Review

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Oxidative Stress in Multiple Myeloma Pathophysiology and Treatment

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Multiple myeloma (MM) is a clonal plasma cell proliferation disease characterized by an abnormal monoclonal protein, leading to specific-organ damage. Nowadays, significant knowledge about the pathophysiology and the treatment of MM has been gained. Unique lesions regarding reactive oxygen species (ROS) and reactive nitrogen species (RNS) production in MM's pathobiology have been reported due to new technologies. On the one hand, in most stages of MM, an overproduction of free radicals and a deregulation of the human antioxidant system can be found, leading to intense myeloma cell proliferation. On the other hand, in advanced disease with comorbidities, oxidative stress suppression leads to further growth of neoplastic clone. Novel agents that have been emerged for MM treatment, such as proteasome inhibitors, immunomodulatory drugs, epigenetic drugs and monoclonal antibodies have improved patients' survival and quality of life. These drugs increase oxidative stress, resulting in myeloma cell apoptosis, via activation of a molecular pathway called Unfolded Protein Response (UPR). Nowadays, the research focuses on the discovery of novel factors that can enhance their anti-myeloma effects, by modulation of oxidative stress.

Keywords: multiple myeloma; oxidative stress; pathophysiology; treatment

Introduction

Multiple myeloma (MM) or Kahler's disease, is the second most common hematological malignancy, following Non Hodgkin Lymphomas (NHL), accounting for 10% of hematological malignancies and 1% of cancers, with an incidence rate of 10 cases out of 100,000 people per year [1,2]. MM belongs to a large group of diseases characterized as plasma cell dyscrasias, along with Waldenstrom's macroglobulinemia, primary amyloidosis and polyneuropathy, organomegaly, endocrinopathy, monoclonal plasma cell disorder, skin changes syndrome (POEMS) [2]. It is characterized by the clonal proliferation of a plasma cell line and the overproduction of an immunoglobulin or a part of it, called M protein, leading to specific end-organ damage [3].

The progression of MM can be subdivided in different stages [1,2]. Actually, monoclonal gammopathy of undetermined significance (MGUS) is a stage in the spectrum of monoclonal gammopathy, a pre-malignant, asymptomatic phase of clonal phase cell growth [1,2]. MGUS is delineated by less than 30 g/L of M protein in the serum or/and less than 10% plasma cells in the bone marrow and the absence of Calcium elevation, Renal failure, Anemia, Bone disease (CRAB) criteria, which are equal to end-organ damage [1,2]. The second stage is called smoldering multiple myeloma (SMM) and it is defined by 30 g/L or more of M protein in the serum or/and 10% or more of plasma cells in the bone marrow, with the simultaneous absence of original CRAB criteria [1,2]. 10% of SMM cases progress to MM per year [1,2]. MM is characterized by the same criteria as SMM, but there is a great difference; MM is accompanied by end-organ damage [1,2]. New diagnostic criteria according to the Revised International Myeloma Working Group include any one or more of the following biomarkers of malignancy or myeloma-defining events (MDEs): clonal bone marrow plasma cells $\geq$60%, involved/uninvolved serum free light chain ratio $\geq$100, more than one focal lesions on MRI $\geq$5 mm or greater in size [1,2]. The risk of MGUS progression to MM is approximately 1% per year [1,2].

Oxidative stress is a biological condition caused by an imbalance between production and accumulation of reactive species in cells and tissues with a concomitant abolishment of the body's antioxidants systems [4,5]. Oxidative stress can be both harmful for the human organism, taking part in the pathogenesis of various diseases and helpful, during acute inflammation response [4,5]. Novel studies reveal a complex relationship between myeloma pathobiology and oxidative stress [6].

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The aim of this study is to demonstrate the role of oxidative stress in MM pathophysiology and treatment. Seventy six references were reviewed. Sources were English articles from Google Scholar, PubMed and Scopus from 2011 to 2023. There were not set any population or geographic limits. The type of publications preferred were randomized-control trials, systematic reviews, meta-analyses and other reviews. The keywords used were: multiple myeloma, oxidative stress, pathophysiology, treatment.

Results

Oxidative Stress in Multiple Myeloma Pathophysiology

There is a sensitive and fragile relationship between oxidative stress and MM [6,7]. During most stages of MM, especially in early stages as the monoclonal gammopathy of undetermined significance (MGUS) an increase in oxidative stress markers such as advanced glycation end products (AGEs), adenosine deaminase, malondialdehyde and 8-isoprostane can be detected in blood and in urine [6–8]. This condition reflects an overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in myeloma cells [9,10]. According to immunochemistry analysis 80% of ROS and RNS are produced by mitochondria, mainly by Fe-S centers of the hydrophilic arm of complex I (Reduced Nicotinamide Adenine Dinucleotide (NADH)-dehydrogenase) and by ubiquinone oxidation system in complex III (cytochrome b-c1 redox) [6,11]. The remaining 20% is produced by endoplasmic reticulum (ER) where the oxidative folding of proteins occurs (formation of S-S bonds) and by peroxisomes, where the oxidation of fatty acids occurs [7,11]. It is remarkable that radicals' production begins in the ER and then it is extended to mitochondria and to peroxisomes [6,12]. Namely, through a complex pathway free radicals oxidize and activate an ER kinase called Protein Kinase RNA-Like ER Kinase (PERK), which catalyzes the transfer of phosphate groups from high-energy, phosphate-donating molecules to a mitochondria ATPase called sarcoendoplasmic reticulum calcium ATPase (SERCa) [6,12]. Through SERCa activation, a depolarization in mitochondrial membrane is occurred, so these membrane-bound cell organelles called mitochondria release a great amount of reactive species [6, 12]. Furthermore, oxidative stress deregulates an endoplasmic reticulum stress-regulated transmembrane transcription factor, called activating transcription factor 6a (ATF6a) [7,12]. This factor induces the expression of two proteins that control energy homeostasis in peroxisomes, peroxisome proliferator-activated receptor (PPAR) and ATP-binding cassette sub-family D member 3 (ABCD3) [6,12]. These two proteins are interrelated with a great release of ROS from peroxisomes [13]. Simultaneously, there is a depletion of the body's antioxidant systems in myeloma cells, such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), catalase and thioredoxin, a situation that further enhances oxidative stress conditions in myeloma cells [9,13].

Nevertheless, the fundamental question that preoccupies scientists' research work is the discovery of the trigger factor that leads to these ROS and RNS overproduction [13,14]. Initially, an increase in ROS, RNS synthesis occurs due to the hypermetabolic conditions and the overproduction-overaccumulation of misfolded immunoglobulins in cancer cells' ER [13,14]. Secondly, production ROS and RNS production is amplified by the deregulation of iron (Fe) metabolism, due to ferroportin's suppression [14]. Ferroportin is an enzyme that extracts iron from cancer cells [14]. Ferroportin's action suppression, results in increased levels of Fe^{2+} detected in the neoplastic cells, as shown by FeRhoNox-1 staining [14,15]. Increased Fe^{2+} levels result to hydroxide radicals' production via Fenton reaction [15]. Thirdly, these oxidative stress conditions lead to a further ROS and RNS production [13,16]. On the one hand, many oxidative stress markers activate specific receptors via a lock and key model, leading to direct activation of specific transcriptional factors that induce silencing of antioxidant genes and deregulation of mitochondrial respiratory chain [13,16]. For example, AGE binds to AGE receptor (RAGE) and induces transcription factor Wnt-Dikkopf-1 [10]. This interaction enhances further bone osteolysis and oxidative stress intensification [10]. On the other hand, these oxidative stress conditions, lead to oxidative modification of proteins, carbohydrates and DNA, which activate ER stress sensors' such as inositol-requiring enzyme 1a [(IRE1a-IRE1a is activated through sulfonylation)], PERK-ATF6 [(PERK-ATF6 are activated through phosphorylation and dimerization)] [16,17]. These oxidative stress sensors activate Unfolded Protein Response (UPR) pathway that promotes survival and expansion of myeloma cells and enhance silencing of the antioxidants genes through the effect of a transcriptional factor called, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [13,16,17] (Fig. 1). Despite this advantageous impingement of the oxidative stress vicious cycle to the proliferation and the survival of myeloma cells, a great increase in ROS and RNS production (as it is generated by some antimyeloma agents), leads to the activation of B-cell lymphoma 2 protein (Bcl-2) associated agonist of cell death (Bad) and Bcl-2-associated X protein (Bax) proapoptotic factors and subsequently, to the activation of the endogenous and exogenous pathway of apoptosis [6,16] (Fig. 1).

In advanced stages of the disease and, especially, in patients with MM that their karyotype presents the t(4;14) translocation, which is associated with poor prognosis, there is a suppression of oxidative stress [6,7,17]. Oxidative stress suppression is caused by the induction of the transcription factor nuclear factor erythroid 2-related fac-

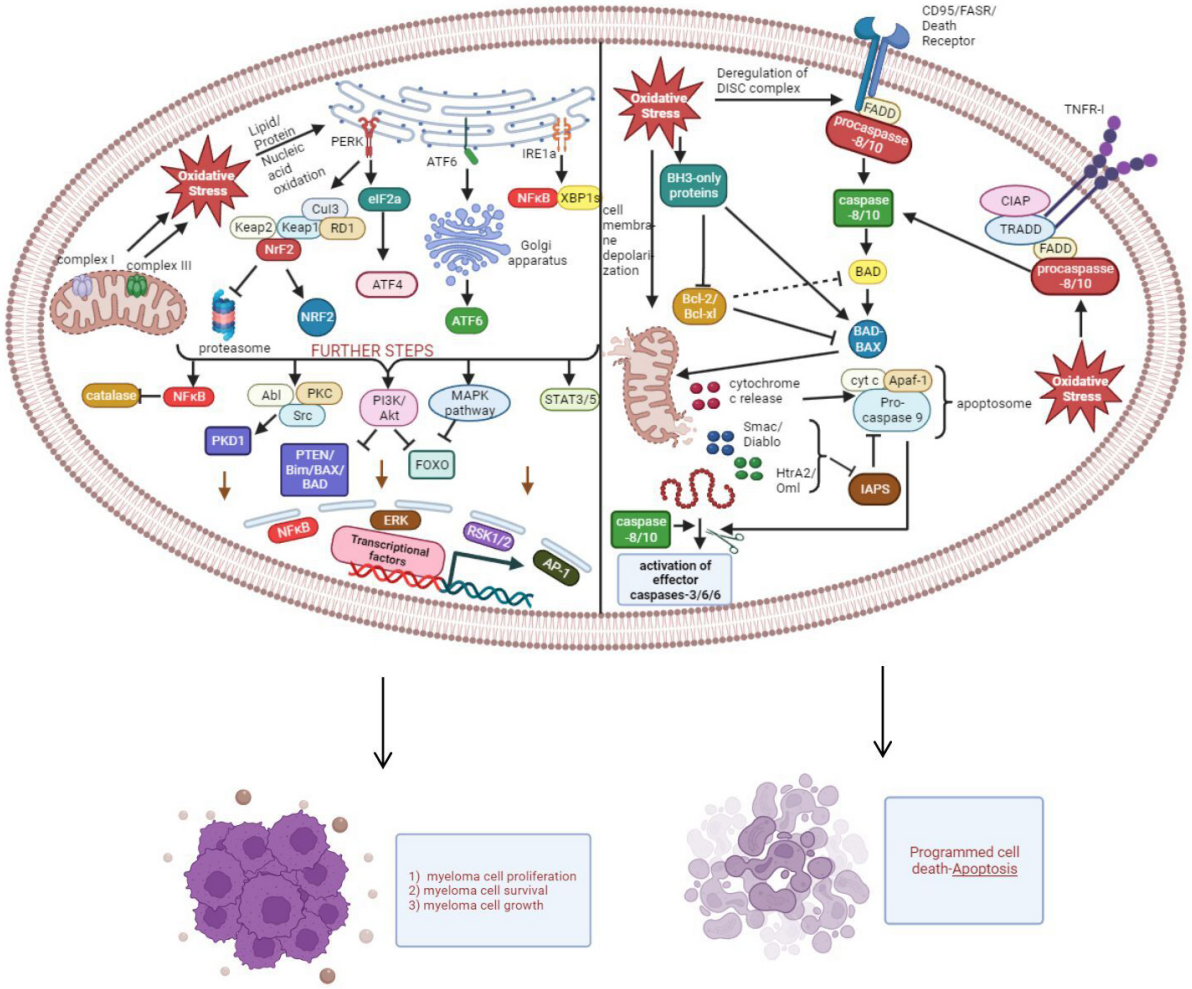

Fig. 1. The dual role of oxidative stress in Multiple myeloma (MM). Abl, Abelson protein; AP-1, Activator protein 1; Apaf1, Apoptotic protease activating factor 1; ATF4, Activating transcription factor 4; Bcl-2, B-cell lymphoma 2 protein; Bad, Bcl-2 associated agonist of cell death; Bax, Bcl-2-associated X protein; Bcl-xl, B-cell lymphoma-extra-large; BIM, Bcl-2 interacting mediator of cell death; CD95, Cluster of differentiation 95; CIAP, Cellular inhibitor of apoptosis protein 1; cyt c, cytochrome c; DISC, Death-inducing signaling complex; eIF2A, eukaryotic translation initiation factor 2A; ERK, Extracellular signal-regulated kinases; FADD, FAS-associated death domain protein; FASR, FAS receptor; FOXO, forkhead box O; HtrA or Omi, Human high temperature requirement A2; IAPs, Inhibitors of apoptosis proteins; IRE1a, inositol-requiring enzyme 1a; Keap1, Kelch-like ECH-associated protein 1; MAPK, mitogen activated protein kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NrF2, nuclear factor erythroid 2-related factor 2; PERK, Protein Kinase RNA-Like ER Kinase; PI3K, phosphatidyl inositol 3-kinase; PKC, protein kinase C; PKD1, Polycystic kidney disease 1; PTEN, Phosphatase and tensin homolog; RSK1/2, Ribosomal s6 kinase 1/2; Smac/DIABLO, second mitochondria-derived activator of caspases; Src, Sarcoma rous homology; STAT, signal transducer and activator of transcription; TNFR-I, Tumor necrosis factor receptor I; TRADD, Tumor necrosis factor receptor type 1-associated DEATH domain protein; XBP1s, X-box binding proteins 1. This picture was drawn by Thomas Achladas (TA) and Giorgos Koktsidis (GK). The program used was https://www.biorender.com/.

tor 2 (NrF2) with unclarified mechanism [18,19]. NrF2 transcription factor encodes antioxidant genes such as GSH and catalase, encodes DNA repair factors and encodes proteins that "supervise" DNA's correct folding such as Wolf-Hirschhorn syndrome candidate gene-1 (WHSC1) and auto-inhibited Ca^{2+}-ATPase 11 (ACA11) [18–20]. Moreover, NrF2 activates a protein acting as an ER radical scavenger, which is called scavenger receptor class A member 3 (SCARA3) [19,20]. Frequently, multiple myeloma co-exists with diabetes and metabolic syndrome [21]. According to genome sequencing in patients with metabolic syndrome a loss of four box C/D small nucleolar RNAs (snoRNAs) (U32a, U33, U34, U35a) encoded in the ribosomal protein L13a (rpL13a) locus, which are associated with unknown mechanism with lipotoxic stress reinforcement, has been established (snoRNAs are a class of small RNA

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molecules that primarily guide chemical modifications of other RNAs, mainly ribosomal RNAs, transfer RNAs and small nuclear RNAs) [21]. This deregulation at the introns 2, 4, 5, 6 located in in the rpl13a genetic locus leads to a further resistance to lipotoxic and oxidative stress, so the neoplastic cells can survive [21].

Molecular Mechanisms Linking Oxidative Stress with Multiple Myeloma

Gene-Transcriptional-Protein Changes

High levels of oxidative stress are linked with the activation of UPR pathway (Fig. 2). UPR via deregulation of signaling cascades such as mitogen activated protein kinase (MAPK) pathway, phosphatidyl inositol 3-kinase-Ak strain transforming (PI3K-Akt) axis, signal transducer and activator of transcription (STAT)3/5 axis and interleukin-6 (IL-6) pathway lead to intense cell proliferation, apoptosis suppression and deregulation of cell differentiation [22–24]. Moreover, during these complex molecular pathways a deregulation of Rho proteins is detected [25,26]. The Rho family of GTPases is a family of small (~21 kDa) signaling G proteins that are associated with many aspects of intracellular actin dynamics [26]. Specifically, Rho proteins regulate organelle development, cytoskeletal dynamics and cell movement [26] (Fig. 2). In MM, a hyperactivation of Rho cascade (Rho-Wiskott Aldrich-Rock proteins) results in alteration of cell polarity, development of filopodia, alteration of the interaction between the neoplastic plasma cells and the peripheral cells of bone marrow stroma, development of new sites of establishment in the extracellular matrix and myeloma cells migration [22,25,26]. This Rho deregulation is caused by oxidative deregulation of focal adhesion kinase (FAK), due to oxidation at a cysteine center (FAK is a crucial Rho pathway's regulator) and by hyperactivation of the Rho-Wiskott Aldrich-Rock GTPase system due to oxidative modification of the allosteric sites of Rho and oxidative inactivation of Rho's inhibitory phosphatase, low molecular weight protein-tyrosine phosphatase (LMW-PTP) [11,26].

Metabolic Deregulation

Activation of the Warburg effect is established, due to ROS-induced alteration of transcription factors expression such as peroxisome proliferator-activated receptor-gamma coactivator 1a (PGC-1a), paired box transcription factor 5 (PAX5) and hypoxia inducible factor (HIF) [27–29] (Fig. 2). Warburg's effect activation leads to the induction of the anaerobic glucose metabolism and consequently, to malignant cells survival [28,29]. Specifically, anaerobic metabolism is enhanced by inhibition of pyruvate dehydrogenase, by enhancement of lactate dehydrogenase, by increased glucose uptake due to overexpression of glucose transporter (GLUT)1/4/8/11 and by increased angiogenesis through the production of vascular endothelial growth factor (VEGF) [28,29]. All these pathways lead to lactic acid overproduction [27,29]. Lactic acid can be also used to the lipid-amino acid-nucleotide biosynthesis pathway [27–29]. The main transcription factor activated in Warburg effect is HIF [28,29]. HIF's activation occurs in three ways, through the UPR pathway cascade, through direct excitatory oxidation of a serine center and indirectly, through the inhibition via oxidation of the HIF ubiquitination factor, Von Hippel-Lindau [27]. Simultaneously, genes epigenetic modification is also deregulated, due to acetyl-CoA production suppression (pyruvate dehydrogenase is inhibited) and deacetylases hyperactivation, because nicotinamide adenine dinucleotide (NAD)+ is overproduced in the context of anaerobic glycolysis [27–29]. The inverse of the Warburg effect is also present [27]. The increased free radicals' production by peripheral myeloma cells leads to their uptake by neighboring fibroblasts [27,28] (Fig. 2). As a result, fibroblasts are under oxidative stress, increased catabolism, impaired mitochondrial function and overproduction of lactic acid [27,28]. Lactic acid is then exported by the monocarboxylate transporter 4, taken up by the tumour cells and further enhances oxidative stress in the myeloma cells [27,28].

Epigenetic Alterations

Epigenetic changes take place in MM both at the level of chromatin condensation and at the level of miRNA biogenesis, mainly through NF-κB transcription factor, but also through nuclear factor erythroid 2-related factor 2 (NrF2) and Kelch-like ECH-associated protein 1 (Keap1) transcription factors (Table 1, Ref. [18,30–33]) [30–32]. The aforementioned transcription factors are deregulated because of UPR cascade [30,31] (Fig. 2). The miRNAs that are usually deregulated-overexpressed are those that are responsible for p53 inactivation [33]. This fact clarifies the extremely low somatic mutation rate of the p53 tumor suppressor gene in MM, in contrast with the other cancers, where the "guardian of the genome" is frequently mutated [33].

Oxidative Stress and Myeloma Treatment

During the last two decades, the outcome of patients with MM has drastically improved [1,2]. The development of proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) has revolutionized the treatment paradigm for these patients and their combinations comprise the backbone of antimyeloma therapy, at all stages of myeloma [1,2]. Oxidative stress can be exploited in two ways in the treatment of MM [34]. Either with novel agents that greatly increase it's levels within the neoplastic cells and lead them to death, or with novel agents that significantly reduce it's levels and prevent malignant cells' expansion [34].

Oxidative Stress Inducers

Proteasome Inhibitors (PIs). Bortezomib is a key factor regarding MM treatment [35,36]. Bortezomib is the first se-

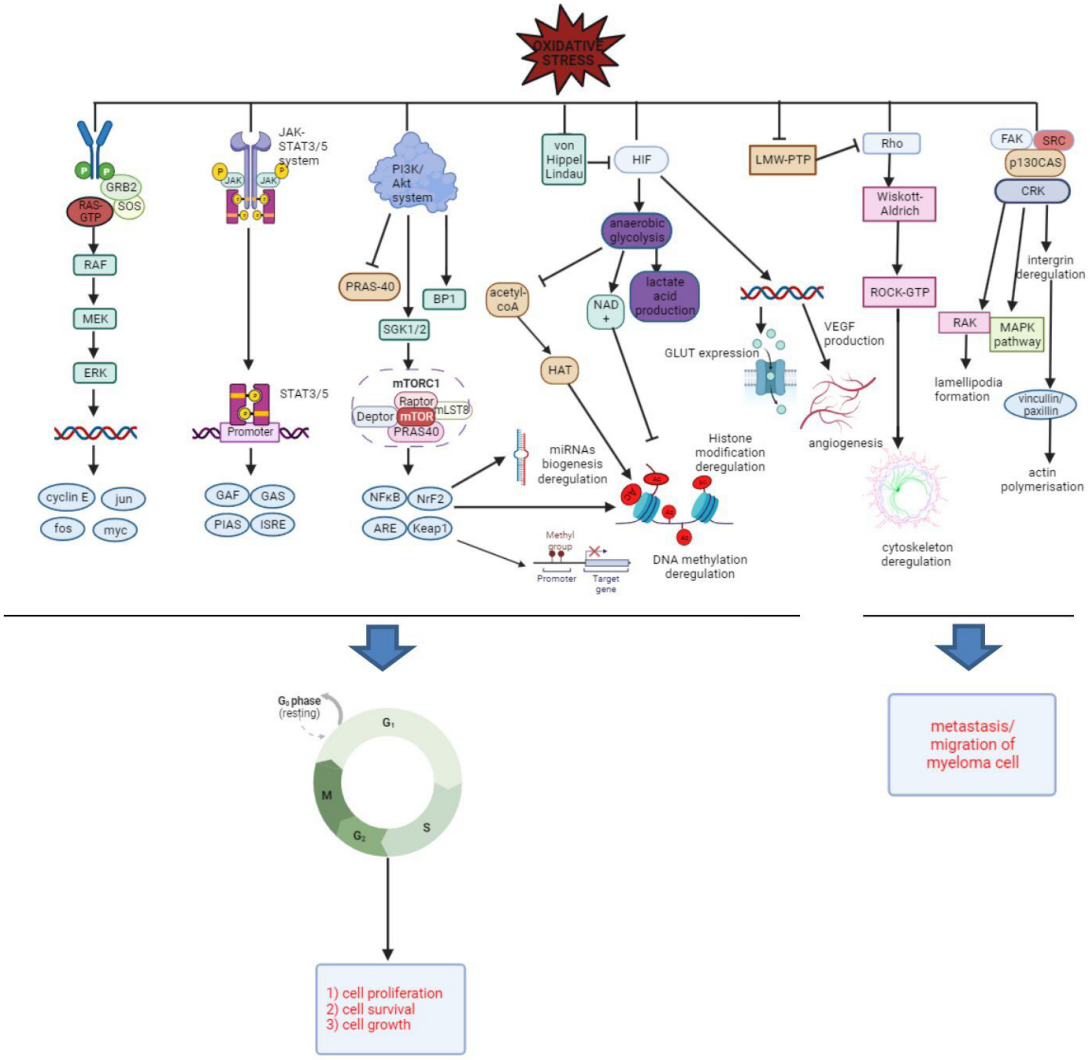

Fig. 2. Deregulated molecular mechanisms due to oxidative stress. BP1, BAH-PHD protein 1; FAK, Focal Adhesion Kinase; GAF, cGMP-specific phosphodiesterases, adenylyl cyclases and FhlA; GLUT, glucose transporter; GAS, Growth arrest specific; GRB2, Growth factor receptor-bound protein 2; HAT, Histone acetyltransferase; ISRE, Interferon-stimulated regulatory element; JAK, Janus kinase; Jun, Ju-nana; LMW-PTP, low molecular weight protein-tyrosine phosphatase; MEK, Mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; NAD, nicotinamide adenine dinucleotide; PIAS, Protein inhibitor of activated STAT; PRAS, P rat sarcoma virus; p130CAS, p130 CRISPR-associated; RAF, Rapidly accelerated fibrosarcoma; Raptor, Regulatory-associated protein of mTOR; SGK1/2, Serine/threonine-protein kinases; ROCK, Rho-associated protein kinase; SOS, Son of sevenless; VEGF, vascular endothelial growth factor. This picture was drawn by Thomas Achladas (TA) and Giorgos Koktsidis (GK). The program used was https://www.biorender.com/.

lective PI approved for the treatment of MM [35]. Although approved in 2003, it was only in 2018 that the contribution of oxidative stress to it's mechanism of action was specified [35,36]. Particularly, bortezomib inhibits the molecular apparatus that degrades misfolded or unfolded proteins, previously labeled with ubiquitin molecules by the homologous ligase [37,38]. Immunoglobulin damaged protein molecules accumulate in the ER, inducing mainly, the C/EBP homologous protein pathway (CHOP pathway) and thus the proteins: protein disulfide isomerase (PDI), endoplasmic reticulum oxidoreductin 1 (ERO-1), NADPH oxidase (NOX) complexes and mitochondrial electron transport enzymes that are associated with ROS and RNS production [35,37,38]. Additionally, misfolded proteins enhance the action of the transcription factor Kruppel-like Factor 9 (KLF9). KLF9 inhibits antioxidant enzymes, mainly thioredoxin [35]. Thus an intracellular state of intense oxidative stress and cytotoxicity is generated [35]. Metformin co-treatment with bortezomib, suppresses induction of the critical UPR effector glucose-regulated protein 78 (GRP78) and impairs autophagosome formation and enhances ROS-mediated apoptosis [39]. This drug

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Table 1. miRNAs that are deregulated in MM, due to oxidative stress.

miRNAs	Signaling pathways that lead to the dysregulation of miRNAs	Signaling factors that are disarranged by microRNAs and favour MM development	References
let-7 miRNA inhibition	Nrf2/Keap1/ARE	RAS, HMGA2, c-MYC, CDC25A, CDK6, cyclin D2 stimulation	[18,30–33]
miRNA-21 stimulation	NF-κB	NADH-dehydrogenase, BIM, PTEN, PDCD4, mapsine inhibition	[18,30–33]
miRNA-34a/137/687 stimulation	HIF	HBP1, SIN3B, BTG1, miRNA-34a/137/15a, SLC1A5, GOT1, erastine inhibition	[18,30–33]
(1) miRNA-17 stimulation (2) miRNA-17-p stimulation	(1) c-MYC (2) Ferroportin	(1) HBP1, SIN3B, BTG1, miRNA-34a/137/15a, SLC1A5, GOT1, erastine inhibition (2) NrF2-miRNA-7-p axis modullation	[18,30–33]
miRNA-200a/200b/200c stimulation	SIP1, ZEB1, HIF1	stimulation of e-cadherin pathway and epithelial mesenchymal transition of the neoplasm	[18,30–33]
miRNA-125/200a/141 stimulation	Nrf2/Keap1/ARE	superoxide dismutase, p38a antioxidants inhibition	[18,30–33]

Abbreviations: BTG1, B cell translocation gene 1; CDC25A, Cell division cycle 25 homolog A; CDK6, Cyclin dependent kinase 6; c-MYC, cellular-myelocytomatosis; GOT1, Glutamic-oxaloacetic transaminase 1; HBP1, Hmg-box transcription factor protein 1; HIF, hypoxia inducible factor; HMGA2, High-mobility group AT-hook 2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PDCD4, Programmed cell death protein 4; RAS, rat sarcoma virus; SIN3B, SIN3 transcription regulator family member B; SIP1, Smad interacting protein 1; SLC1A5, Solute carrier family 1 member 5; ZEB1, Zinc finger E-box binding homeobox 1; Reduced Nicotinamide Adenine Dinucleotide (NADH).

combination is significantly effective against bortezomib-resistant MM [39]. Except from Bortezomib, other PIs can be used against MM, through activation of oxidative stress, such as Carfilzomib [2,40]. A low dose of Carfilzomib, a novel second generation PI, is frequently combined with a low concentration of resveratrol [40]. This co-treatment induces in a dose and time dependent manner the release of second mitochondria-derived activator of caspase (Smac), the down regulation of a stress sensor sirtuin1 (SIRT1), with deacetylase enzyme activity and consequently, the inhibition of autophagy and the induction of ROS toxicity [40].

Immunomodulatory Drugs (IMiDs). Thalidomide, Lenalidomide and Pomalidomide are IMiDs-antiangiogenic agents [41,42]. Novel studies show that these drugs inhibit the antioxidant enzyme thioredoxin reductase and induce the endoplasmic reticulum enzyme flavoprotein endoplasmic reticulum oxidoreductin 1 (ERO-1) by unknown mechanism [41,42]. ERO-1 is involved in disulfide bond formation and releases H202 [41,42]. These pathways lead to the accumulation of ROS, H2O2 and dimeric λ-light chains in the cytoplasm, thus enhancing the action of the neprilysisn metalloendopeptidase protein, leading to the activation of the programmed cell death pathway [41,42]. It is very important to notice that ROS production was the pathogenetic mechanism that caused teratogenesis in the newborns (limb malformations-phocomelia) after thalidomide administration to pregnant women as antiemetic [41,42]. Thalidomide inhibits angiogenesis in the fetal limbs [41,42].

Alkylating Agents. Melphalan has been the main drug used in MM management for many years in the past [43]. Melphalan in combination with prednisone has been recommended as a treatment since 1964 [43]. The increase in mitochondrial metabolism leads to the overproduction of hydrogen peroxide tributyl radicals [43]. As a consequence, the DNA double helix breaks and the tumor cells are destroyed [43].

Histone Deacetylase Inhibitors. They are a relatively new class of antimyeloma agents, including Vorinostat and Panobinostat, targeting enzymes involved in epigenetic regulation of gene expression [44,45]. A novel inhibitor is belinostat or PXD101 or N-hydroxy-3-phenylsulfamoylphenyl acrylamide [44,45]. Belinostat is a low molecular weight inhibitor of the deacetylation of histones H3 and H4 which, in combination with the PI bortezomib, potently induces apoptosis and osteolysis reduction by CD138+ myeloma cells [44,45]. This is an epigenetic drug leading to transcriptional silencing of certain genes [44,45]. Consequently, the caspases 3, 8, 9 are activated, leading to ROS release from mitochondria, as it is proved by Western blot, immunohistochemistry and polymerase chain reaction (PCR) [44,45]. ROS release leads to breaks in DNA as it is demonstrated by *in vitro* studies with radioactive H3-thymidine and by elevated H2A histone family member X (H2AX) marker which is associated with DNA breakage [44,45]. Simultaneously, there is a ROS-induced activation of p53 and consequently of mitogen activated protein kinase receptor 38 (MAPR38), p27, cyclin-dependent kinase inhibitor p21, due to increased phosphorylation at ser15 [44,45]. These kinases in turn inhibit the anti-apoptotic proteins B-cell lymphoma 2 protein (Bcl-2) and B-cell lymphoma extra-large (Bcl-xl) and promote the pro-apoptotic Bcl-2 interacting mediator of cell death (BIM) and inhibitor of cyclin dependent kinase 1a (Icdk1a) [44,45]. Finally, they inhibit the mitotic activity of myeloma cells by enhanc-

ing the acetylation of tubulin [44,45]. Tubulin is a protein of the spindle microtubules [44,45]. Tubulin's acetylation prevents their polymerization of microtubules for spindle formation [44,45]. Eventually all the above lead to cell death [44,45].

Monoclonal Antibodies (MoAbs). In contrast with the aforementioned drugs, the correlation between MoAbs and oxidative stress mediated toxicity is still unclear [46,47]. Anti-CD38 MoAbs represent the backbone for treatment of newly diagnosed and relapsed MM [46,47]. CD-38 is a surface NAD+-degrading electroenzyme (NAD+ase), which is overexpressed in myeloma cells [46]. Using an anti-CD38 novel agent the breakdown of NAD+ is prevented and thus the level of NAD+ is increased [46]. This biological condition leads to the deregulation of the mitochondrial axis pten-induced kinase 1 (Pink1)/Parkin/ring-type E3 ubiquitin ligase/presenilin-associated rhomboid-like protease and consequently to the deregulation of slowly degraded mitochondrial matrix proteins, isocitrate dehydrogenase 2 (Idh2) and heat shock protein 78 (Hsp78) and the rapidly degraded proteins isocitrate dehydrogenase protein 1 (Idp1) and 1-aminocyclopropane-1-carboxylic acid oxidase 2 (ACO2), which are associated with mitochondrial dynamics [46,48]. The loss of mitochondrial dynamics leads to a significantly slower onset of mitophagy, the autophagic clearance of defective mitochondria and thus to ROS production [46,48]. This oxidative stress enhancement triggers apoptosis, antibody-dependent cellular cytotoxicity, antibody-dependent cellular phagocytosis, complement-dependent cytotoxicity and a further sensitivity to PIs and dexamethasone action in myeloma cells [46–49]. Very important to notice that only Isatuximab has been shown to target ectoenzymatic function of CD38 protein [46,47]. Daratumumab and the other anti-CD38 MABs do not affect NAD+ase activity of the enzyme [46]. Isatuximab co-treatment with agents like all-trans retinoic acid, IMiDs and nicotinamide phosphoribosyltransferase inhibitors (NAMPT inhibitors) such as FK866 increases MM cell's surface CD38 antigen and contributes to the eradication of low proliferative or dormant MM-initiating cells or drug-resistant clones and consequently prevents from minimal residual disease [46,49]. A more comprehensive functional analysis about the impact of Isatuximab on cell biology and oxidative stress is indispensable [46,49]. A correlation between Elotuzumab, a signaling lymphocytic activation molecule 7 (SLAM7) inhibitor and oxidative stress has not been proved [46].

Clinical trials play a crucial role in evaluating the safety and efficacy of new treatment approaches, including drug combinations that incorporate agents with oxidative stress inducers. These trials aim to improve outcomes for patients with myeloma by exploring novel therapeutic strategies that target specific pathways involved in cancer progression. Table 2 (Ref. [50–64]) summarizes the most recent, significant Phase II or III multiple myeloma clinical trials containing regimens with drug combinations inducing Oxidative Stress.

New Experimental Drugs. Phenethyl ester of caffeic acid, formonetin, CP-31398, fangchinoline (they act through modulation of oncogenic STAT3 signaling pathway), long-chain fatty acid analogs of the MEDICA series (they promote mitochondrial stress and inhibit cholesterol biosynthesis) and miRNAs belong to the new experimental antimyeloma agents (Table 3, Ref. [6,26,32]) [30,31,65–70]. Regarding miRNAs, miR-144, miR-28, miR-200a and miR-93 selectively inhibit the antioxidant pathway of NrF2 and miR-125b targets-inhibits the mRNAs of the antioxidant proteins NAD(P)H quinone, oxidoreductase 1, heme oxygenase-1 (HO-1) and peroxiredoxin like 2A (PRXL2A) [6,18,26,32]. There are various challenges for the implementation of new experimental drugs in clinical use [31]. Nevertheless, although they show "encouraging" results in eliminating myeloma cell lines in preclinical *in vitro* models, clinical trials have not been conducted, yet [32]. As a result, interactions with other drugs, contraindications, effective drug's dose concentration and the ability of these agents to infiltrate myeloma cells' microenvironment and finally, to kill *in vivo* tumor cells, has not been classified [68,69]. The greatest challenge concerns epigenetic drugs and, especially, miRNAs in clinical use [30]. Off-target effects of these epigenetic modifiers (a miRNA can suppress more than one mRNA), low isoform selectivity (the effects of different isoforms in tumorgenesis are still unidentified) and efficient transport and delivery systems, which can specifically target myeloma cells must be taken into consideration [32]. Clinical trials and new molecular technologies such as optogenetics and transcriptional activator-like effectors approach will clarify, on the one hand, novel agents' effectiveness and on the other hand, the exact myeloma cells' epigenetic profile and potential epigenetic targets [31]. Results of various preclinical studies advocate that these new experimental drugs cannot become multiple myeloma's backbone treatment, yet [30,69]. However, synergistically with existing therapies such as PIs and MoAbs, newest experimental drugs could be effective in myeloma cells' elimination [30,68].

Oxidative Stress Inhibitors

Deferasirox. As already mentioned, there is impairment in ferroportin function in patients with MM, which leads to Fe accumulation and ROS production [14,71]. Deferasirox is a chelating agent, binds to Fe and creates an inert complex [14,71]. Consequently, the number of ROS is significantly altered after deferasirox administration [14,71]. ROS number alteration stops the activating phosphorylation of proline-rich tyrosine kinase 2 (Pyk2), so the glycogen synthase kinase 3β (GSK-3β) protein that degrades β-catenin is activated and thus, the Wnt/β-catenin/PI3K-

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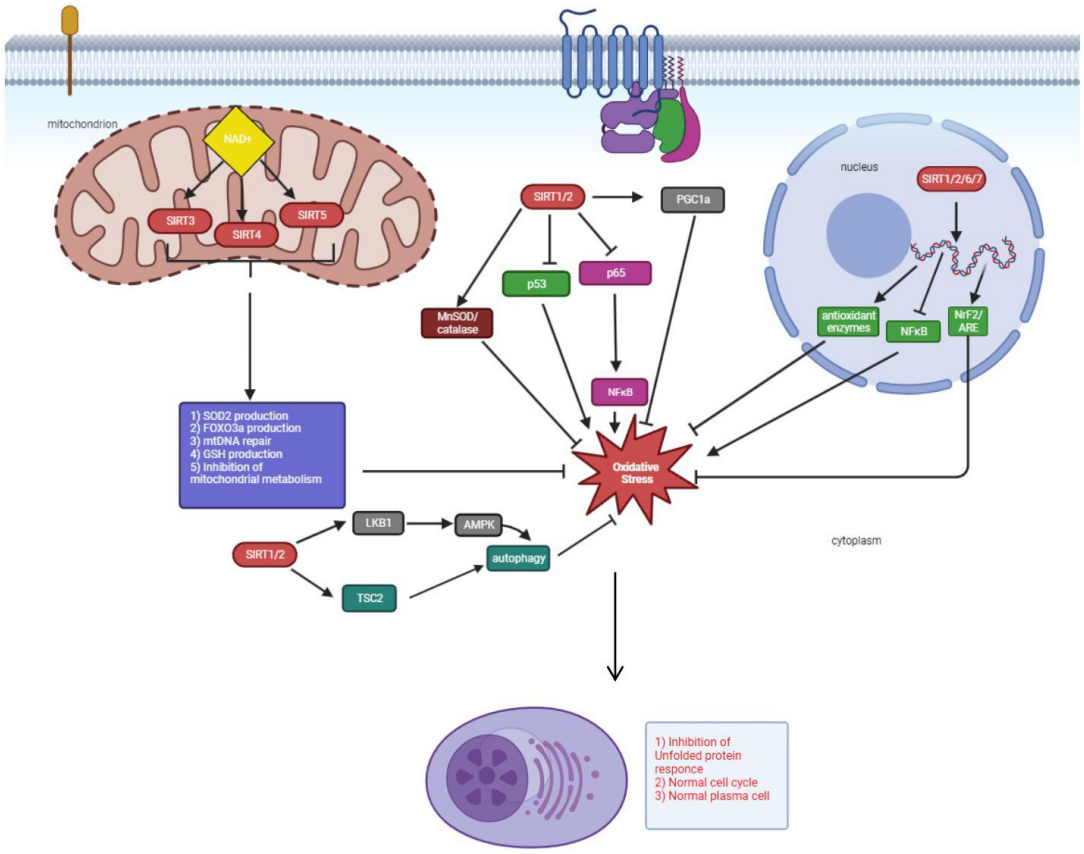

Fig. 3. The complex action of sirtuins. AMPK, AMP-activated protein kinase; GSH, Glutathione; FOXO3a, Forkhead Box O3a; LKB1, Liver kinase b1; mtDNA, mitochondrial deoxyribonucleic acid; PGC1a, peroxisome proliferator-activated receptor-gamma coactivator 1a; SIRT1, Sirtuin 1; SOD2, Superoxide dismutase 2; TSC2, Tuberous sclerosis complex 2. This picture was drawn by Thomas Achladas (TA) and Giorgos Koktsidis (GK). The program used was https://www.biorender.com/.

Akt/cyclin D1/axis inhibition protein 2 (Axin-2), cellular-myelocytomatosis (c-Myc) oncogenic pathways are inactivated and the abnormal plasma cells are led to apoptosis [14,71]. Moreover, deferasirox contributes to apoptosis induction by inhibiting bone marrow stroma cells that feed cancer cells, by inducing the caspases 9 and 3 and by enhancing bortezomib's action [14]. Although deferasirox is associated with increased nephrotoxicity, the proximal renal tubular injury is reversible [14].

Experimental Treatments Related to Antioxidant Genes Overexpression, such as the Overexpression of Manganese Superoxide Dismutase (MnSOD). The researchers succeeded in this endeavor applying the technology of antagomirs [31]. These are *in vitro* engineered, complementary oligonucleotides, mainly, for the 3 untranslated region [31]. They are stabilized after 2-O-methylation or 2-O-methoxyethylation or 2-O-methylenation on ribose sugar and inhibit miRNA binding to the target mRNA [31]. Nevertheless this method has several limitations [31]. Despite advances in bioinformatics we do not know exactly all the miRNAs targets [31]. Also, no suitable mode of administration of these molecules has been developed, since high-pressure injection and electrodrilling cause tissue damage, the use of viral vectors sometimes incorporates unwanted mutations into the genome and finally, liposomal transporters lead to severe immune responses [31].

Oxidative Stress and Bone Marrow Transplantation in Patients with MM

The standard of care for fit MM patients is to receive high-dose chemotherapy with autologous stem cell rescue, known as autologous stem cell transplantation, after induction therapy [72–74]. In order to mobilize CD34+ stem cells from the peripheral blood, hematopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF) and chemokinetic agents are administered [75]. During bone marrow stimulation, hematopoietic cells are exposed to an increased amount of oxygen and nutrients, resulting in a significant increase in mitochondrial oxidative metabolism and ROS production [75]. This leads to either apoptosis of large numbers of cells (40% of patients fail to collect sufficient number of CD34+ stem cells) or the cells exhibit deregulated pathways of proliferation, differentiation and immune response, leading to a high risk of graft versus host disease after transplantation [75]. The researchers treated

Table 2. Most recent, significant Phase II or III multiple myeloma clinical trials containing regimens with drug combinations inducing Oxidative Stress.

Trial	Regimen	Phase/primary endpoint	Median follow-up	Patient population	Number of patients	Responses	PFS, mo (95% CI)	OS, mo (95% CI)	Ref.
IsKia EMN24	Isa-KRd vs. KRd	Phase III/MRD negativity by NGS post-ASCT consolidation	21 mo	Transplant-Eligible Patients With Newly Diagnosed MM	302	Post consolidation response - ≥VGPR, 94% in both arms - CR, 74% vs. 72% - sCR, 64% vs. 67%	95% at 1 year in both arms	NR	[50]
CENTAURUS	Dara Monotherapy	Phase II, open-label/≥ CR, PD, or death	85.2 mo (range: 0–94.3 mo)	Intermediate- or High-Risk Smoldering MM	123	ORR% in dosing schedules - intense: 58.5% - intermediate: 53.7% - short: 37.5%	Median PFS, (95% CI) including extension phase for the different dosing schedules - intense: NR - intermediate: 84.4 mo - short: 74.1 mo	In different dosing schedules - intense: NR - intermediate: NR - short NR	[51,52]
GRIFFIN	D-RVd vs. RVd	Phase II/sCR at the end of post-ASCT cons	49.6 mo	Newly Diagnosed Transplant-Eligible Patients	207	sCR: 67% vs. 48% [95% CI 1.22–3.89], $p = 0.0079$	4-yr PFS rates: 87.2% vs. 70.0%	For either group NR (HR 0.90 [95% CI 0.31–2.56], $p = 0.84$)	[53]
	VenDd vs. DVd	Phase I/II PFS, response rates (ORR, ≥VGPR, ≥CR)		R/R MM with t(11;14)	81	ORR: 96.4% vs. 65.4%, VGPR: 25.5% vs. 19.2%, CR: 27.3 vs. 7.7	Median PFS, mo (95% CI): 46.1% vs. 15.5%	NR for both arms	[54]
PERSEUS	D-VRd vs. VRd	Phase III	47.5 mo	Newly Diagnosed Transplant-Eligible Patients	709	≥CR, 87.9% vs. 70.1%	4-yr PFS rates: 84.3% vs. 67.7% (HR, 0.42), Median PFS was not reached in either arm	Overall survival immature	[55]
IFM 2018-04	D-KRd Induction and Consolidation with Tandem Transplant	Phase II	32 mo	High-Risk Newly Diagnosed Myeloma Patients	50	ORR (before maintenance): 100%, CR: 81%	24-months PFS: 87% (78–87%)	24-months OS: 94% (87–100%)	[56]
Response Adaptive D-KRd	D-KRd MRD adapted KRd consolidation +/- ASCT and maintenance vs. observation	Phase II	36 mo	Patients with Newly Diagnosed Multiple Myeloma	39	End of induction, ≥CR: 56% (95% CI: 42%–70%) CR: 44%, VGPR: 36%, PR: 3%	2-year PFS 85.3% (95% CI: 71.6%– 99.1%) median PFS: NR		[57]
GMMG-HD6	RVd/R maintenance RVd/E-R maintenance E-RVd/R maintenance E-RVd/E-R maintenance	Phase III	60.8 mo	Newly Diagnosed Multiple Myeloma Transplant-Eligible Patients	555	*CR rates* RVd/R: 50% RVd/E-R: 44% E-RVd/R: 51% E-RVd/E-R: 49% *VGPR rates* RVd/R: 8% RVd/E-R: 11% E-RVd/R: 11% E-RVd/E-R: 8% *PR rates* RVd/R: 9% RVd/E-R: 8% E-RVd/R: 7% E-RVd/E-R: 8%	Median PFS RVd/R: NR (95% CI 45.5 mo) RVd/E-R: 60.8 mo (50.3-NR) E-RVd/R: 56.6 mo (50.0-NR) E-RVd/E-R: NR (45.6 mo-NR)	Median overall survival was not reached in either treatment group	[58]

Table 2. Continued.

Trial	Regimen	Phase/primary end-point	Median follow-up	Patient population	Number of patients	Responses	PFS, mo (95% CI)	OS, mo (95% CI)	Ref.
CASTOR	D-Vd vs. Vd	Randomized, Open-Label, Phase III Trial	72.6 mo (0.0–79.8)	R/R MM	241			Median OS, D-Vd: 49.6 mo vs. Vd: 38.5 mo (hazard ratio, 74; 95% CI 0.59–0.92) ($p = 0.0075$)	[59]
EMN20 Trial	KRd vs. Rd	Phase III	24.9 mo	Newly Diagnosed Fit or Intermediate-Fit Multiple Myeloma Patients Not Eligible for ASCT	82	MRD negativity 10^{-5} - At 1 yr of treatment, KRd: 50% vs. Rd: 0% ($p < 0.0001$) - At 2 yrs of treatment, KRd: 55% vs. Rd: 0% ($p < 0.0001$) - Sustained MRD Neg at 2 yr, KRd: 38% vs. Rd: 0% ($p < 0.0001$)	Median PFS KRd: not reached, Rd: 20.9 mo (HR 0.29, 95% CI 0.13–0.64) ($p = 0.002$)	2-yr OS, KRd: 89% vs. Rd: 74% (HR 0.36, 95% CI 0.11–1.17) ($p = 0.09$)	[60]
CASSIOPEIA	D-VTd vs. VTd	Phase III/sCR at 100 d post-ASCT and PFS from second randomization	35.4 mo	Newly Diagnosed Multiple Myeloma Transplant-Eligible Patients	886	CR or better D-VTd: 39% vs. VTd: 26% ($p < 0.0001$) MRD negativity 10^{-5} D-VTd: 64% vs. VTd: 44% ($p < 0.001$)	Median PFS D-VTd: NR VTd: 46.7 mo (HR 0.53, 95% CI 0.42–0.68) ($p < 0.0001$)		[61]
POLLUX	D-Rd vs. Rd	Phase III	79.7 mo (0.0–86.5)	Previously Treated Multiple Myeloma	296	ORR (92.9 vs. 76.4%; $p < 0.0001$) CR or better: 56.6 vs. 23.2% ($p < 0.0001$) MRD negativity 10^{-5} 30.4 vs. 5.3% ($p < 0.0001$)	Median PFS D-Rd: NR Rd: 31.7 mo (HR, 0.53; 95% CI, 0.42–0.68) ($p < 0.0001$)	Median OS, D-Rd: 67.6 mo vs. Rd: 51.8 mo (hazard ratio, 0.73; 95% CI 0.58–0.91) ($p = 0.0044$)	[62,63]
PERSEUS	D-VRd followed by Len+D Maintenance vs. VRd followed by Len Maintenance	Phase III	47.5 mo	Transplantation-Eligible Patients with Newly Diagnosed Multiple Myeloma	709	CR or better D-VRd: 87.9% vs. VRd: 70.1% ($p < 0.001$) MRD-negative status D-VRd: 75.2% vs. VRd: 47.5% ($p < 0.001$)	48 mo PFS D-VRd: 84.3% vs. VRd: 67.7% (HR, 0.42; 95% CI 0.30–0.59) ($p < 0.001$)	NR	[64]

Abbreviations: ASCT, Autologous Stem Cell Transplantation; CR, Complete Response; d, Dexamethasone; Dara/D, Daratumumab; E, Elotuzumab; Isa, Isatuximab; K, Carfilzomib; MRD, Minimal Residual Disease; NR, Not Reached; ORR, Overall Response Rate; OS, Overall Survival; PFS, Progression Free Survival; PR, Partial Response; R, Lenalidomide; Ref., Reference; R/R, Relapsed Refractory; sCR, stringent Complete Response; T, Thalidomide; V, Bortezomib; Ven, Venetoclax; VGPR, Very Good Partial Response.

Table 3. miRNAs as novel agents against MM.

miRNA	Target	References
miR-144/28/200a/93	NrF2	[6,26,32]
miR-125b	NAD(P)H quinone, oxidoreductase 1, HO-1, PRXL2A	[6,32]
miR-210	ISCU1/2	[6,32]
miR-450a	ACO2, TIMMDC1, ATP5B $\kappa\alpha\iota$ MT-ND2 (glycolysis-gloutaminolysis inhibition)	[6,32]
miRNA-17-3p	MnSOD, GPX2, TrxR2 antioxidants- increase in MM cell's radiosensitivity	[6,32]

Some of the aforementioned miRNAs have been used as novel agents with a great success against other cancers, such as colon and ovarian cancer. A group of scientists believe that they can be also used against other type of malignancies, such as MM. Abbreviations: ACO2, 1-aminocyclopropane-1-carboxylic acid oxidase 2; ATP5B, ATP synthase F1 subunit beta; GPX2, Glutathione peroxidase 2; HO-1, heme oxigenase-1; ISCU1/2, Iron-sulfur cluster assembly enzyme 1/2; MT-ND2, Mitochondrially encoded nadh dehydrogenase 2; PRXL2A, peroxiredoxin like 2A; TIMMDC1, Translocase of inner mitochondrial membrane domain containing 1; TrxR2, Mitochondrial thioredoxin reductase system 2; MnSOD, manganese superoxide dismutase.

the complications of oxidative stress by administering non-steroidal anti-inflammatory drugs (NSAIDs) [75]. Meloxicam is an NSAID drug that suppresses oxidative phosphorylation by reducing electron flow in the respiratory chain and activates the sirtuin antioxidant pathway [75]. The sirtuin antioxidant pathway is a family of 7 enzymes first discovered in saccharomyces cerevisiae [76]. They act as histone deacetylases-acetyltransferases that are dependent on the nicotinamide adenine dinucleotide (NAD) [75,76]. Sirtuins regulate via stress response elements, such as ARE gene loci, the Nrf2 antioxidant transcription factor, increase the expression of antioxidant factors such as catalase, forkhead box protein O3a (FOXO3a), glutathione system and activate proteins that protect DNA and mRNA from oxidative damage, such as enzymes that repair and stabilize genetic material e.g., human antigen R (HuR) (Fig. 3) [71]. Finally, sirtuins inhibit ADP-ribosyl glutamate dehydrogenase and thus mitochondrial metabolism [76] (Fig. 3).

Discussion

MM is a highly heterogeneous hematologic malignancy and the clinical outcomes of MM patients vary widely [1]. MM is characterized by the clonal proliferation of a plasma cell line with overproduction of a monoclonal immunoglobulin or a part of it [2]. Tumorigenesis, proliferation, migration but also apoptosis of myeloma cells are linked with redox signaling and especially with oxidative stress deregulation [4]. The balance between expansion of the neoplastic clone and programmed cell death is friable [5]. On the one hand, in most stages of the disease, especially in early stages, due to increased metabolism and protein synthesis and inflammation, the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) increases [7,8]. The production of ROS and RNS is induced by respiratory chain complexes I and III in mitochondria, by peroxisomes and by endoplasmic reticulum (ER), with concomitant depletion of body's antioxidant systems (glutathione system, catalase, thioredoxin) [7,9]. These oxidative stress conditions promote myeloma cell genomic instability and favor it's nourishment, by modifying metabolism (Warburg effect - the reverse of the Warburg effect) [11,15,27]. The oxidative stress, also, promotes neoplastic cells' migration to distant sites, into the bone marrow and other organs by changing cytoskeleton of the malignant cell and its interaction with extracellular matrix, through G-protein Rho and FAK kinase [26]. A great increase in ROS and RNS production (as it is generated by some antimyeloma agents) leads to myeloma programmed cell death [13,16]. On the other hand, in advanced stages of MM and in MM cases carrying the unfavorable prognosis t(4;14) translocation, oxidative metabolism is suppressed, due to the Nrf2 factor, protecting myeloma cell from apoptosis, necroptosis and ferroptosis [17,21]. As a result, there is further growth of the neoplastic clone [17,21].

Nevertheless, an alteration of the oxidative status is not only responsible for the onset of multiple myeloma and its progression, but it also appears pivotal for the therapeutic response and for developing any chemo-resistance [34]. There are two drug categories neutralizing myeloma cells through oxidative stress [34,35]. The first category includes proteasome inhibitors, histone deacetylases inhibitors and immunomodulatory drugs [34,36,37]. These antimyeloma agents provoke an overproduction of free radicals, leading to irreversible cell injury and activation of significant tumor suppressor genes such as p53, p21, PUMA [34,36,37]. These tumor suppressor genes cause the actuation of initiator caspases 9, 3, which are critical to the apoptotic pathway [44]. The second drug category includes agents such as deferasirox (a chelating agent) and novel epigenetic modulating agents, called antagomirs that lead to antioxidant genes overexpression [31,71]. miRNA that silence MnSOD expression are antagomir's most important target [31]. Last but not least, ROS and RNS overproduction obstruct autologous' and allogeneic's stem cell transplantation prosecution [73,75]. Namely, oxidative stress reduces the number of CD34+ stem cells that are selected during bone marrow's stimulation with chemo-kinetic agents and increases the risk for graft versus host disease during allogeneic stem cell transplantation [75]. Meloxicam, a NSAID confronts the aforementioned obstacles [76].

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As science is just beginning to appreciate that redox homeostasis is of critical importance in cancer, there are critical issues that need to be addressed. First of all, the ROS threshold that activates individual members of the network and the extent to which thresholds for individual transcription factors change during the expansion of the myeloma clone is still unclear. Moreover, the exact signaling cascades of the UPR pathway and the crosstalk between myeloma cells and bone marrow stromal cells, as far as redox signaling is concerned need to be clarified. Clinical trials must be conducted in order to combine effectively and safely antimyeloma agents that target oxidative stress, with other backbone antimyeloma therapies, such as monoclonal antibodies. Further molecular analysis is necessary in order to ascertain a correlation between newer, breakthroughs, bispecific MoAbs such as elranatamab and teclistamab used in relapsed/refractory myeloma with oxidative stress mediated cellular cytotoxicity. Implementation of newer technologies in myeloma complex metabolic pathways will feature newer molecular drug targets. Consequently, the concept of personalized medicine is coming closer.

The main limitation of this review is that the role of oxidative stress in MM pathophysiology and treatment is a constantly evolving field, introducing new discoveries and innovations. An every year "supplementation" of the article, with the new data will be a significant way to address this limitation.

Conclusion

There is a sensitive and fragile balance between oxidative stress and MM. ROS and RNS production enhances cellular growth, survival, and expansion of myeloma cells. Nevertheless, ROS and RNS overproduction, due to novel agents' administration activate both endogenous and exogenous apoptosis. In advanced disease, oxidative stress suppression leads to the further growth of neoplastic clone. A further study of the correlation between molecular mechanisms leading to myeloma cell proliferation and apoptosis and oxidative stress, using gene editing technologies such as clustered regularly interspaced short palindromic repeats (CRISPR-Cas) system and massive parallel sequencing, is required. A comprehensive understanding of these interactions will likely lead to the development of innovative, targeted, and consequently, more efficacious treatment modalities with reduced adverse effects. Integrating novel treatments with current or emerging therapies is anticipated to enhance both the survival and quality of life for patients with MM. Overall, the results from recent clinical trials suggest that drug combinations containing agents with oxidative stress inducers hold promise as a therapeutic strategy for patients with myeloma. Further research is needed to optimize these treatment regimens, identify predictive biomarkers, and understand the mechanisms underlying their efficacy.

Availability of Data and Materials

Not applicable.

Author Contributions

TA designed the research study. TA, KL and KK performed the literature research and data presentation. TA, KT, EM analyzed the literature. KT, EM, TD and AB provided data selection and curation. TA wrote the first draft of the manuscript. TA and GK designed the figures. KT, EM, GK and CL performed the critical analysis of the data, review and editing. All authors contributed to the correction of the paper. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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