-apoptotic members of the bcl-2 family (Urra et al., 2013). Furthermore, it can also enhance the activity of the death receptor 5 (DR5) (Lu et al., 2014) although it is unclear whether this mechanism is involved in ER stress-mediated apoptosis: recently it has been shown that misfolded proteins can directly bind and activate DR5 (Lam et al., 2020). Eventually, translation must be resumed, because a prolonged block would lead to cell death, and this is obtained by a negative feedback loop. Indeed, CHOP activates transcription of the protein phosphatase GADD34, which de-phosphorylates eIF2α allowing resumption of general translation. However, this must occur when the cell is ready to cope with increased folding demands. Premature resumption of protein translation causes excessive load and overwhelming levels of oxidative stress leading to cell death (Han et al., 2013). Indeed, inhibitors of GADD34 are highly beneficial in pathological states linked to protein misfolding, in which cell death is caused by hyper-activation of the UPR rather than by protein toxicity itself (Das et al., 2015). Cell fate, survival versus apoptosis, is linked to the intensity and duration of stress and is determined by integration of the different UPR pathways. The mechanisms that shift pro-survival toward pro-apoptotic UPR are not completely defined. Nonetheless, many studies are shedding light on the matter, hinting to a complex, interconnected and timely regulated response, extensively discussed by Hetz and Papa (Hetz and Papa, 2018), that involves also pathways linked to JNK and NF-κB. There is a high degree of crosstalk among the pathways constituting the UPR and it plays a main role in determining the final outcome of the response. Just to mention a couple of examples, CHOP expression is regulated not only by ATF4, downstream of PERK, but also by ATF6 (Yang et al., 2020) and ATF6 heterodimerizes with sXBP1.

The UPR belongs to an intricate network that controls proteostasis by directing the processes related to protein biogenesis, maturation and degradation (Brehme et al., 2019). Whereas the core proteostasis network consists of the mechanisms driving protein translation, folding and degradation (including ubiquitin-proteasome system, lysosomes and autophagy), stress response pathways such as the UPR, the Heat Shock Response (HSR), the Integrated Stress Response (ISR) and the oxidative stress response add levels of regulation that allow the cell to cope with and survive situations that unbalance proteostasis. Thus, cell stress responses are tightly linked and activation of one has consequences on the others, especially if the source of stress is intense or prolonged in time. In particular, phosphorylation of eIF2α is central to the ISR, of which the UPR is only one of the components. Very different conditions of stress, (ER stress, starvation, oxidation, heavy metals, viral infection), that share the consequence of perturbing proteostasis, converge on eIF2α phosphorylation via four kinases (PERK, GCN2, PKR and HRI) (Costa-Mattioli and Walter, 2020). There is an obvious level of crosstalk among the different pathways constituting the proteostasis network: for example, accumulation of misfolded proteins in the cytosol activates the HSR and requires increased proteasome activity, that, if the load is excessive, hampers ERAD and activates the UPR. Besides this kind of interaction however, there is also direct crosstalk, for example between the UPR and autophagy, as well as between the UPR and oxidative stress (Senft and Ronai, 2015; Rashid et al., 2015; Zhang et al., 2019a). Indeed, migration of ATF6 to the Golgi apparatus requires formation of a disulfide bond (Oka et al., 2022), that is favored by an oxidative environment and PERK activates NRF-2, the master controller of the oxidative stress response (del Vecchio et al., 2014; Cullinan et al., 2003). These considerations underline that it is very important to acknowledge that the UPR is not an isolated system.

3. ER stress and the UPR in HSC

Hematopoietic Stem Cells (HSCs) are the life-long reservoir that sustains multilineage hematopoiesis (Bao et al., 2019), hence preservation of their integrity is of pivotal importance. They are nested in niches in the bone marrow (BM) in a quiescent state, which helps reducing genotoxic and metabolic stresses due to cell division and active metabolism (Sigurdsson and Miharada, 2018). Low protein synthesis rates sustain self-renewal capacity and preserve integrity of HSCs reservoir by maintaining a high quality proteome, with low amounts of misfolded and unfolded proteins (Hidalgo San Jose et al., 2020). Fetal liver (FL) is the only known site where HSCs actively expand and present increased protein production rate. Nevertheless, FL-HSCs do not show any signs of ER stress activation because bile acids, secreted from maternal and fetal liver, serve as chemical chaperones (Sigurdsson et al., 2016). Balance between pro-survival and pro-apoptotic pathways of the UPR is essential for preservation of homeostasis in normal and stress conditions, in order to block progression of damaged HSCs and prevent leukemogenesis. It has been suggested that the UPR could contribute to avoid expansion of damaged HSCs by differential activation of pro-apoptotic pathways. HSCs are characterized by increased activity of PERK branch and decreased signaling by IRE1α at steady state and upon pharmacological induction of ER stress, in the latter case leading to apoptosis. On the contrary, downstream progenitors preferentially activate the IRE1α pathway and are more resistant to ER stress (Van Galen et al., 2014). In any case, ATF4, downstream of PERK pathway and of the IRS, is essential for HSCs to respond to physiological levels of stress, normally occurring in the BM, like in case of fasting for example (van Galen et al., 2018). Different studies point to a cytoprotective role for IRE1α branch in HSCs. Liu and colleagues showed that inflammation, induced by injection of lipopolysaccharide in vivo, increases IRE1α-XBP1 activity in HSCs cells, thus preserving their clonogenic and reconstitution potential (Liu et al., 2019). DDRGK is an ER membrane associated protein, it is induced upon ER stress and it is essential for the ubiquitin-like modification UFMylation (Banerjee et al., 2020; Gerakis et al., 2019). In this respect, UFM1 is a ubiquitin-like protein which is conjugated to target proteins via an E1-like activating enzyme UBA5 and E2-like conjugating enzyme UFC1 (Banerjee et al., 2020; Gerakis et al., 2019). It has been shown that DDRGK stabilizes IRE1α. DDRGK

depletion, or depletion of RACD, a protein important for stabilizing the complex to which DDRGK belongs, strongly affects HSCs reconstitution capacity in irradiated recipient mice. Analyses of DDRGK depleted HSCs recovered from mouse BM, after transplantation and engraftment, show decreased IRE1α protein and increased PERK phosphorylation, associated with higher rates of apoptosis relatively to control cells (Liu et al., 2017; Zhang et al., 2015).

It has been hinted that hormetic effect of the response to ER stress can play an important role in HSCs preservation (Luchsinger, 2021). Hormesis is an exposure of cells to harmful conditions of mild intensity that precondition cells to activate resistance mechanisms and, as a consequence, prepare them to cope with higher amounts of stress without suffering damages. It is not fully understood how the UPR is activated in adult HSCs in steady-state; however, physiological perturbations, like blood nutrient level oscillations, activate stress responses strictly interconnected with the UPR (van Galen et al., 2018; Ho et al., 2017). Another source of physiological stress could be the hypoxic environment of the BM. Indeed, a characteristic feature of BM is low concentration of oxygen that decreases from about 4% around vessels to about 1% in the endosteum and this is a physiologic condition in HSCs homeostasis (Bruno et al., 2021a). It is known that hypoxic conditions significantly trigger the UPR (Díaz-Bulnes et al., 2020). Indeed, hypoxia inducible factors (HIFs) are critical for HSCs maintenance in the BM niche and depletion of HIF2α in human cord blood $CD34^{+}$ cells leads to increased amounts of reactive oxygen species (ROS) and activation of the UPR and apoptosis (Rouault-Pierre et al., 2013). The ER participates to proteostasis preservation also through the process of ER associated degradation (ERAD), in which misfolded proteins accumulated in the ER lumen are recognized, ubiquitylated and retrotranslocated into the cytosol, where they are degraded by the proteasome. Two studies demonstrate that ERAD is essential for retaining HSCs quiescence and self-renewal capacity, identifying different ERAD targets involved (Liu et al., 2020; Xu et al., 2020). Interestingly, one of these, MPL (the cell surface receptor for thrombopoietin), is also essential for proper localization of HSCs in the bone marrow niche (Xu et al., 2020). Recently, it has been proposed that the mitochondrial unfolded protein response (UPR^{mt}), a protective program controlling proteostasis in mitochondria, could be a main regulatory mechanism for maintenance of adult stem cells (Mohrin et al., 2018). It has been suggested that the UPR^{mt} is activated when stem cells shift from quiescence to proliferation state, however further research is certainly needed.

The BMM plays a critical role in maintaining HSCs in a proper state. Physiologically, BMM can be divided into the endosteal and vascular niches. The first one mainly contains osteoblasts, which take part in supporting quiescence and pluripotent state of HSCs. The second one contains endothelial cells, the main role of which is to support HSCs proliferation and differentiation (Bruno et al., 2021a). The UPR is involved in bone homeostasis, thus participating in maintenance of a healthy BMM. Indeed, physiological ER stress is encountered while osteoblasts and chondrocytes differentiate, because of production of bone and cartilage extracellular matrix components (Tavasolian et al., 2020) and it is necessary for cell differentiation. In particular, PERK is a main regulator of osteoblast proliferation and differentiation (Tavasolian et al., 2020; Guo et al., 2021, 2020).

4. ER stress and the UPR in AML

The UPR plays a major role in leukemogenesis, favoring leukemic cell survival and resistance to therapy. First evidence supporting a role of the UPR in hematologic malignancies arose from the increased expression of the spliced form of XBP1 in $CD138^{+}$ primary myeloma cells collected from patients (Schardt et al., 2011). Leukemia is supported by leukemic stem cells (LSCs), which have properties distinct from the bulk leukemia cells, including capacity to self-renew and develop drug resistance (Khateb and Ronai, 2020a). The advantage provided by UPR activation in LSCs is to enable them to handle increased metabolic demands related to higher rates of cell proliferation. Furthermore, leukemic cells are subjected to different intrinsic sources of proteotoxic stress. Indeed, leukemia cells produce high amounts of ROS (Sillar and DeIuliis, 2019) and are often characterized by expression of mutant proteins that in some cases are misfolded or misplaced in cell compartments (Schmidt-Arras et al., 2005; Manara et al., 2014). Pabst and colleagues found activation of the UPR in about 25% of 122 AML patients and showed increased levels of sXBP1. The presence of sXBP1 correlated with higher expression of the ER chaperones BiP, calreticulin and PDI, which are UPR target genes. They also showed, in vitro, that calreticulin and PDI form a complex able to inhibit translation of C/EBPα, a main hematopoietic transcription factor that takes part in maintaining LT-HSC reservoir and is essential for myeloid differentiation. Accordingly, AML patients with higher sXBP1 and PDI levels presented lower amounts of C/EBPα protein. UPR activation was more frequent in AML subtypes M2 and M3 (FAB classification) (Haefliger et al., 2011). Other studies detected UPR activation in AML; however, there is no clear correlation with genetic features or prognosis (Tanimura et al., 2011; Sun et al., 2016). Different studies point to an important role of the IRE-1α branch for AML cell survival and expansion. Zhou and colleagues identified the transcription factor Jun as a direct regulator of UPR target genes and Jun expression is significatively increased in AML driven by different genetic defects, like t(8;21), t(15;17), inv(16), and 11q23 translocations, as well as in complex or normal karyotypes. The authors demonstrated that Jun directly binds to the promoters of XBP1, ATF4 and CHOP augmenting their expression and that Jun is up-regulated in AML cells upon ER stress induction in vitro. Importantly, silencing ATF4 or XBP1 in a murine model of AML driven by the MLL-AF9 fusion protein, significantly prolonged survival of engrafted syngeneic mice (Zhou et al., 2017). Murine pre-leukemic stem cells, generated by conditional expression of the oncogene $NRAS^{G12D}$, showed increased resistance to ER stress in vivo and higher reconstitution potential in irradiated mice relative to normal HSCs. Loss of one IRE-1α allele impaired $NRAS^{G12D}$ pre-LCS resistance to ER stress and competitive reconstitution advantage (Liu et al., 2019). Further support to the hypothesis that the IRE-1α-XBP1 pathway sustains AML cells is provided by the observation that the XBP1 promoter is highly hypomethylated in AML samples, in correlation with high expression levels (Sun et al., 2016).

Leukemic cells must cope with imbalance of redox homeostasis and it is well established that the UPR and the oxidative stress response are tightly related, each one triggering the other (Zhang et al., 2019a). We have already mentioned that leukemic cells produce high amounts of ROS. Furthermore, the hypoxic environment of the BM is a physiological condition for HSC, hence adaptation to hypoxia is essential for AML cells. It is established that HIF-1α is vital for AML LSCs (Wang et al., 2011). Acute promyelocytic leukemia (APL) LSCs express high levels of HIF-1α, which regulates genes involved in cell migration, chemotaxis, neo-angiogenesis and self-renewal (Percio et al., 2014; Coltella et al., 2014). The link between hypoxia, oxidative stress and the UPR in AML is supported by a study in which HIF-2α was depleted: silencing of HIF2α in human primary AML cells significantly impaired their engraftment capacity in an orthotopic mouse model. *Ex vivo* experiments demonstrated that HIF2α-depleted primary AML cells are more sensitive to ER stress induced apoptosis and such sensitivity is due to generation of high levels of oxidative stress (Rouault-Pierre et al., 2013). Chemotherapeutic agents generate oxidative stress (Irwin et al., 2013) and the UPR has been linked to development of drug resistance in different types of cancer (Bahar et al., 2019). Besides re-establishing protein homeostasis altered by hyper-oxidative conditions, the UPR participates in reduction of oxidative stress by direct modulation of the antioxidant response master regulator Nrf-2 (del Vecchio et al., 2014; Cullinan et al., 2003). For example, the PERK-Nrf2 pathway participates to development of resistance to inhibitors of histone methyltransferase G9a. G9a inhibitors or genetic depletion of G9a induce apoptosis in AML cell lines and reduce the frequency of LSCs in AML mouse models (Lehnertz et al.,

2014; Kondengaden et al., 2016). It has been shown that G9a regulates tolerance to oxidative stress, by avoiding over-activation of the response. The LSC-like AML cell line KG1 is particularly resistant to G9a inhibitors, due to activation of the PERK-Nrf2 pathway that suppresses excessive oxidative stress and inhibition of PERK repristinates sensitivity to G9a inhibitors.

5. Targeting the UPR as a therapeutic strategy for AML

Prognosis of AML patients is poor, with a 5 year survival rate below 30%. Although in the past 50 years no new drug was approved for AML treatment (Levin et al., 2021), in the last few years FDA approved nine novel agents both for newly diagnosed and relapsed/refractory AML (Ahmadmehrabi et al., 2021). However, conventional chemotherapy is still the most common approach and prognosis of AML patients remains dismal because they very often suffer from relapse of the disease (van Gils et al., 2021a). In the case of a relapse, it is essential to repeat mutational screening and cytogenetic analysis because of clonal evolution of the disease (Thol and Ganser, 2020). The secondary cancer does not respond to the drugs used in the first line of treatment due to resistance of cancer cells which survived and gained the ability to evade or cope with therapeutic agents. The UPR is activated by many chemotherapeutic drugs and it also takes important part in chemoresistance (Bahar et al., 2019). Cancer cells are able to survive under prolonged conditions of ER stress, due to unfavorable environment, hypoxic and poor in nutrients, through activation of pro-survival UPR. Indeed, drug resistant tumor cells are additionally resistant to ER stress-induced cell death (Salaroglio et al., 2017). These considerations, together with the role played by the UPR in survival and proliferation of AML cells and in crosstalk with the BM niche (reviewed below), lead to the conclusion that targeting UPR could be a successful strategy to induce death of AML cells. Two approaches are possible, depending on the context: inhibition of adaptive UPR or activation of terminal UPR (Fig. 1).

As discussed above, evidence points to the IRE1α pathway as a key factor for AML cells survival and expansion. Different IRE1α inhibitors have been developed and have been tested in models of multiple myeloma, B-acute lymphoblastic leukemia and chronic lymphatic leukemia suggesting a possible efficacy of this strategy (reviewed in (Khateb and Ronai, 2020a)). It has also been shown that IRE1α inhibitors exhibit cytotoxicity against AML cell lines and primary blasts at concentrations that did not affect healthy bone marrow mononucleated cells (Sun et al., 2016). However, the matter must be further investigated and currently there are no studies assaying IRE1α inhibitors in AML in vivo models. Accordingly, silencing of BiP in the APL cell line NB4 increased sensitivity to cytosine arabinoside (Wey et al., 2012).

It is unlikely that any UPR inhibitor will show sufficient efficacy against AML cells when used alone. However, on the one hand, inhibition of specific elements of the UPR could be useful to potentiate the effects of drugs targeting other pathways that also activate the UPR as a protective response. On the other hand, drugs or intrinsic features like expression of mutant proteins, that generate proteotoxic stress, could render AML cells more sensitive to pharmacological activation of the UPR, tipping the balance toward apoptosis. Thus, below we discuss drugs that exhibit cytotoxic activity in AML models, that are not specific UPR inducers but that, besides other effects, affect the UPR. Indeed, they could be candidates for a combined therapy with drugs blocking the UPR. Sorafenib is a multikinase inhibitor, effective in AML, which activates the UPR. Initially, it was developed as a specific inhibitor of C-Raf and B- Raf, but it was shown to inhibit other tyrosine kinases which are involved in tumor progression, for example VEGFR-2,3, PDGFR-β, c-Kit and FLT3 (Rahmani et al., 2007a). Internal tandem duplication of

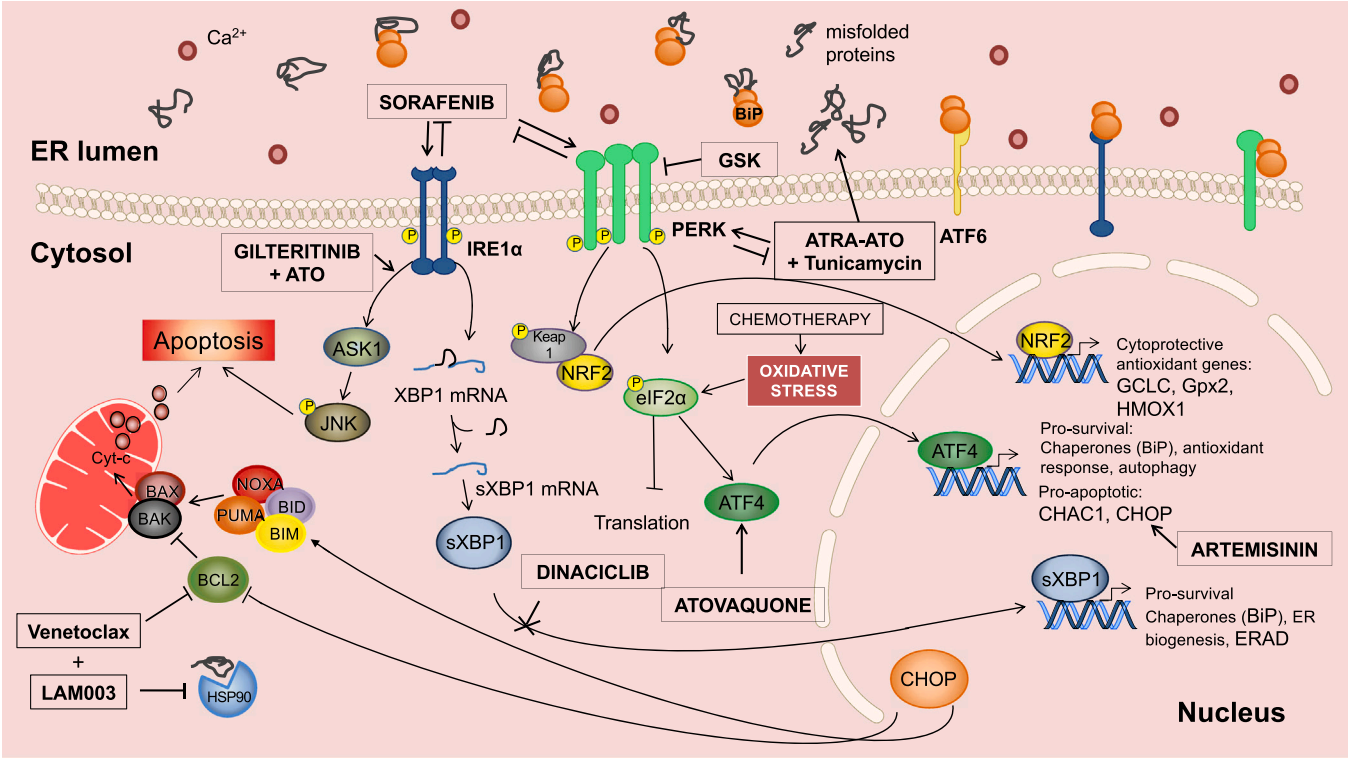

Fig. 1. The UPR and the IRS are part of the cell responses to drugs. Different branches of the UPR play a protective or a pro-apoptotic role in response to treatments aimed at inducing AML cell death. Understanding these mechanisms is of paramount importance to manipulate the stress responses in order to increase treatment efficacy. For example, sorafenib cytotoxicity is reduced by the pro-survival activity of IRE1α and repression of this pathway increases sorafenib action. A combination of gilteritinib and ATO, on the contrary, exploits the pro-apoptotic signaling of IRE1α. Chemotherapeutic drugs increase oxidative stress and activate the IRS through phosphorylation of eIF2α, thus inhibition of this pathway could ameliorate their potency. Indeed, PERK pathway inhibition boosts the cytotoxic effects of the combination of RA, tunicamycin (Tm) and ATO, the efficacy of which relies on the generation of ER and oxidative stress.

FLT3 (FLT3-ITD) is a mutation present in about 25% of AML patients and it is associated with poor prognosis and high relapse rate. Sorafenib has been extensively studied as a first generation FLT3 inhibitor: in combination with upfront chemotherapy, it improved survival without relapse in young patients, but not in elderly ones (Röllig et al., 2015). Efficacy of monotherapy with sorafenib in patients with relapsed/refractory (r/r) FLT3-ITD$^+$ AML, administered as palliative therapy, is restricted by development of tyrosine-kinase inhibitor resistance (Metzelder et al., 2012). However, two independent clinical trials recently demonstrated that sorafenib prevents AML relapses after allo-hematopoietic stem cell transplantation (HSCT) in patients carrying FLT3-ITD mutation, thus it is very efficient for maintenance therapy (Xuan and Liu, 2021; Burchert et al., 2020). Sorafenib administration in human U937 leukemic cell line induced ER stress as shown by phosphorylation of PERK and IRE1α, XBP1 splicing, phosphorylation of eIF2α and inhibition of protein translation. Notably, hindrance of the UPR by silencing or expression of dominant negative constructs of PERK or XBP1 in U937 and K562 cells increased sensitivity to sorafenib; on the contrary, overexpression of IRE1α in K562 augmented resistance Rahmani et al., 2007a. Sorafenib has been shown to induce the UPR also in hepatic stellate cells and in hepatocellular carcinoma cells (Sharma et al., 2021; Lin et al., 2021). Thus, pharmacological interference with the UPR by the use of PERK or Ire1α inhibitors could increase FLT-ITD$^+$ AML cell sensitivity to sorafenib. Accordingly, Moses and colleagues showed that sorafenib efficacy is synergistically increased by an analog of the antimalarial drug artemisinin (ART838), which increases ROS and expression of CHOP. Importantly, they showed that the triple combination of sorafenib, ART838 and the anti-apoptotic protein BCL-2 inhibitor venetoclax, significantly prolonged survival of an in vivo orthotopic AML model (Moses et al., 2021). AML cell lines and primary blasts resistant to sorafenib show activated PI3K/mTOR pathway and a PI3K/mTOR inhibitor, Gedatolisib, effectively blocked growth of resistant clones both in vitro and in vivo (Lindblad et al., 2016). Moreover, genome wide CRISPR screening of FLT3-ITD$^+$ AML primary cells identified negative regulators of MAPK and mTOR pathways as a mediator of resistance to sorafenib (Damnernsawad et al., 2020). Intriguingly, recent studies indicate that the mTOR pathway can activate the UPR (Fang et al., 2021; Gaudette et al., 2020).

Gilteritinib, formerly known as ASP2215, is a potent and selective second generation FLT3 inhibitor. As a single agent it improves mean survival of patients with relapsed or refractory FLT3 mutated AML compared with conventional chemotherapy (Levis and Perl, 2020a; Perl et al., 2019). Gilteritinib is a type I inhibitor and as such it mimics ATP, thus its binding is less dependent on conformation of the activation loop than type II inhibitors. It is a multi-target drug, but it inhibits FLT3 more specifically than other kinases. Interestingly, it also has an inhibitory effect against AXL, which contributes to chemoresistance in AML (Park et al., 2015). During first few days of Gilteritinib administration, blasts are cleared from peripheral blood, but bone marrow response occurs slowly Levis and Perl, 2020a. FLT3 inhibitors used as monotherapy caused initial remission but ended in disappointing final results with relapses emerging in a short time. Studies aimed at elucidating the mechanism of resistance showed that it is due not only to additional mutations in FLT3 but also to development of clones with non-related mutations (for example involving the RAS and MAPK pathways) McMahon et al., 2019a. In order to prevent polyclonal drug resistance in r/r AML patients, gliteritinib may be combined with other therapeutic agents, such as for example the oxidative stress inducer arsenic trioxide (ATO). Hu et al (Hu et al., 2020)., showed that the combination of gilteritinib and low doses of ATO has synergistic effect on reduction of proliferation and increased rate of apoptosis in vitro and in vivo in FLT3-ITD$^+$ AML cell lines. This effect is dependent on IRE1α-JNK signaling indicating that in this context activation of the UPR IRE1α branch upon treatment promotes AML apoptosis (Hu et al., 2020).

Another interesting drug, which partially hinders the UPR, is dinaciclib. Dinaciclib (SCH727965) is an inhibitor of cyclin-dependent kinases (CDKs) 1,2,5 and 9. Deregulation of cell cycle control caused by abnormal CDKs activity is observed in most cancers, including AML (Cucchi et al., 2020; Šimoničová et al., 2022; Grant and Roberts, 2003). Exposure of human leukemic and myeloma cells to nanomolar concentrations of dinaciclib inhibited sXBP-1 nuclear localization and expression of BiP, which are prompted in response to treatment with the ER stress inducers tunicamycin and thapsigargin, increasing their cytotoxic effects (Nguyen and Grant, 2014). However, dinaciclib does not affect IRE1α activation upon tunicamycin or thapsigargin exposure, thus it hinders sXBP1 accumulation through a mechanism that remains to be clarified. Interestingly, silencing CDK1 and CDK5 by shRNA resulted in the same effects upon ER stress induction by Tunicamycin or Thapsigargin. These findings support the hypothesis that specific CDKs could be key components linking cell cycle regulation and the UPR. Furthermore, dinaciclib can activate the immune system to recognize and eliminate solid cancer cells (Md Sakib Hossain et al., 2018) and Yun et al., reported that it enhances natural killer cells' ability to target AML cells (Yun et al., 2019).

The anti-parasitic drug atovaquone induces apoptosis in AML cell line and primary blasts both in vitro and in vivo, in an orthotopic AML mouse model, with negligible effects on normal bone marrow cells. Stevens and colleagues demonstrated that micromolar concentrations of atovaquone activate the phospho-eIF2α/ATF4 axes, with consequently increased expression of the pro-apoptotic CHOP and CHAC1 genes. Another ATF4 target, which was shown to be up-regulated by atovaquone is REDD1, that in turn negatively regulates the mTOR pathway. Interestingly, atovaquone inhibits both mTOR and mitochondrial respiration, key elements for survival of chemotherapy-resistant AML cells (Stevens et al., 2019).

Asperuloside is extracted from different traditional Chinese medical plants and it is known for its anti-tumor and anti-inflammation properties. It has been recently shown that asperuloside leads AML cell lines and human primary blasts to apoptotic cell death and reduces tumor growth in a xenograft model obtained by subcutaneous injection of U937 AML cells. Asperuloside activates the UPR, as indicated by increased phosphorylation of PERK, eIF2α and IRE1α and by up-regulated expression of ATF6, sXBP1, BiP and CHOP. Downregulation of BiP by shRNA in U937 cells, partially reduced UPR activation upon treatment with asperuloside and reduced its cytotoxic effects, suggesting a pro-apoptotic role of the UPR in this context (Rong et al., 2020).

The focus of the current review is on the ER stress response but it must be kept in mind that proteome homeostasis relies on the crosstalk among many different cell pathways, among which, apart from the UPR, there are the heat shock response, the mitochondrial UPR, autophagy, the proteasome/ubiquitin system as well as the oxidative stress response. These pathways are tightly related and each one can influence the others. Various studies point to the promising strategy of aggravating cell stress responses to proteostasis imbalance to induce AML cell death. In order to generate proteotoxic stress and redirect AML cells toward terminal UPR, we proposed the use of a combination of the differentiating agent retinoic acid (RA), the ER stress inducer tunicamycin and the oxidative stress inducer ATO. RA is known to be highly effective as a cure for acute promyelocytic leukemia (APL) especially when it is associated with chemotherapy or with ATO. RA is a differentiating agent and at pharmacological doses it is able to resume APL blast granulocytic differentiation and partially re-activate cell metabolism in other types of AML. We found that APL cell lines and primary blasts undergoing RA-mediated differentiation are more sensitive to tunicamycin-induced ER stress and incur in cell death at tunicamycin doses that are not effective in the absence of RA. Importantly, addition of the oxidative stress inducer ATO showed a synergistic effect with RA and tunicamycin both in RA-sensitive and RA-resistant NB4 cell line, resulting in stronger cytotoxicity (Masciarelli et al., 2018). We have shown that activation of the PERK/P-eIF2α/AFT4/CHOP pathway is essential to counteract the toxic effects of the combination. Indeed, inhibition of PERK phosphorylation by the inhibitor GSK2606414

markedly increased cell death upon treatment, whereas the GADD34 inhibitor Guanabenz, that leads to prolonged phosphorylation of eIF2α, completely blunted the effects of the combination. Moreover, the combination of RA, tunicamycin and ATO is also highly effective against FLT3-ITD$^+$ AML primary blasts and AML cell lines expressing MLL-AF6, MLL-AF9 and FLT3-ITD oncogenic proteins. These mutant proteins generate an intrinsic proteotoxic stress that renders the cells specifically sensitive to pharmacological induction of the same kind of stress, allowing the use of low doses of each of the drugs used in combination, with the possible advantage of modest general toxicity. Indeed, the combination of RA, tunicamycin and ATO showed negligible effects on normal bone marrow cells (Masciarelli et al., 2019). Liang and colleagues showed, by molecular docking analysis, that both RA and ATO can bind FLT3-ITD specific residues, but not wt FLT3. Treatment of FLT3-ITD$^+$ AML cells with high doses of RA plus ATO inhibits FLT3 phosphorylation and induces its degradation via the ubiquitin-proteasome system (Liang et al., 2020).

In the context of UPR induction and alteration of proteostasis in AML, one must also consider the Heat Shock Protein Response. In particular, one should mention the HSP90 chaperone. Heat shock protein 90 is an adenosine triphosphate-dependent chaperone and it is part of the chaperome, a big family of proteins, including chaperones, co-chaperones and adaptors, required for maintenance of proteostasis. Interestingly, HSP90 has recently been shown to be the key component of a dynamic multiprotein complex called “epichaperome”, a spatially and functionally integrated network in which mutant oncoproteins are stabilized, resulting in enhanced tumor cell survival (Rodina et al., 2017). For this reason, different HSP90 inhibitors have been developed, and expectedly since they perturb proteostasis, it has been shown that they can activate the UPR (Uddin et al., 2021; Kubra et al., 2020). Interestingly, the HSP90 inhibitor LAM003 shows high anti-leukemic activity specifically against FLT3-ITD$^+$ AML cell lines and human primary blasts in vitro and in an AML orthotopic mouse model in vivo. Indeed, the FLT3-ITD oncoprotein relies on HSP90 for its stability in order to avoid degradation (Yu et al., 2014). Importantly, LAM003 displayed synergistic activity with chemotherapeutic drugs, FLT3 inhibitors (FLT3i) and Venetoclax (Beeharry et al., 2019a). This study points out that LAM003 is promising for the treatment of relapsed/refractory (r/r) AML patients after FLT3 inhibitor therapy. Indeed, it is effective against FLT3i resistant cells, demonstrating that HSP90 has a pivotal role in stabilization of mutant proteins. Furthermore, resistance to FLT3i is due also to the contribution of bone marrow stromal cells, which support AML blasts, dramatically affecting drug efficacy, and LAM003 was shown to overcome this mechanism of drug resistance. The points of strength of this research article are the attention given to the different mechanisms conferring resistance and the conclusion that pharmacological perturbation of proteostasis, in combination with agents interfering with survival pathways, can overcome resistance mechanisms.

It is well known that nicotinamide adenine dinucleotide (NAD$^+$) regulates growth and metastatic potential of many cancer cells. Since also leukemic cells are characterized by higher levels of NAD$^+$ compared to healthy cells, it was proposed to use inhibitors of nicotinamide phosphoribosyltransferase (NAMPT), a rate-limiting enzyme in the biosynthesis of NAD$^+$ from nicotinamide, including for example APO866. Cagnetta and colleagues showed that even though APO866 was active, it showed limited cytotoxic effects on patient derived AML cells. Since membrane transporter proteins play a major role in multidrug resistance, they propose to inhibit the activity of a key efflux transporter of this family, P-glycoprotein (Pgp), in order to enhance the cytotoxic activity of APO866 on AML cells. Interestingly for the aim of this review, APO866 and Pgp inhibitors synergistically lead to an increase in ER stress, ATP shortage, and cell death of AML cell lines and primary blasts, without affecting healthy leukocytes and HSCs (Cagnetta et al., 2015). It has also been shown that APO866 causes leukemia cell death by increasing reactive oxygen/nitrogen species, in a PARP1-dependent manner and that the combination of APO866 with oxidative agents (H_2O_2) or with DNA damaging agents (e.g., etoposide) shows synergistic, cytotoxic effects (Cloux et al., 2019). Furthermore, Jones and colleagues showed that the APO866-dependent decrease in the amount of NAD$^+$ restores sensitivity of r/r LSCs to the combination of venetoclax and azacitdine. They found that r/r LSCs rely on NAD$^+$ to increase their amino acid metabolism and fatty acid oxidation to sustain OXPHOS, thus overcoming the effects of venetoclax and azacytidine (Jones et al., 2020a). In conclusion, inhibition of the biosynthetic pathways of NAD+ with drugs like APO866 could be very promising to combat AML, also in combination with drugs that perturb redox state and proteostasis, like oxidative agents or ER stress inducers.

Finally, in the same context, it is important to note that chemotherapeutic drugs used in AML induce oxidative stress (Hosseini et al., 2019; Li et al., 2020) and activate the integrated stress response (IRS) (Williams et al., 2020) providing support for the suggestion that their combination with drugs that perturb proteostasis could be a viable therapeutic strategy for AML.

6. Targeting the UPR to prevent the protective role of the BM niche toward AML cells

The bone marrow microenvironment of leukemia patients undergoes a complex process of specific adaptations which exert important effects on the hematopoietic stem cell compartment, creating a favorable habitat for LSCs and increasing drug resistance. Functional changes in stromal components in the bone marrow must be considered as one of the aspects of leukemia biogenesis, because they create an aberrant microenvironment which supports survival and expansion of leukemic cells (Doron et al., 2018a; Méndez-Ferrer et al., 2020a). Thus, investigation of leukemia must take into account the BMM. Numerous studies conducted on cell lines or primary AML blasts in culture, suggest possible treatments to eliminate AML cells, but the efficacy of many of these could be strongly reduced by the conditions created by the crosstalk between AML cells and the BMM Beeharry et al., 2019a. Full understanding of intercellular communication between AML cells and their niche is important to identify new therapeutic targets. Various reviews readily covered the most recent discoveries concerning the crosstalk between AML and the BMM and the role of the BM niche in supporting AML (Nehrbas et al., 2020; Bruno et al., 2021a; Doron et al., 2018a; Méndez-Ferrer et al., 2020a). Here we review the main points, mostly focusing on findings showing how the connections between AML and stromal cells involve proteostasis (Fig. 2).

The BMM is composed of different cell populations: mesenchymal stem cells (MSCs), BM stromal cells (BMSC), osteoblasts, endothelial cells, adipocytes, peripheral neurons and Schwann cells. Single cell RNA sequencing in AML bearing mice identified a number of subpopulations, among the populations just mentioned, that are affected by AML (Baryawno et al., 2019). Under physiological conditions, the BMM maintains the hematopoietic stem cell compartment and promotes lineage differentiation. Rapid growth of leukemia disrupts stromal functions and results in pancytopenia caused by many direct and indirect effects on HSCs.

Cells communicate through secretion of cytokines, neurotransmitters, peptides (hormones and growth factors), and small nucleic acids. Important means of communication are exosomes, membrane derived extracellular vesicles (EVs) with a small diameter, ranging between 30 and 150 nm, able to traffic proteins, lipids, cytokines and a variety of non-coding RNAs and DNAs among cells (Bernardi and Farina, 2021). Exosomes can influence response of the immune system, creating a favorable microenvironment for cancer cells and modulate angiogenesis. AML-derived exosomes affect the immune response, for example by decreasing natural killer (NK) cells cytotoxicity in vitro (Szczepanski et al., 2011) and interfering with cellular immunotherapy via delivery of immunosuppressive molecules (Hong et al., 2017). EVs released by AML cells create a favorable environment for leukemia development by

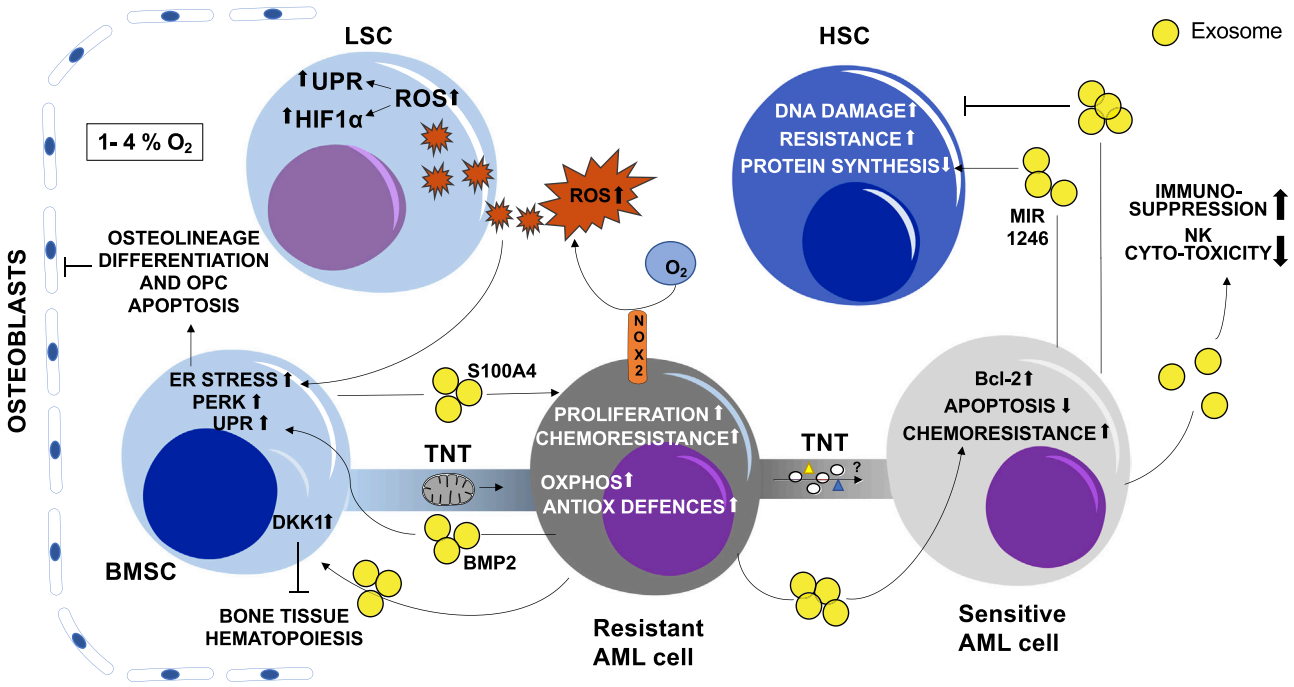

Fig. 2. *Crosstalk in the bone marrow niche.* BMSCs and MSCs deliver a variety of molecules (among which are ncRNA and proteins) and even mitochondria to AML cells by extracellular vesicles and tunneling nanotubes (TNT), increasing their proliferation rate, metabolic activity (OXPHOS), resistance to oxidative stress as well as chemoresistance. AML cells, on the other hand, release factors that transform the niche to their advantage. They are able to repress the immune response, inhibit normal hematopoiesis and osteogenesis. Osteogenesis inhibition is dependent on generation of ER stress and activation of the UPR, "transferred" from AML cells to MSCs via extracellular vesicles. AML clones that developed higher resistance to apoptosis can transfer such resistance to sensitive cells.

directly inhibiting hematopoiesis by different mechanisms (Baryawno et al., 2019; Hornick et al., 2016; Horiguchi et al., 2016), among which is impairment of protein synthesis. EVs are able to inhibit the mTOR pathway by delivering miR-1246, thus reducing protein synthesis in long term HSC (LT-HSC) and rendering them more resistant toward competition with leukemic cells (Abdelhamed et al., 2019). As discussed above, under physiological conditions, reduced protein synthesis promote HSC self-renewal and preservation (Sigurdsson and Miharada, 2018). However, Abdelhamed and colleagues found that, in the presence of AML-derived EVs, quiescent LT-HSC accumulate DNA damage, favoring the development of novel leukemic clones with consequent increased possibility of relapses. These findings may pave the way toward potential novel targets, involved in regulation of protein synthesis, to promote residual physiological hematopoiesis and to reduce the risk of relapses (Abdelhamed et al., 2019). A recent study showed that BMSCs from healthy donors produce exosomes that increase proliferation, migration capability and resistance to chemotherapy of AML cell lines and that these effects are, at least in part, due to increased expression of the calcium binding protein S100A4 (Lyu et al., 2021). Although these findings were obtained in cell lines and must be further investigated, they suggest that BMSCs can transfer factors that increase tumorigenicity even if not induced by AML cells. Furthermore, various studies report that exosomes released by AML cells transform the BM niche. Primary AML cells and cell lines produce exosomes able to alter growth factor and cytokines secretion by BM stromal cells (Huan et al., 2013, 2015). It is well defined that exosomes secreted by AML cells play a key role in transforming the BM niche to favor leukemia growth and proliferation, increasing MSCs and decreasing osteoblast progenitors and bone formation. Different underlying molecular mechanisms have been described. Kumar and colleagues reported that preconditioning of mice with AML-derived exosomes accelerated AML engraftment in an orthotopic humanized mouse model. Intravenous injection of AML exosomes induced expression of DKK1 in the BM stromal compartment, leading to impaired hematopoiesis and bone loss. Indeed, DKK1 is an inhibitor of the Wnt signaling pathway that is important for osteoblast differentiation and hematopoiesis (Kumar et al., 2018). Interestingly, Wnt pathway down-regulation is related to ER stress and UPR activation in preadipocytes (Zhang et al., 2018) and DKK1 over-expression induces ER stress by down-regulating Wnt pathway and causing JNK phosphorylation in endothelial cells (Di et al., 2017). Activation of the eIF2α branch of the UPR in endothelial cells, following over-expression of DKK1, leads to apoptosis and this effect is reversed by treatment with the eIF2α phosphorylation inhibitor salubrinal (Di et al., 2017). It is worth to speculate that, if increased expression of DKK1 in BM stromal cells, mediated by AML-released exosomes, activates the eIF2α pathway as well, inhibition of this pathway can render the BMM less hospitable for leukemic cells. Importantly, it has been shown that ER stress and the UPR in the BM niche contribute to AML-driven changes in stromal cells. Doron and colleagues (Doron et al., 2017) demonstrated that EVs, produced by AML cell lines engrafted in an orthotopic mouse model, are able to transfer ER stress to MSCs and osteoblastic progenitor cells (OPCs) and trigger the UPR. Activation of the UPR in MSCs promoted osteolineage differentiation, in accordance with previous literature, at the same time increasing apoptosis rate of OPCs, thus changing the BMM composition. The authors suggest that ER stress is transmitted to stromal cells by EVs carrying bone morphogenic protein 2 (BMP2), a strong osteogenic cue (Doron et al., 2017). Furthermore, human AML cells isolated from mouse BM after leukemia engraftment and development, exhibited activated UPR and induction of ER stress in AML cell lines by thapsigargin was sufficient to increase secretion of EVs enriched in BMP2. Thus, this study suggests that AML cells exploit the UPR to adapt to metabolic stress to which they are exposed in the BM niche and ER stress is then transmitted to the stromal compartment by EVs to render the niche more hospitable.

It has been shown that relative levels of anti-apoptotic and pro-apoptotic members of the bcl-2 family are a determinant of resistance to apoptosis and are associated with AML prognosis. Even though the main site of action of the bcl-2 family is mitochondria, many of these proteins, among which Bcl-2 itself, localize at the ER where they integrate stress signaling networks and regulate cell death by controlling

calcium release from the ER and by modulating the UPR and autophagy. Bcl-2 interacts with inositol 1,4,5-triphosphate receptors (IP_3Rs), which are the ER IP_3-gated Ca^{++} channels, thus impeding IP_3Rs-mediated Ca^{++} release and inhibiting apoptosis. Furthermore, Bcl-2 family proteins modulate UPR activation by interaction with IRE1α, fine tuning the threshold to activate the response (Pihán et al., 2017a). The UPR can also regulate the expression of members of the Bcl-2 family. It has been shown that CHOP inhibits the expression of the anti-apoptotic protein Bcl-2 while increasing that of the pro-apoptotic BIM (Urra et al., 2013). Moreover, other pro-apoptotic Bcl-2 family members (BID, BIM, NOXA and PUMA) are up-regulated by the UPR (Pihán et al., 2017a). Importantly, it has been shown that apoptosis-resistant human primary leukemic blasts were able to increase the expression of the anti-apoptotic protein Bcl-2 in apoptosis-sensitive blasts in an experimental co-culture system. Moreover, Bcl-2 family profile of normal lymphocytes and leukemic blasts within the bone marrow of AML patients positively correlated, suggesting that AML cells can transfer apoptosis resistance to their environment. Indeed, comparative proteomic analysis of the secretome of AML primary blasts with high versus low apoptotic resistance, showed significant differences, especially in factors related to RNA processing (Wojtuszkiewicz et al., 2016). In 2018, the FDA approved the use of the Bcl-2 inhibitor Venetoclax, alone or in combination with standard chemotherapy or hypomethylating agents. Unfortunately, as for other therapies that will be discussed below, these treatments result in high rate of relapses (Zhang et al., 2022), thus different combination strategies must be developed. In this context, it could be worth to take into account the activity of the Bcl-2 family at the ER and its crosstalk with the UPR.

Until now we discussed the crosstalk between AML cells and their environment via EVs. Another important means of communication between leukemic cells and BM stromal cells is direct cell-cell interaction through gap junctions, endocytosis and tunneling nanotubes (TNT) (Kolba et al., 2019). TNTs are thin tunnels, embedded in the plasma membrane and sustained by F-actin backbone, that provide a path for a direct cell to cell transfer of vesicles, organelles, ncRNAs, proteins and viral particles (Rustom et al., 2004). The number of TNTs increases under stress conditions and improves cell survival (Pasquier et al., 2013; Ariazi et al., 2017). AML cells communicate with each other and with bone marrow stromal cells via TNTs, transferring, for example, chemotherapeutic drugs (Omsland et al., 2017) and mitochondria (Marlein et al., 2017). Recent studies from different laboratories demonstrate that mitochondria transfer is a crucial mechanism through which BMSC protect and support AML cells and that this is a process tightly related to redox balance. Marlein and colleagues (Marlein et al., 2017) showed that AML cells, but not nonmalignant $CD34^+$ cells, prompted mitochondria transfer from BMCS to AML cells via TNTs, resulting in increased ATP production. Mitochondria transfer is stimulated by ROS increase in BMCS, due to superoxide produced by NOX2 in AML cells. Indeed, immunocompromised NSG mice implanted with AML cells where NOX2 was stably knocked-down, exhibited prolonged survival and NOX2-knockdown AML cells, recovered from the BM of engrafted mice, possessed less mitochondria than control AML cells (Marlein et al., 2017). Mitochondria are transferred by BM stromal cells to AML cells also through microtubule-dependent endocytic pathway. Moschoi and colleagues (Moschoi et al., 2016) demonstrated that direct transfer of mitochondria from stromal cells to AML cells is enhanced when leukemic cells are exposed to chemotherapy and augments AML cell resistance to chemotherapeutic drugs like cytarabine. Mitochondria transfer is not erratic, but specific toward AML cells, not involving, for example, $CD3^+$ T cells. CD38 is a newly described, clinically relevant target in AML (Naik et al., 2019), which is also essential for mitochondria transfer from BMSCs to AML cells. A monoclonal anti-CD38 antibody, daratumumab, which is approved for treatment of multiple myeloma, is able to inhibit the transfer of mitochondria from mesenchymal stromal cells (Mistry et al., 2021). Importantly, NSG mice engrafted with AML cells and treated with daratumumab survived substantially longer than control ones (Mistry et al., 2021). Increased mitochondria mass results in enhanced oxidative phosphorylation, with higher production of ROS. This could be detrimental for AML cells that already have to cope with a more oxidative environment than nonmalignant cells. Forte and colleagues confirmed, in vitro and in vivo (Forte et al., 2020), that BMSCs transfer mitochondria to AML cells and increase their oxidative phosphorylation capability, especially upon chemotherapeutic treatment with cytarabine. Very importantly, at the same time, BMSCs increase AML cell antioxidant defenses augmenting the expression of proteins involved in detoxification and/or glutathione metabolism. Both in vitro and in vivo, pharmacological inhibition of elements involved in the antioxidant response synergized with chemotherapy, abolishing the protective effects of BMSCs toward AML cells (Forte et al., 2020). At the moment, there are no studies concerning the involvement of the UPR in the defense from increased oxidative stress related to augmented oxidative phosphorylation. However, considering the tight crosstalk between oxidative stress and the UPR, it is possible to imagine a connection and it remains an open question to be investigated.

Taken all together the evidence described herein point to the UPR as a possible target to prevent the protective role of the BM niche toward AML cells. This strategy could be successful to enhance the effects of treatments like chemotherapy that strongly perturb proteostasis.

7. Conclusions

For many decades AML has been treated with intensive chemotherapy, based on cycles of a combination of cytarabine and anthracycline. However, this regimen is successful in a small percentage of cases and more than half of AML patients are elderly and thus not suitable for such an intensive chemotherapeutic protocol. As a result, the long-term disease free survival rate remains below 30% (DiNardo and Perl, 2019). In recent years striking progress has been made in identifying molecular target drugs and many have been approved, alone or in combination with conventional chemotherapy, providing encouraging results (Kayser and Levis, 2022). Nonetheless, relapses remain a major impediment that reduce the efficacy of such treatments, mainly due to development of multiple resistant clones, characterized by novel mutations not present at diagnosis (van Gils et al., 2021a). The most recent literature suggests that a possible strategy is to combine multiple approaches targeting diverse adaptive pathways (Farge et al., 2017; Ramsey et al., 2018; Jones et al., 2020a). The findings reviewed herein reveal that interfering with the proteostasis network, of which the UPR is a major component, is a promising strategy to increase the efficacy of chemotherapy and of molecular target drugs. Furthermore, this approach could also be beneficial to reduce the protective effects of the BM niche.

Funding

AIRC IG 2018 - ID. 21406 project, 'Progetti Ateneo' Sapienza University of Rome to F.F.; the fellowship of FL was supported by the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) Foundation "Fund for Ideas 2018" grant.

Authors' contributions

MS, FL wrote the initial draft of the manuscript. All the authors edited the manuscript and approved the final version for publication.

Competing interests

The authors declare that they have no competing interests.

References

Abdelhamed, S., Butler, J.T., Doron, B., et al., 2019. Extracellular vesicles impose quiescence on residual hematopoietic stem cells in the leukemic niche. EMBO Rep. 20 (7).

Acosta-Alvear, D., Zhou, Y., Blais, A., et al., 2007. XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks. Mol. Cell 27 (1), 53–66.

Ahmadmehrabi, K., Haque, A.R., Aleem, A., Griffiths, E.A., Roloff, G.W., 2021. Targeted therapies for the evolving molecular landscape of acute myeloid leukemia. Cancers 13 (18), 1–15.

Almanza, A., Carlesso, A., Chintha, C., et al., 2019. Endoplasmic reticulum stress signalling – from basic mechanisms to clinical applications. FEBS J. 286 (2).

Ariazi, J., Benowitz, A., de Biasi, V., et al., 2017. Tunneling nanotubes and gap junctions–their role in long-range intercellular communication during development, health, and disease conditions. Front. Mol. Neurosci. 10.

Bahar, E., Kim, J.-Y., Yoon, H., 2019. Chemotherapy resistance explained through endoplasmic reticulum stress-dependent signaling. Cancers 11 (3), 338.

Banerjee, S., Kumar, M., Wiener, R., 2020. Decrypting UFMylation: how proteins are modified with UFM1. Biomolecules 10 (10).

Bao, E.L., Cheng, A.N., Sankaran, V.G., 2019. The genetics of human hematopoiesis and its disruption in disease. EMBO Mol. Med. 11 (8).

Baryawno, N., Przybylski, D., Kowalczyk, M.S., et al., 2019. A cellular taxonomy of the bone marrow stroma in homeostasis and leukemia. Cell 177 (7), 1915–1932.e16.

Beeharry, N., Landrette, S., Gayle, S., et al., 2019a. LAM-003, a new drug for treatment of tyrosine kinase inhibitor–resistant FLT3-ITD–positive AML. Blood Adv. 3 (22), 3661–3673.

Bernardi, S., Farina, M., 2021. Exosomes and extracellular vesicles in myeloid Neoplasia: the multiple and complex roles played by these “magic bullets”. Biol. (Basel) 10 (2).

Bhattarai, K.R., Chaudhary, M., Kim, H.R., Chae, H.J., 2020. Endoplasmic reticulum (ER) stress response failure in diseases. Trends Cell Biol. 30 (9).

Brehme, M., Sverchkova, A., Voisine, C., 2019. Proteostasis network deregulation signatures as biomarkers for pharmacological disease intervention. Curr. Opin. Syst. Biol. 15, 74–81.

Bruno, S., Mancini, M., de Santis, S., et al., 2021a. The role of hypoxic bone marrow microenvironment in acute myeloid leukemia and future therapeutic opportunities. Int. J. Mol. Sci. 22 (13).

Burchert, A., Bug, G., Fritz v., L., et al., 2020. Sorafenib maintenance after allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia with *FLT3* –internal tandem duplication mutation (SORMAIN). J. Clin. Oncol. 38 (26), 2993–3002.

Cagnetta, A., Caffa, I., Acharya, C., et al., 2015. APO866 increases antitumor activity of cyclosporin - a by inducing mitochondrial and endoplasmic reticulum stress in leukemia cells. Clin. Cancer Res. 21 (17).

Chen, X., Cubillos-Ruiz, J.R., 2021. Endoplasmic reticulum stress signals in the tumour and its microenvironment. Nat. Rev. Cancer 21 (2), 71–88.

Choi, J.A., Song, C.H., 2020. Insights into the role of endoplasmic reticulum stress in infectious diseases. Front. Immunol. 10.

Christianson, J.C., Carvalho, P., 2022. Order through destruction: how ER-associated protein degradation contributes to organelle homeostasis. EMBO J.

Cloux, A.J., Aubry, D., Heulot, M., et al., 2019. Reactive oxygen/nitrogen species contribute substantially to the antileukemia effect of APO866, a NAD lowering agent. Oncotarget 10 (62).

Coltella, N., Percio, S., Valsecchi, R., et al., 2014. <scp>HIF</scp> factors cooperate with <scp>PML</scp> - <scp>RAR</scp> α to promote acute promyelocytic leukemia progression and relapse. EMBO Mol. Med. 6 (5), 640–650.

Costa-Mattioli, M., Walter, P., 2020. The integrated stress response: from mechanism to disease. Science 368 (6489).

Cucchi, D.G.J., Groen, R.W.J., Janssen, J.J.W.M., Cloos, J., 2020. Ex vivo cultures and drug testing of primary acute myeloid leukemia samples: current techniques and implications for experimental design and outcome. Drug Resist Updat 53, 100730.

Cullinan, S.B., Zhang, D., Hannink, M., et al., 2003. Nrf2 Is a direct PERK substrate and effector of PERK-dependent cell survival. Mol. Cell. Biol. 23 (20), 7198–7209.

Damnernsawad, A., Bottomly, D., Kurtz, S.E., et al., 2020. A genome-wide CRISPR screen identifies regulators of MAPK and MTOR pathways that mediate resistance to sorafenib in acute myeloid leukemia. Haematologica 107 (1), 77–85.

Das, I., Krzyzosiak, A., Schneider, K., et al., 2015. Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. Science 348 (6231), 239–242.

De Kouchkovsky, I., Abdul-Hay, M., 2016. ‘Acute myeloid leukemia: a comprehensive review and 2016 update’. Blood Cancer J.

Di, M., Wang, L., Li, M., 2017. Dickkopf1 destabilizes atherosclerotic plaques and promotes plaque formation by inducing apoptosis of endothelial cells through activation of ER stress. Cell Death Dis. 8 (7) e2917–e2917.

Díaz-Bulnes, P., Saiz, M.L., López-Larrea, C., Rodríguez, R.M., 2020. Crosstalk between hypoxia and ER stress response: a key regulator of macrophage polarization. Front. Immunol. 10.

DiNardo, C.D., Perl, A.E., 2019. Advances in patient care through increasingly individualized therapy. Nat. Rev. Clin. Oncol. 16 (2), 73–74.

Döhner, H., Estey, E., Grimwade, D., et al., 2017. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 129 (4), 424–447.

Doron, B., Abdelhamed, S., Butler, J., et al., 2017. Transmissible ER stress reconfigures the AML bone marrow compartment. Leukemia 33 (4), 918–930.

Doron, B., Handu, M., Kurre, P., 2018a. Concise review: adaptation of the bone marrow stroma in hematopoietic malignancies: current concepts and models. Stem Cells 36 (3).

Fang, C., Weng, T., Hu, S., et al., 2021. IFN-γ-induced ER stress impairs autophagy and triggers apoptosis in lung cancer cells. OncoImmunology 10 (1).

Farge, T., Saland, E., de Toni, F., et al., 2017. Chemotherapy-resistant human acute myeloid leukemia cells are not enriched for leukemic stem cells but require oxidative metabolism. Cancer Discov. 7 (7), 716–735.

Féral, K., Jaud, M., Philippe, C., et al., 2021. ER stress and unfolded protein response in leukemia: friend, foe, or both? Biomolecules 11 (2), 1–31.

Forte, D., García-Fernández, M., Sánchez-Aguilera, A., et al., 2020. Bone marrow mesenchymal stem cells support acute myeloid leukemia bioenergetics and enhance antioxidant defense and escape from chemotherapy. Cell Metab. 32 (5), 829–843.e9.

van Galen, P., Mbong, N., Kreso, A., 2018. Integrated stress response activity marks stem cells in normal hematopoiesis and leukemia. Cell Rep. 25 (5).

Gaudette, B.T., Jones, D.D., Bortnick, A., Argon, Y., Allman, D., 2020. mTORC1 coordinates an immediate unfolded protein response-related transcriptome in activated B cells preceding antibody secretion. Nat. Commun. 11 (1), 723.

Gerakis, Y., Quintero, M., Li, H., Hetz, C., 2019. The UFMylation system in proteostasis and beyond. Trends Cell Biol. 29 (12), 974–986.

Ghemrawi, R., Khair, M., 2020. Endoplasmic reticulum stress and unfolded protein response in neurodegenerative diseases. Int. J. Mol. Sci. 21 (17).

Ghosh, R., Wang, L., Wang, E.S., et al., 2014. Allosteric inhibition of the IRE1α RNase preserves cell viability and function during endoplasmic reticulum stress. Cell 158 (3), 534–548.

van Gils, N., Denkers, F., Smit, L., 2021a. Escape from treatment; the different faces of leukemic stem cells and therapy resistance in acute myeloid leukemia. Front. Oncol. 11.

Gómora-García, J.C., Gerónimo-Olvera, C., Pérez-Martínez, X., Massieu, L., 2021. IRE1α RIDD activity induced under ER stress drives neuronal death by the degradation of 14-3-3 θ mRNA in cortical neurons during glucose deprivation. Cell Death Discov. 7 (1).

Grant, S., Roberts, J.D., 2003. The use of cyclin-dependent kinase inhibitors alone or in combination with established cytotoxic drugs in cancer chemotherapy. Drug Resist Update 6 (1), 15–26.

Guo, J., Ren, R., Sun, K., et al., 2020. PERK controls bone homeostasis through the regulation of osteoclast differentiation and function. Cell Death Dis. 11 (10).

Guo, J., Ren, R., Sun, K., He, J., Shao, J., 2021. PERK signaling pathway in bone metabolism: Friend or foe? Cell Prolif. 54 (4).

Haefliger, S., Klebig, C., Schaubitzer, K., et al., 2011. Protein disulfide isomerase blocks CEBPA translation and is up-regulated during the unfolded protein response in AML. Blood 117 (22), 5931–5940.

Han, D., Lerner, A.G., vande Walle, L., 2009. IRE1α kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. Cell 138 (3), 562–575.

Han, J., Back, S.H., Hur, J., et al., 2013. ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. Nat. Cell Biol. 15 (5), 481–490.

Harding, H.P., Zhang, Y., Ron, D., 1999. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature 397 (6716), 271–274.

Harding, H.P., Zhang, Y., Zeng, H., et al., 2003. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol. Cell 11 (3), 619–633.

Hetz, C., Papa, F.R., 2018. The unfolded protein response and cell fate control. Mol. Cell 69 (2), 169–181.

Hidalgo San Jose, L., Sunshine, M.J., Dillingham, C.H., et al., 2020. Modest declines in proteome quality impair hematopoietic stem cell self-renewal. Cell Rep. 30 (1).

Ho, T.T., Warr, M.R., Adelman, E.R., 2017. Autophagy maintains the metabolism and function of young and old stem cells. Nature 543 (7644).

Hong, C.-S., Sharma, P., Yerneni, S.S., et al., 2017. Circulating exosomes carrying an immunosuppressive cargo interfere with cellular immunotherapy in acute myeloid leukemia. Sci. Rep. 7 (1), 14684.

Horiguchi, H., Kobune, M., Kikuchi, S., et al., 2016. Extracellular vesicle miR-7977 is involved in hematopoietic dysfunction of mesenchymal stromal cells via poly(rC) binding protein 1 reduction in myeloid neoplasms. Haematologica 101 (4).

Hornick, N.I., Doron, B., Abdelhamed, S., et al., 2016. AML suppresses hematopoiesis by releasing exosomes that contain microRNAs targeting c-MYB. Sci. Signal. 9 (444).

Hosseini, M., Rezvani, H.R., Aroua, N., et al., 2019. Targeting myeloperoxidase disrupts mitochondrial redox balance and overcomes cytarabine resistance in human acute myeloid leukemia. Cancer Res. 79 (20), 5191–5203.

Hu, X., Cai, J., Zhu, J., et al., 2020. Arsenic trioxide potentiates Gilteritinib-induced apoptosis in FLT3-ITD positive leukemic cells via IRE1a-JNK-mediated endoplasmic reticulum stress. Cancer Cell Int. 20 (1), 250.

Huan, J., Hornick, N.I., Shurtleff, M.J., 2013. RNA trafficking by acute myelogenous leukemia exosomes. Cancer Res. 73 (2), 918–929.

Huan, J., Hornick, N.I., Goloviznina, N.A., 2015. Coordinate regulation of residual bone marrow function by paracrine trafficking of AML exosomes. Leukemia 29 (12), 2285–2295.

Irwin, M.E., Rivera-Del Valle, N., Chandra, J., 2013. Redox control of leukemia: from molecular mechanisms to therapeutic opportunities. Antioxid. Redox Signal. 18 (11), 1349–1383.

Jabbour, E., Kantarjian, H., 2018. Chronic myeloid leukemia: 2018 update on diagnosis, therapy and monitoring. Am. J. Hematol. 93 (3).

Janssens, S., Pulendran, B., Lambrecht, B.N., 2014. Emerging functions of the unfolded protein response in immunity. Nat. Immunol. 15 (10), 910–919.

Jimenez, J.J., Chale, R.S., Abad, A.C., Schally, A.V., 2020. Acute promyelocytic leukemia (APL): a review of the literature. Oncotarget 11 (11).

Jones, C.L., Stevens, B.M., Pollyea, D.A., et al., 2020a. Nicotinamide metabolism mediates resistance to venetoclax in relapsed acute myeloid leukemia stem cells. Cell Stem Cell 27 (5), 748–764.e4.

Kayser, S., Levis, M.J., 2022. Updates on targeted therapies for acute myeloid leukaemia. Br. J. Haematol. 196 (2), 316–328.
Khateb, A., Ronai, Z.A., 2020a. Unfolded protein response in leukemia: from basic understanding to therapeutic opportunities. Trends Cancer 6 (11).
Kolba, M.D., Dudka, W., Zaręba-Kozioł, M., et al., 2019. Tunneling nanotube-mediated intercellular vesicle and protein transfer in the stroma-provided imatinib resistance in chronic myeloid leukemia cells. Cell Death Dis. 10 (11), 817.
Kondengaden, S.M., Luo, L., Huang, K., et al., 2016. Discovery of novel small molecule inhibitors of lysine methyltransferase G9a and their mechanism in leukemia cell lines. Eur. J. Med. Chem. 122, 382–393.
Kopp, M.C., Larburu, N., Durairaj, V., Adams, C.J., Ali, M.M.U., 2019. UPR proteins IRE1 and PERK switch BiP from chaperone to ER stress sensor. Nat. Struct. Mol. Biol. 26 (11), 1053–1062.
Kubra, K.T., Uddin, M.A., Akhter, M.S., Barabutis, N., 2020. Hsp90 inhibitors induce the unfolded protein response in bovine and mice lung cells. Cell. Signal. 67, 109500.
Kumar, B., Garcia, M., Weng, L., et al., 2018. Acute myeloid leukemia transforms the bone marrow niche into a leukemia-permissive microenvironment through exosome secretion. Leukemia 32 (3).
Lam, M., Marsters, S.A., Ashkenazi, A., Walter, P., 2020. Misfolded proteins bind and activate death receptor 5 to trigger apoptosis during unresolved endoplasmic reticulum stress. Elife 9.
le Thomas, A., Ferri, E., Marsters, S., et al., 2021. Decoding non-canonical mRNA decay by the endoplasmic-reticulum stress sensor IRE1α. Nat. Commun. 12 (1), 7310.
Lehnertz, B., Pabst, C., Su, L., et al., 2014. The methyltransferase G9a regulates HoxA9-dependent transcription in AML. Genes Dev. 28 (4), 317–327.
Levin, M., Stark, M., Ofran, Y., Assaraf, Y.G., 2021. Deciphering molecular mechanisms underlying chemoresistance in relapsed AML patients: towards precision medicine overcoming drug resistance. Cancer Cell Int. 21 (1).
Levis, M., Perl, A.E., 2020a. Gilteritinib: potent targeting of FLT3 mutations in AML. Blood Adv. 4 (6).
Li, Y., Xue, Z., Dong, X., 2020. Mitochondrial dysfunction and oxidative stress in bone marrow stromal cells induced by daunorubicin leads to DNA damage in hematopoietic cells. Free Radic. Biol. Med. 146, 211–221.
Liang, C., Peng, C.J., Wang, L.N., et al., 2020. Arsenic trioxide and all-trans retinoic acid suppress the expression of FLT3-ITD. Leuk. Lymphoma 61 (11).
Lin, J.-C., Yang, P.-M., Liu, T.-P., 2021. PERK/ATF4-dependent ZFAS1 upregulation is associated with sorafenib resistance in hepatocellular carcinoma cells. Int. J. Mol. Sci. 22 (11), 5848.
Lindblad, O., Cordero, E., Puissant, A., et al., 2016. Aberrant activation of the PI3K/mTOR pathway promotes resistance to sorafenib in AML. Oncogene 35 (39).
Liu, J., Wang, Y., Song, L., et al., 2017. A critical role of DDRGK1 in endoplasmic reticulum homoeostasis via regulation of IRE1α stability. Nat. Commun. 8 (1).
Liu, L., Zhao, M., Jin, X., et al., 2019. Adaptive endoplasmic reticulum stress signalling via IRE1α–XBP1 preserves self-renewal of haematopoietic and pre-leukaemic stem cells. Nat. Cell Biol. 21 (3).
Liu, L., Inoki, A., Fan, K., et al., 2020. ER-associated degradation preserves hematopoietic stem cell quiescence and self-renewal by restricting mTOR activity. Blood 136 (26).
Long, L., Assaraf, Y.G., Lei, Z.-N., et al., 2020. Genetic biomarkers of drug resistance: a compass of prognosis and targeted therapy in acute myeloid leukemia. Drug Resist Updat 52, 100703.
Lu, M., Lawrence, D.A., Marsters, S., et al., 2014. Opposing unfolded-protein-response signals converge on death receptor 5 to control apoptosis. Science 345 (6192), 98–101.
Luchsinger, L.L., 2021. Hormetic endoplasmic reticulum stress in hematopoietic stem cells. Curr. Opin. Hematol. 28, 1–7.
Lyu, T., Wang, Y., Li, D., et al., 2021. Exosomes from BM-MSCs promote acute myeloid leukemia cell proliferation, invasion and chemoresistance via upregulation of S100A4. Exp. Hematol. Oncol. 10 (1).
Madhusudhan, T., Wang, H., Dong, W., et al., 2015. Defective podocyte insulin signalling through p85-XBP1 promotes ATF6-dependent maladaptive ER-stress response in diabetic nephropathy. Nat. Commun. 6.
Magagnin, M.G., Koritzinsky, M., Wouters, B.G., 2006. Patterns of tumor oxygenation and their influence on the cellular hypoxic response and hypoxia-directed therapies. Drug Resist Updat 9 (4–5), 185–197 (Aug-Oct).
Manara, E., Baron, E., Tregnago, C., et al., 2014. MLL-AF6 fusion oncogene sequesters AF6 into the nucleus to trigger RAS activation in myeloid leukemia. Blood 124 (2), 263–272.
Marciniak, S.J., Yun, C.Y., Oyadomari, S., et al., 2004. CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. Genes Dev. 18 (24), 3066–3077.
Marlein, C.R., Zaitseva, L., Piddock, R.E., et al., 2017. NADPH oxidase-2 derived superoxide drives mitochondrial transfer from bone marrow stromal cells to leukemic blasts. Blood 130 (14).
Martelli, A.M., Paganelli, F., Chiarini, F., Evangelisti, C., McCubrey, J.A., 2020. The unfolded protein response: a novel therapeutic target in acute leukemias. Cancers 12 (2).
Masciarelli, S., Capuano, E., Ottone, T., et al., 2018. Retinoic acid and arsenic trioxide sensitize acute promyelocytic leukemia cells to ER stress. Leukemia 32 (2), 285–294.
Masciarelli, S., Capuano, E., Ottone, T., et al., 2019. Retinoic acid synergizes with the unfolded protein response and oxidative stress to induce cell death in FLT3-ITD+ AML. Blood Adv. 3 (24), 4155–4160.
Maurel, M., Chevet, E., Tavernier, J., Gerlo, S., 2014. Getting RIDD of RNA: IRE1 in cell fate regulation. Trends Biochem. Sci. 39 (5), 245–254.
McMahon, C.M., Ferng, T., Canaani, J., et al., 2019a. Clonal selection with RAS pathway activation mediates secondary clinical resistance to selective FLT3 inhibition in acute myeloid leukemia. Cancer Discov. 9 (8), 1050–1063.
Md Sakib Hossain, D., Javaid, S., Cai, M., et al., 2018. Dinaciclib induces immunogenic cell death and enhances anti- PD1–mediated tumor suppression. J. Clin. Investig. 128 (2).
Méndez-Ferrer, S., Bonnet, D., Steensma, D.P., et al., 2020a. Bone marrow niches in haematological malignancies. Nat. Rev. Cancer 20 (5), 285–298.
Metzelder, S.K., Schroeder, T., Finck, A., et al., 2012. High activity of sorafenib in FLT3-ITD-positive acute myeloid leukemia synergizes with allo-immune effects to induce sustained responses. Leukemia 26 (11), 2353–2359.
Mistry, J.J., Moore, J.A., Kumar, P., et al., 2021. Daratumumab inhibits acute myeloid leukemia metabolic capacity by blocking mitochondrial transfer from mesenchymal stromal cells. Haematologica 106 (2).
Mohrin, M., Widjaja, A., Liu, Y., Luo, H., Chen, D., 2018. The mitochondrial unfolded protein response is activated upon hematopoietic stem cell exit from quiescence. Aging Cell 17 (3).
Moschoi, R., Imbert, V., Nebout, M., et al., 2016. Protective mitochondrial transfer from bone marrow stromal cells to acute myeloid leukemic cells during chemotherapy. Blood 128 (2).
Moses, B.S., McCullough, S., Fox, J.M., et al., 2021. Antileukemic efficacy of a potent artemisinin combined with sorafenib and venetoclax. Blood Adv. 5 (3), 711–724.
Naik, J., Themeli, M., de Jong-Korlaar, R., et al., 2019. CD38 as a therapeutic target for adult acute myeloid leukemia and T-cell acute lymphoblastic leukemia. Haematologica 104 (3).
Nehrbas, J., Butler, J.T., Chen, D.W., Kurre, P., 2020. Extracellular vesicles and chemotherapy resistance in the AML microenvironment. Front. Oncol. 10.
Newell, L.F., Cook, R.J., 2021. Advances in acute myeloid leukemia. BMJ n2026.
Nguyen, T.K., Grant, S., 2014. Dinaciclib (SCH727965) inhibits the unfolded protein response through a CDK1- And 5-dependent mechanism. Mol. Cancer Ther. 13 (3).
Niewerth, D., Jansen, G., Assaraf, Y.G., et al., 2015. Molecular basis of resistance to proteasome inhibitors in hematological malignancies. Drug Resist Updat 18, 18–35.
Oakes, S.A., 2020. Endoplasmic reticulum stress signaling in cancer cells. Am. J. Pathol. 190 (5).
Oka, O.B.V., Pierre, A.S., Pringle, M.A., et al., 2022. Activation of the UPR sensor ATF6α is regulated by its redox-dependent dimerization and ER retention by ERp18. Proc. Natl. Acad. Sci. USA 119 (12).
Omsland, M., Bruserud, Ø., Gjertsen, B.T., Andresen, V., 2017. Tunneling nanotube (TNT) formation is downregulated by cytarabine and NF-κB inhibition in acute myeloid leukemia (AML). Oncotarget 8 (5).
Papp, B., Launay, S., Gélébart, P., et al., 2020. Endoplasmic reticulum calcium pumps and tumor cell differentiation. Int. J. Mol. Sci. 21 (9).
Park, I.K., Mundy-Bosse, B., Whitman, S.P., et al., 2015. Receptor tyrosine kinase Axl is required for resistance of leukemic cells to FLT3-targeted therapy in acute myeloid leukemia. Leukemia 29 (12).
Pasquier, J., Guerrouahen, B.S., al Thawadi, H., et al., 2013. Preferential transfer of mitochondria from endothelial to cancer cells through tunneling nanotubes modulates chemoresistance. J. Transl. Med. 11 (1), 94.
Pelcovits, A., Niroula, R., 2020. Acute myeloid leukemia: a review. R. I Med J. 103 (3), 38–40.
Percio, S., Coltella, N., Grisanti, S., Bernardi, R., Pattini, L., 2014. A HIF-1 network reveals characteristics of epithelial-mesenchymal transition in acute promyelocytic leukemia. Genome Med. 6 (12).
Perl, A.E., 2017. The role of targeted therapy in the management of patients with AML. Blood Adv. 1 (24), 2281–2294.
Perl, A.E., Martinelli, G., Cortes, J.E., et al., 2019. Gilteritinib or chemotherapy for relapsed or refractory FLT3 -mutated AML. N. Engl. J. Med. 381 (18).
Pihán, P., Carreras-Sureda, A., Hetz, C., 2017a. BCL-2 family: integrating stress responses at the ER to control cell demise. Cell Death Differ. 24 (9), 1478–1487.
Porter, K.R., Claude, A., Fullam, E.F., 1945. A study of tissue culture cells by electron microscopy: Methods and preliminary observations. J. Exp. Med. 81 (3).
Rahmani, M., Davis, E.M., Crabtree, T.R., et al., 2007a. The kinase inhibitor sorafenib induces cell death through a process involving induction of endoplasmic reticulum stress. Mol. Cell. Biol. 27 (15).
Ramsey, H.E., Fischer, M.A., Lee, T., et al., 2018. A novel MCL1 inhibitor combined with venetoclax rescues venetoclax-resistant acute myelogenous leukemia. Cancer Discov. 8 (12), 1566–1581.
Rashid, H.-O., Yadav, R.K., Kim, H.-R., Chae, H.-J., 2015. ER stress: autophagy induction, inhibition and selection. Autophagy 11 (11), 1956–1977.
Rodina, A., Wang, T., Yan, P., et al., 2017. The epichaperome is an integrated chaperome network that facilitates tumour survival. Nature 538 (7625), 397–401.
Röllig, C., Serve, H., Hüttmann, A., et al., 2015. Addition of sorafenib versus placebo to standard therapy in patients aged 60 years or younger with newly diagnosed acute myeloid leukaemia (SORAML): a multicentre, phase 2, randomised controlled trial. Lancet Oncol. 16 (16), 1691–1699.
Rong, C., Wei, W., Yu-Hong, T., 2020. Asperuloside exhibits a novel anti-leukemic activity by triggering ER stress-regulated apoptosis via targeting GRP78. Biomed. Pharmacother. 125, 0–6.
Rouault-Pierre, K., Lopez-Onieva, L., Foster, K., et al., 2013. HIF-2α protects human hematopoietic stem/progenitors and acute myeloid leukemic cells from apoptosis induced by endoplasmic reticulum stress. Cell Stem Cell 13 (5).
Rustom, A., Saffrich, R., Markovic, I., Walther, P., Gerdes, H.-H., 2004. Nanotubular highways for intercellular organelle transport. Science 303 (5660), 1007–1010.
Salaroglio, I.C., Panada, E., Moiso, E., et al., 2017. PERK induces resistance to cell death elicited by endoplasmic reticulum stress and chemotherapy. Mol. Cancer 16 (1).
Saultz, J., Garzon, R., 2016. Acute myeloid leukemia: a concise review. J. Clin. Med. 5, 3.
Schardt, J.A., Mueller, B.U., Pabst, T., 2011. Activation of the unfolded protein response in human acute myeloid leukemia. Methods Enzymol. 489 (C).

Schmidt-Arras, D.-E., Böhmer, A., Markova, B., et al., 2005. Tyrosine phosphorylation regulates maturation of receptor tyrosine kinases. Mol. Cell. Biol. 25 (9), 3690–3703.
Senft, D., Ronai, Z.A., 2015. UPR, autophagy, and mitochondria crosstalk underlies the ER stress response. Trends Biochem. Sci. 40 (3), 141–148.
Sharma, S., Ghufran, S.M., Ghose, S., Biswas, S., 2021. Cytoplasmic vacuolation with endoplasmic reticulum stress directs sorafenib induced non-apoptotic cell death in hepatic stellate cells. Sci. Rep. 11 (1), 3089.
Shoulders, M.D., Ryno, L.M., Genereux, J.C., et al., 2013. Stress-independent activation of XBP1s and/or ATF6 reveals three functionally diverse ER Proteostasis Environments. Cell Rep. 3 (4), 1279–1292.
Sigurdsson, V., Miharada, K., 2018. Regulation of unfolded protein response in hematopoietic stem cells. Int. J. Hematol. 107 (6).
Sigurdsson, V., Takei, H., Soboleva, S., et al., 2016. Bile acids protect expanding hematopoietic stem cells from unfolded protein stress in fetal liver. Cell Stem Cell 18 (4).
Sillar, Germon, DeIuliis, Dun, 2019. The role of reactive oxygen species in acute myeloid leukaemia. Int. J. Mol. Sci. 20 (23), 6003.
Šimoničová, K., Janotka, Ľ., Kavcová, H., et al., 2022. Different mechanisms of drug resistance to hypomethylating agents in the treatment of myelodysplastic syndromes and acute myeloid leukemia. Drug Resist Updat 61, 100805.
Stevens, A.M., Xiang, M., Heppler, L.N., et al., 2019. Atovaquone is active against AML by upregulating the integrated stress pathway and suppressing oxidative phosphorylation. Blood Adv. 3 (24), 4215–4227.
Sun, H., Lin, D.-C., Guo, X., et al., 2016. Inhibition of IRE1α-driven pro-survival pathways is a promising therapeutic application in acute myeloid leukemia. Oncotarget 7 (14), 18736–18749.
Szczepanski, M.J., Szajnik, M., Welsh, A., Whiteside, T.L., Boyiadzis, M., 2011. Blast-derived microvesicles in sera from patients with acute myeloid leukemia suppress natural killer cell function via membrane-associated transforming growth factor-β1. Haematologica 96 (9).
Tanimura, A., Yujiri, T., Tanaka, Y., et al., 2011. Activation of the unfolded protein response in primary acute myeloid leukemia cells. Int. J. Hematol. 94 (3), 300–302.
Tavasolian, F., Hosseini, A.Z., Mirzaei, A., et al., 2020. Unfolded protein response-mediated modulation of mesenchymal stem cells. IUBMB Life 72 (2).
Thol, F., Ganser, A., 2020. Treatment of relapsed acute myeloid leukemia. Curr. Treat. Options Oncol. 21 (8).
Uddin, M.A., Kubra, K., Sonju, J.J., et al., 2021. Effects of heat shock protein 90 inhibition in the lungs. Med. Drug Discov. 1–17.
Urra, H., Dufey, E., Lisbona, F., Rojas-Rivera, D., Hetz, C., 2013. When ER stress reaches a dead end. Biochim. Et. Biophys. Acta (BBA) Mol. Cell Res. 1833 (12), 3507–3517.
Urra, H., Dufey, E., Avril, T., Chevet, E., Hetz, C., 2016. Endoplasmic reticulum stress and the hallmarks of cancer. Trends Cancer 2 (5), 252–262.
Van Galen, P., Kreso, A., Mbong, N., et al., 2014. The unfolded protein response governs integrity of the haematopoietic stem-cell pool during stress. Nature 510 (7504).
del Vecchio, C.A., Feng, Y., Sokol, E.S., 2014. De-Differentiation confers multidrug resistance via noncanonical PERK-Nrf2 signaling. PLoS Biol. 12 (9), e1001945.
Wang, Y., Liu, Y., Malek, S.N., Zheng, P., Liu, Y., 2011. Targeting HIF1α eliminates cancer stem cells in hematological malignancies. Cell Stem Cell 8 (4), 399–411.
Wey, S., Luo, B., Tseng, C.-C., et al., 2012. Inducible knockout of GRP78/BiP in the hematopoietic system suppresses Pten-null leukemogenesis and AKT oncogenic signaling. Blood 119 (3), 817–825.
Williams, M.S., Amaral, F.M.R., Simeoni, F., Somervaille, T.C.P., 2020. A stress-responsive enhancer induces dynamic drug resistance in acute myeloid leukemia. J. Clin. Investig. 130 (3), 1217–1232.
Wojtuszkiewicz, A., Schuurhuis, G.J., Kessler, F.L., et al., 2016. Exosomes secreted by apoptosis-resistant Acute Myeloid Leukemia (AML) blasts harbor regulatory network proteins potentially involved in antagonism of apoptosis. Mol. Cell. Proteom. 15 (4).
Xu, L., Liu, X., Peng, F., et al., 2020. Protein quality control through endoplasmic reticulum-associated degradation maintains haematopoietic stem cell identity and niche interactions. Nat. Cell Biol. 22 (10).
Xuan, L., Liu, Q., 2021. Maintenance therapy in acute myeloid leukemia after allogeneic hematopoietic stem cell transplantation. J. Hematol. Oncol. 14 (1), 4.
Yang, H., Niemeijer, M., van de Water, B., Beltman, J.B., 2020. ATF6 Is a critical determinant of CHOP dynamics during the unfolded protein response. iScience 23 (2), 100860.
Yu, C., Kancha, R.K., Duyste, J., 2014. Targeting oncoprotein stability overcomes drug resistance caused by FLT3 kinase domain mutations. PLoS One 9 (5), 1–5.
Yun, H.D., Schirm, D.K., Felices, M., Miller, J.S., Eckfeldt, C.E., 2019. Dinaciclib enhances natural killer cell cytotoxicity against acute myelogenous leukemia. Blood Adv. 3 (16).
Zhang, M., Zhu, X., Zhang, Y., et al., 2015. RCAD/Ufl1, a Ufm1 E3 ligase, is essential for hematopoietic stem cell function and murine hematopoiesis. Cell Death Differ. 22 (12).
Zhang, Q., Riley-Gillis, B., Han, L., et al., 2022. Activation of RAS/MAPK pathway confers MCL-1 mediated acquired resistance to BCL-2 inhibitor venetoclax in acute myeloid leukemia. Signal Transduct. Target. Ther. 7 (1), 51.
Zhang, Z., Zhang, L., Zhou, L., et al., 2019a. Redox signaling and unfolded protein response coordinate cell fate decisions under ER stress. Redox Biol. 25, 101047.
Zhang, Z., Wu, S., Muhammad, S., Ren, Q., Sun, C., 2018. miR-103/107 promote ER stress-mediated apoptosis via targeting the Wnt3a/β-catenin/ATF6 pathway in preadipocytes. J. Lipid Res. 59 (5), 843–853.
Zhou, C., Martinez, E., di Marcantonio, D., et al., 2017. JUN is a key transcriptional regulator of the unfolded protein response in acute myeloid leukemia. Leukemia 31 (5), 1196–1205.