

FORUM REVIEW ARTICLE

Hematopoietic Stem Cells: Normal *Versus* Malignant

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Abstract

Significance: The long-term hematopoietic stem cell (LT-HSC) demonstrates characteristics of self-renewal and the ability to manage expansion of the hematopoietic compartment while maintaining the capacity for differentiation into hematopoietic stem/progenitor cell (HSPC) and terminal subpopulations. Deregulation of the HSPC redox environment results in loss of signaling that normally controls HSPC fate, leading to a loss of HSPC function and exhaustion. The characteristics of HSPC exhaustion *via* redox stress closely mirror phenotypic traits of hematopoietic malignancies and the leukemic stem cell (LSC). These facets elucidate the HSC/LSC redox environment as a druggable target and a growing area of cancer research.

Recent Advances: Although myelosuppression and exhaustion of the hematopoietic niche are detrimental side effects of classical chemotherapies, new agents that modify the HSPC/LSC redox environment have demonstrated the potential for protection of normal HSPC function while inducing cytotoxicity within malignant populations.

Critical Issues: New therapies must preserve, or only slightly disturb normal HSPC redox balance and function, while simultaneously altering the malignant cellular redox state. The cascade nature of redox damage makes this a critical and delicate line for the development of a redox-based therapeutic index.

Future Directions: Recent evidence demonstrates the potential for redox-based therapies to impact metabolic and epigenetic factors that could contribute to initial LSC transformation. This is balanced by the development of therapies that protect HSPC function. This pushes toward therapies that may alter the HSC/LSC redox state but lead to initiation cell fate signaling lost in malignant transformation while protecting normal HSPC function. *Antioxid. Redox Signal.* 29, 1612–1632.

Keywords: HSC, stem cell function, redox-active compound, LSC, hematopoiesis

Introduction

THE HEMATOPOIETIC COMPARTMENT contains a tightly regulated hierarchy of cell types responsible for the function and maintenance of the bone marrow (BM) and blood systems. All peripheral blood cells, both the myeloid and lymphoid lineage, differentiate from a common pool of hematopoietic stem and progenitor cells (HSPCs) that reside within the BM (54, 138). This pool of HSPCs can be broken down into three subsets of cell types: the long-term hematopoietic stem cell (LT-HSC), short-term hematopoietic stem cell (ST-HSPC), and the multipotent progenitor cell (MPP). Two distinct progenitor cell populations emerge from the

MPP pool: the common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). Differentiation from these progenitor cell populations eventually results in the establishment of peripheral effector cell populations within each lineage, such as B and T cells within the lymphoid lineage or erythrocytes and neutrophils within the myeloid lineage (1, 54, 137). There are several phenotypic and functional facets that define the HSPC populations individually and as a whole. The central defining characteristics of hematopoietic stem cells (HSCs), particularly those within the long-term population, are the ability to self-renew while, at the same time, limit the size of the HSPC population and demonstrate the potential to differentiate into any mature cell

type within the hematopoietic system (Fig. 1) (1, 11, 54, 97, 137, 138).

ST-HSPC and progenitor populations have the capacity to maintain normal hematopoiesis for a period of 6–8 weeks. Serial transplant studies *in vivo* have identified the LT-HSC as the population and cell type that can sustain normal hematopoiesis throughout an organism's entire lifespan. This fact demonstrates a loss of HSC self-renewal capacity as a function of increased cellular differentiation. For these reasons, normal LT-HSC function must be maintained throughout the lifespan of an organism. This elucidates the LT-HSCs as the only population that holds true characteristics of the HSC. Because self-renewal and differentiation of LT-HSPC and ST-HSPC and MPP populations are essential to normal hematopoietic function, we define this entire population as the HSPCs and reserve the term HSC for the true LT-HSC populations.

Loss of normal LT-HSC and ST-HSPC function is a hallmark of natural stem cell aging and several hematopoietic disorders, most notably the development and progression of hematopoietic malignancies (1, 4, 11, 54, 65, 90, 97, 137, 138, 156, 165, 173). Within these cancers, normal hematopoietic regulation is lost, yet disease still progresses through the differentiation and clonal expansion of progenitor cell pools, eventually leading to a lack of terminal differentiation to functional cell types within the periphery. This observation led to the identification of the cancer stem cell (CSC) or more specifically the leukemic stem cell (LSC) (2, 65, 66, 119, 128,

130, 143, 156, 173). Although we know that hematopoietic neoplasms are driven by LSC populations, developing therapies that treat LSC pools as entities separate from normal HSPCs has been difficult. Thus, little progress has been made in the development of therapies that both eradicate malignant HSPCs while, at the same time, protect or pose no detriment to healthy HSPC populations within a single patient.

There is a heterogeneous and diverse set of cytogenetic abnormalities within various hematopoietic cancers that, in some cases, may lend themselves to personalized treatment plans. However, intrinsic characteristics that separate normal HSPCs from their malignant counterparts are becoming more relevant (7, 12, 13, 16, 77, 105, 121, 150). The identification of these differences will lead to the development of safe therapeutics that have broad implications for treatment of several hematopoietic neoplasms across patient populations. Chief in the differences between normal and malignant HSPCs is the generation of reactive species and the management of the cellular redox environment (5, 22, 67, 75, 82, 106, 107, 119, 128, 129, 143, 150, 155, 159). It has been well established that cancer cells demonstrate elevated levels of reactive species generation and a difference in basal redox environment as compared with their normal counterparts. This difference is heavily rooted in an increased metabolism and production of reactive oxidative species such as superoxide and hydrogen peroxide (H_2O_2), which, in turn, leans on the cellular antioxidant capacity and thus, enhances the need for reducing species such as glutathione (GSH). The result is

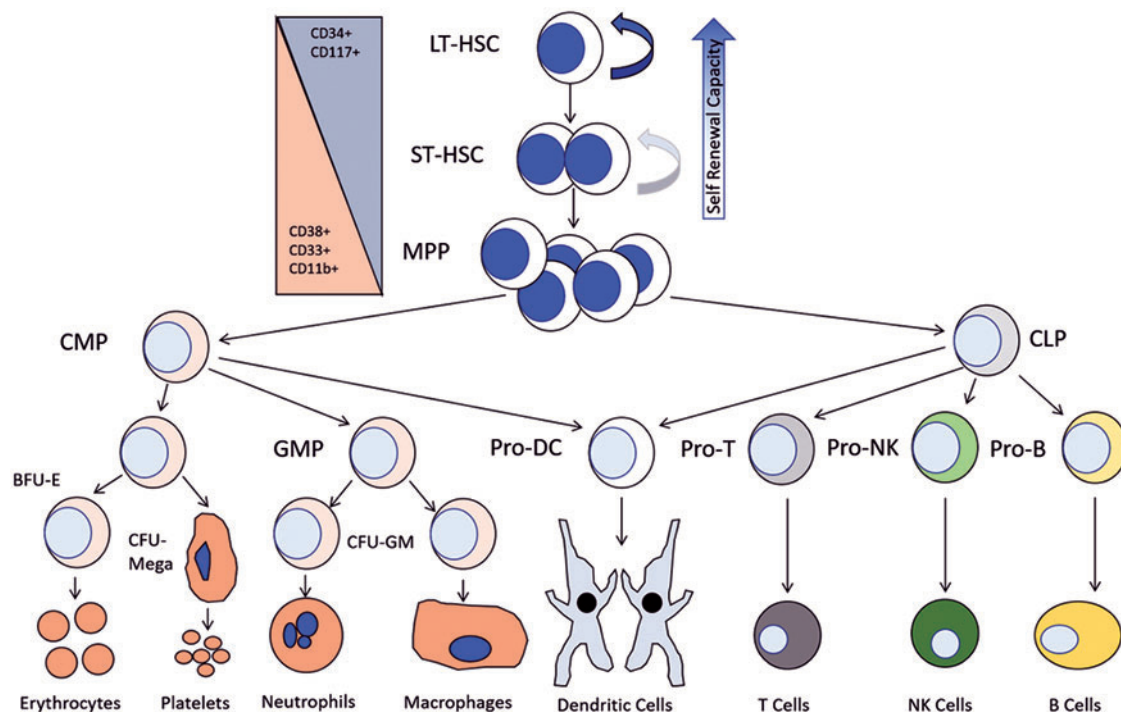


FIG. 1. The bone marrow compartment contains a complex and highly regulated hierarchy of cell types tasked with maintaining the production and balance of all cells in the blood system. Beginning with the most primitive $CD34^+$ LT-HSCs, the ability to self-renew and differentiate to downstream phenotypes defines the LT-HSC pool with a decreased capacity for self-renewal as differentiation occurs. From the MPP pool, both lymphoid and myeloid cell types develop and eventually differentiate into terminal effector cell types seen in the periphery. CD34, cluster of differentiation protein 34; CLP, common lymphoid progenitor; CMP, common myeloid leukemia; LT-HSC, long-term hematopoietic stem cell; MPP, multipotent progenitor cell; ST-HSC, short-term hematopoietic. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

an unbalance in equilibria that stresses both sides of cellular oxidoreduction capacity, herein we refer to this stress imbalance simply as redox stress. In fact, the malignant hematopoietic phenotype mirrors the changes in normal hematopoietic architecture brought on by increased production of redox stress, which results in alterations to the HSPC redox environment (66, 69, 70, 117, 129, 155). This fact has recently presented researchers with a druggable target in which a therapeutic index can be defined that exploits the malignant cell redox environment while leaving normal cell populations unharmed (67). This is accomplished by examining the effects of redox-active compounds in both normal and malignant hematopoietic stem and progenitor cell populations. Redox-active compounds have traditionally been defined as those that can undergo single electron transfers acting as either an oxidizing or reducing agent. These compounds include nitroxides such as tempol, flavonoids such as oxorylin or baicalein, and those with transition metal centers, such as Mn (III) *meso*-tetrakis(N-(n-butoxyethyl)pyridinium-2-yl) porphyrin (MnTnBuOE), capable of cycling through multiple oxidation states acting as active sites for therapeutic efficacy (8–10, 49, 113, 147, 149, 161, 164, 176). Other compounds, such as doxorubicin (DOX), may transition through structural changes as a result of electron exchange *via* metabolism (34, 36, 50, 86, 100, 101, 172). In addition, we consider those compounds that affect the cellular redox environment by actively modifying concentrations of cellular reductants or those that inhibit normal antioxidant enzyme function, such as buthionine sulfoximine (BSO) or parthenolide (PTL), although actual oxidoreduction reactions do not directly result from the chemistry of the molecules themselves (69, 115). In this review, we offer a concise summary of key studies examining the effects of endogenous mechanisms and redox-active compounds resulting in changes to the cellular redox environment of normal and malignant HSPCs. Furthermore, we present the HSPC cellular redox environment as a druggable target that allows for effective intervention within malignant populations while leaving normal HSPC populations unharmed.

Redox Regulation and Redox-Active Compounds Affect Normal HSC Function

The production and elimination of the initial and ensuing reactive species are the cornerstones of cellular redox environment management. Here, metabolic byproducts such as O_2^- (superoxide) and H_2O_2 , as well as $\cdot OH$ (hydroxyl radical), and $ONOO^-$ (peroxynitrite) are the main reactive species responsible for oxidative damage, including DNA oxidation, lipid peroxidation, and protein oxidation as well as nitration (155). As a result, an increase in metabolic activity and reactive species generation is closely associated with an increase in cellular proliferation and differentiation (70, 117, 138, 153). It should come as no surprise then that various concentrations of the original redox-active compound, molecular oxygen (O_2), significantly affect cellular redox status and HSPC proliferation. The BM niche, or extracellular components surrounding HSPCs, and peripheral tissues have oxygen tensions ranging from 1% to 7%. *In vitro* study of primary normal and malignant HSPCs, as well as various culture lines, has demonstrated the beneficial effects of a hypoxic environment (18, 26, 27, 60, 61, 131, 133). Both

normal and malignant HSCs benefit from growth conditions that have oxygen concentrations ranging from 1% to 3%, compared with cultures at normoxic (5%) and atmospheric (21%) O_2 concentrations (29, 70, 116, 144, 152). Interestingly, a low oxygen environment leads to a decrease of GSH levels, as well as antioxidant enzyme expression and function (19, 40, 108). Primitive HSC populations have been thought to be refractory to induction of oxidative stress, due to their quiescent state, antioxidant capacity, and local niche environment. However, their environment may be a key factor in keeping HSCs sensitive to redox stress. This facet of the BM environment has potential in allowing for the development of differential sensitivities to redox insult between normal and malignant HSC as well as HSPC populations (Fig. 2).

Cluster of differentiation protein (CD) 34^+ HSPCs express higher levels of superoxide dismutase (copper-zinc superoxide dismutase [SOD1] and SOD2) and catalase (CAT) relative to other antioxidant enzymes, such as, glutathione peroxidases (GPx), glutathione-S-transferases (GSTs), glutathione reductase (GR), thioredoxin (TXN), and thioredoxin reductase (TXNR) utilized for the removal of H_2O_2 as well as other radicals and reactive species (Fig. 3) (115). However, the expression level of antioxidant defense enzymes such as SOD and CAT is lower in the primitive LT-HSC compartment than in their downstream differentiated progeny (4, 99, 155). This means that if these defense enzymes fail, a significant buildup of primary and secondary reactive species may occur; thus, quickly and, potentially irreversibly, altering the redox environment within HSCs. In addition, hematopoietic malignancies are generally typified by the uncontrolled clonal expansion of HSPC populations. Therefore, primitive malignant populations may, themselves, express low levels of antioxidant enzyme capacity, such as SOD or CAT. This would then result in higher levels of downstream reducing agents such as the GSH/GSSH redox couple.

In the past decade, several key studies have identified management of the HSPC redox environment as a crucial factor in the maintenance of the HSPC population. The HSPC compartment itself contains cell populations that can be separated based on their reactive oxygen species (ROS) levels and redox environments. Using 2'-7'-dichlorofluorescence (DCF) diacetate, Jang and Sharkis were able to separate two HSPC populations characterized by high and low levels of ROS (70). These two populations display distinct differences in HSPC function. Limiting dilution transplantation assays showed that HSPCs with low levels of ROS repopulated recipient mice significantly better than HSCs with high levels of ROS. This characteristic was also carried over to secondary and tertiary transplant recipients. In addition, the "ROS low" population contained more primitive long-term culture-initiating cells (LTC-ICs) and resulted in better multilineage reconstitution (70). Interestingly, upon transplantation the "ROS high" population resulted in lineage reconstitution that favored myeloid cells. Conversely, ROS low cells favored lymphoid lineage development. This indicates a potential for the development of myeloid lineage malignancies upon redox deregulation within the normal HSPC populations. The ROS high population demonstrated elevated levels of p38 activity, elevated expression of p16 and lower levels of p53 expression. The LTC-IC capacity of the ROS high population did respond to treatment with either a p38 mitogen-activated protein kinase (p38 MAPK) inhibitor, the GSH precursor N-acetyl-L-cysteine (NAC), or rapamycin (70). This

FIG. 2. The bone marrow compartment demonstrates oxygen concentrations that range from 1% (furthest for the blood supply) to 7% (closest to the blood supply). Low oxygen concentrations have been referenced as a factor contributing to the protection and quiescence of HSCs. However, upon transformation, LSCs may demonstrate increased levels of metabolism and ROS levels, while maintaining a lower antioxidant capacity than their downstream progeny. This difference, a product of the oxygen environment, may allow for a differential redox insult and targeting of LSCs over normal HSCs driven by the introduction of a redox-active compound. HSC, hematopoietic stem cell; HSPC, hematopoietic stem/progenitor cell; LSC, leukemic stem cell; ROS, reactive oxygen species. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

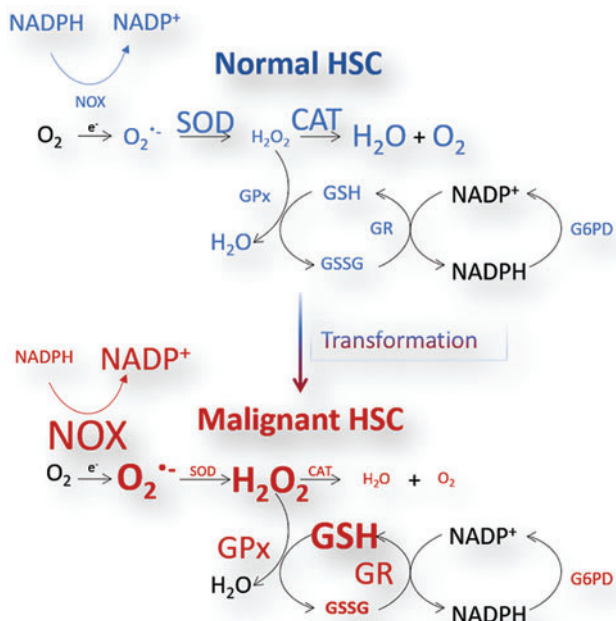
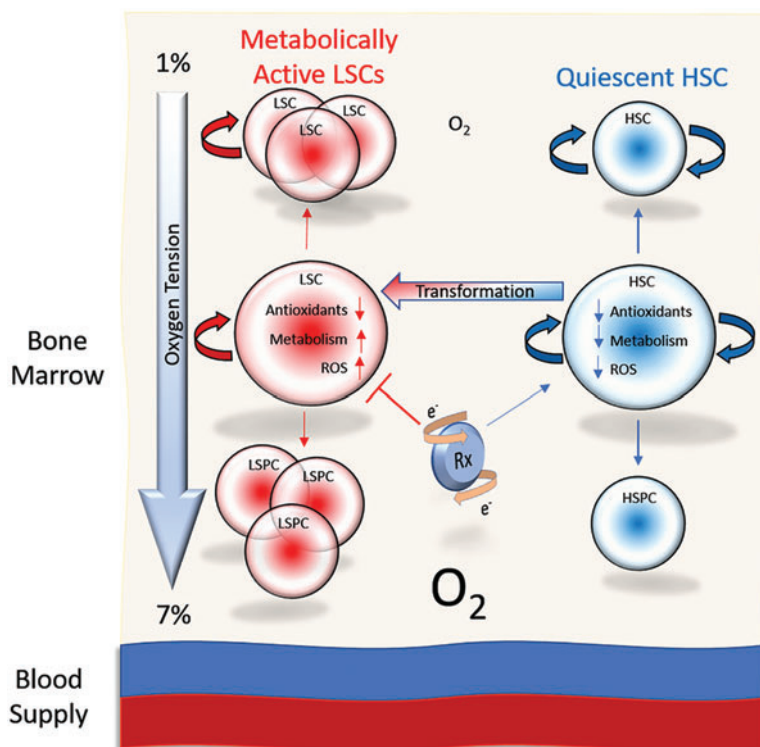


FIG. 3. Normal and malignant HSCs demonstrate differences in the regulation and management of the antioxidant system. Normal HSCs express higher levels of primary antioxidant enzymes SOD and CAT. Malignant HSCs are subject to elevated levels of ROS produced by NOX enzyme and rely on higher cellular concentrations of GSH as well as the GSH metabolic system to manage the malignant HSC redox state. CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; H₂O₂, hydrogen peroxide; NADPH, nicotinamide adenine dinucleotide phosphate; NOX, nicotinamide adenine dinucleotide phosphate oxidase; SOD, superoxide dismutase. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

indicates that buffering the HSPC redox environment can improve normal HSPC function. Thus, as one might expect, NAC treatment aids the engraftment of normal human HSPCs isolated from human cord blood into recipient mice. These results are demonstrated even in the highly enriched lineage (Lin)⁻ CD34⁺ CD38⁻ CD90⁺ CD45 isoform A (CD45RA)⁺ CD49f⁺ Rho GTPase (Rho^{low}) HSC population, indicating that exogenous protection against reactive species challenges and can independently improve HSC function (64).

In 2007, Ito *et al.* utilized the *Atm*^{-/-} mouse model to demonstrate that an elevation of ROS activated p38 MAPK signaling, resulting in a loss of normal HSC maintenance as well as quiescence eventually leading to HSPC senescence (69). They found that depletion of cellular GSH *via* BSO treatment led to an increase in H₂O₂ within the HSPC Lin⁻/stem cell antigen 1 (Sca-1)^{+/c-Kit} (LSK) population, resulting in an increased expression of cell cycle regulators p15^{INK4B} (cyclin-dependent kinase inhibitor B) p16^{INK4A} cyclin-dependent kinase inhibitor 2A, p18^{INK4C} (cyclin-dependent kinase 4 inhibitor C), and p19^{ARF} (cyclin-dependent kinase 4/6 inhibitor). This led to a loss of HSPC function; yet the upregulation of tumor suppressor proteins was reversed by treatment with NAC or a p38 MAPK inhibitor. These effects were not demonstrated in differentiated cell populations (69). Similarly, ROS accumulation, p15^{INK4B}, p16^{INK4A}, p18^{INK4C}, and p19^{ARF} upregulation, and loss of HSPC function were demonstrated in *Atm*^{-/-} mice. Within this model, treatment with a p38 MAPK inhibitor both before and after transplant recovered HSC function in *Atm*^{-/-} mice, compared with wild-type controls. Using NAC and p38 MAPK inhibitor treatments, this study also demonstrated that ROS-mediated p38 activation is responsible for a loss of HSPC quiescence (69). Interestingly, H₂O₂ oxidation of ataxia-telangiectasia mutated

(Atm) results in internal disulfide crosslinking and dimer formation that leads directly to Atm activation independent of DNA double-strand breaks (58). Results found by Ito *et al.* are very similar to those found by Miyamoto *et al.* using a *Foxo3a*^{-/-} mouse model. These studies demonstrate forkhead box O3A (FOXO3A) regulation of HSC function. Importantly FOXO3A operates downstream of phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B (PTEN/PI3K/AKT) pathway, and loss of FOXO3A activity results in HSC exhaustion and activation of the p38 MAPK pathway (102).

Just as these phenomena have been demonstrated in mice, human cells subjected to serial transplantation in mice demonstrate similar characteristics. Serial transplantation of human Lin⁻ CD34⁺ CD38⁻ HSCs loses their ability to repopulate the BM (171). This loss of HSC function in human cells is accompanied by an accumulation of oxidative DNA damage, characterized by an increase in γ H2AX foci, as well as an increase in the expression of cell cycle inhibitors p16^{INK4A}, p14^{ARF} (p14 alternate reading frame tumor suppressor), and p21^{CIP} (cyclin-dependent kinase inhibitor 1) (171). The formation of these DNA damage foci is recapitulated in normal human HSCs treated with BSO. Upon formation, DNA damage repair enzymes, Atm, p53 binding protein (53BP1), CHK2, and FOXO3A are recruited to the foci. Furthermore, examination of the highly enriched Lin⁻ CD34⁺ CD38⁻ CD90⁺ CD45RA⁺ HSC population showed that although damage is seen in both the stem and progenitor cell (CD34⁺ CD38⁺) populations, only the HSC compartment undergoes cell cycle arrest and apoptosis. In addition, HSC populations recover more slowly than progenitor populations after the oxidative insult is removed. Consistent with other studies, addition of NAC as a protecting agent preserves HSC function in correlation with decreased accumulation of DNA damage foci, even in the face of oxidative insult (171). Control of DNA damage response can also be managed *via* the polycomb repressor (BMI1) signaling *via* control of INK4A/ARF (CHK2) proteins. Loss of BMI1 function results in mitochondrial dysfunction and accumulation of ROS, and loss of HSPC self-renewal and differentiation, a phenotype that can be partially restored by treatment with NAC or the deletion of the *Chk2* gene itself (91).

Clearly the accumulation of oxidative damage incurs a detrimental effect on HSC function, and as one may anticipate, nuclear factor (erythroid-derived 2)-like 2 (NRF2) activity has been identified as a major regulator of the HSC redox environment and HSC function (79, 98, 160). Tsai *et al.* were able to utilize the *Nrf2*^{-/-} mouse to demonstrate familiar effects on HSC function. These studies elegantly showed that the loss of NRF2 resulted in an increase in the LSK population, but only within the ST-HSC and MPP compartments, not within the LT-HSC population (153). Furthermore, *Nrf2*^{-/-} mice demonstrated an increase in both the common myeloid (CMP, granulocyte macrophage progenitor, and megakaryocyte-erythroid progenitor) and lymphoid (lymphoid primed multipotent progenitor, CLP-1, and CLP-2) progenitor cell populations. Although progenitor pools underwent significant expansion, the primitive LT-HSC populations were unchanged. However, MKI67 antigen KI-67 analysis, 5-FU pulse experiments, and culture in OP9-DL1 revealed that NRF2 regulates HSPC proliferation and differentiation resulting in diminished quiescence and increased differentiation within the most primitive LT-HSC populations (153). Importantly, the NRF2-deficient HSPCs also significantly underperformed in colony formation

and *in vivo* transplant assays, indicating that not only does NRF2 regulate proliferation and differentiation but also regulate HSPC self-renewal and function. Proliferation analysis *via* coculture of deficient cells with WT counterparts demonstrated a proliferative advantage for the *Nrf2*^{-/-} cells (153). Chimeric studies showed that the proliferative advantage is generated from within the HSPC itself and does not result from signals received from the BM environment. Moreover, the loss of NRF2 results in a decreased BM homing effect, implying that NRF2 even regulates the retention of HSPCs in the BM (153). This homing effect is linked to the loss of chemokine receptor type 4 (CXCR4) expression, resulting in deficient chemotaxis abilities in *Nrf2*^{-/-} HSPCs (43, 153).

The studies already discussed have laid the groundwork for a clear understanding of how the HSPC redox environment affects normal HSPC regulation and function. Ultimately, dysregulation of HSPC redox balance results in well-defined changes to HSPC populations. As previously noted, the resulting alterations to HSPC populations mimic those demonstrated in several disease states, most notably, the myeloproliferative disorders (MPDs). Clearly, regulating the cellular GSH pool is required for faithful management of ROS and HSPC function. HSPCs that undergo redox deregulation resulting in an increase in ROS seem to demonstrate a natural response that functions through the Jun N-terminal kinase (JNK)/MAPK signaling as well as FOXO and Atm DNA damage response pathways (Fig. 4) (69, 70, 102). This results in loss of HSPC quiescence and function. Despite the perception that HSPCs maintain robust function as a result of their protected location and their highly quiescent state, we now know that HSPCs not only rely on intrinsic signaling but also that HSPCs are more sensitive to redox stress than previously thought (171). These facets are highlighted by improving HSPC function through buffering redox capacity but also by the result of losing major regulatory control over redox environment management *via* transcription factors such as NRF2 (64, 153). Thus, redox modulators, such as BSO, which induce strong conditions of oxidative stress, work to the detriment of normal HSPC function. Conversely, compounds such as NAC that provide antioxidant aid improve HSPC function.

Chemotherapeutics and Normal HSCs

The work already summarized demonstrates the importance of HSPC redox regulation in maintaining normal HSPC function. Normal HSPC toxicity and functional exhaustion are a significant problem underlying the use of traditional chemotherapeutics for the treatment of hematopoietic malignancies. Most chemotherapies currently utilized for the treatment of the hematopoietic malignancies are not designed to manipulate the cellular redox environment of the HSPC; rather, they target DNA synthesis or immune function. However, we now know that many cancer therapeutics do in fact impact the redox milieu of target and normal tissues. These redox-active therapeutics illicit detrimental effects on HSCs that echo the results previously discussed. Methotrexate (MTX), a long-approved cancer drug within the antifolate class that inhibits endogenous nucleotide synthesis, has been well documented to induce oxidative stress within target tissues (6, 141). MTX treatment results in an increase of superoxide production while concurrently depleting cellular GSH and total thiol levels by 70%. These MTX-mediated changes in the cellular redox environment are

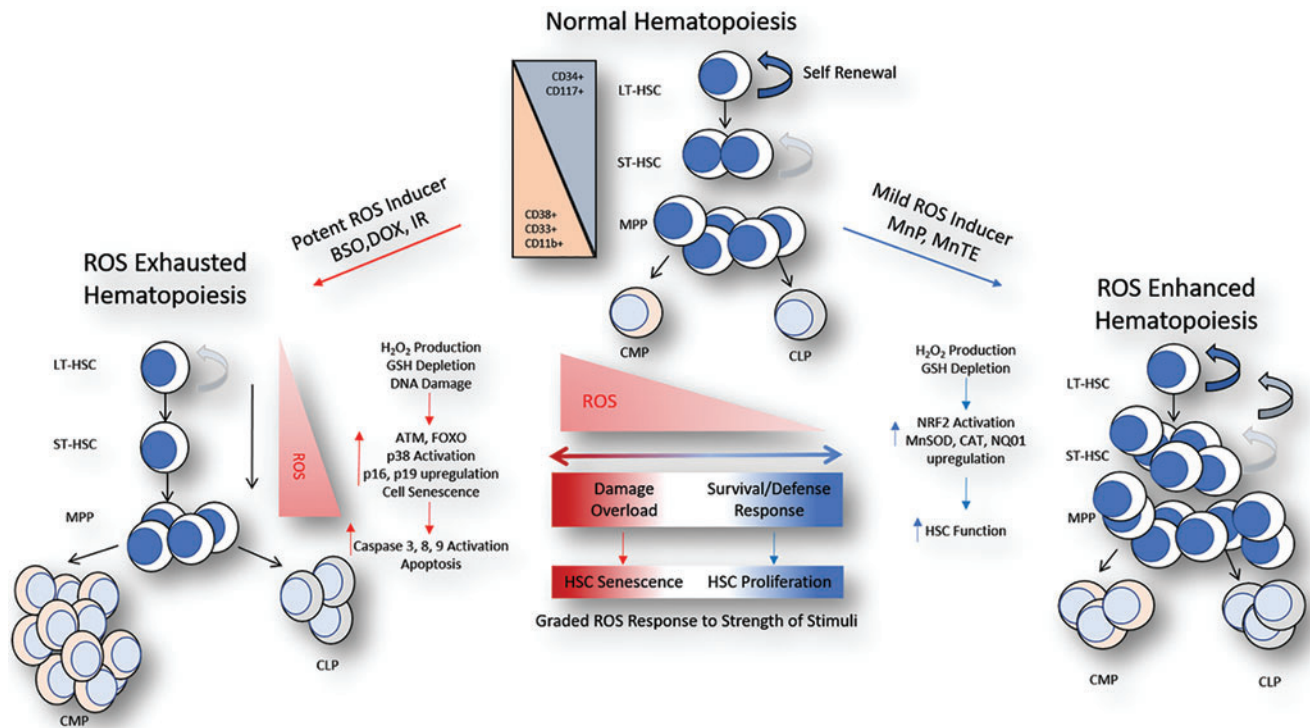


FIG. 4. A schematic diagram demonstrates the differential effect and graded response of HSCs to various redox-active compounds and ROS inducers. Normal hematopoiesis is characterized by primitive LT-HSCs that have the ability to self-renew and differentiate into any other cell type. As differentiation occurs, progenitor cell types lose their ability to self-renew. This process is accelerated in the presence of potent ROS inducers, such as conventional chemotherapeutic agents and ionizing radiation. Response to these stimuli results in DNA damage response and activation of signaling that results in the upregulation of cellular senescence and apoptosis. New evidence now suggests that mild ROS inducers have an opposite effect wherein generation of a mild ROS milieu results in the activation of antioxidant defense pathways leading to an improvement in HSC function. The result is a graded response to different levels of ROS stimulant that leads to variations of HSC function and either exhaustion or strengthening of the hematopoietic niche. Atm, ataxia-telangiectasia mutated; BSO, buthionin sulfoximine; DOX, doxorubicin; MnP, Mn porphyrin; Nq01, NAD(P)H dehydrogenase quinone 1; Nrf2, nuclear factor (erythroid-derived 2)-like 2. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

accomplished *via* inhibition of MnSOD, GPx, GR, and γ -glutamylcysteine synthetase (6). Although chemotherapeutics such as MTX target dividing cells, quiescent HSC populations seem to remain refractory to MTX treatment even in the face of cell cycle inducers. However, inhibition of nucleoside transport allows for HSPC sensitization to MTX cytotoxicity, resulting in a loss of HSPC function (3). Although antifolate drugs mainly target DNA synthesis, their effects on redox management cannot be ignored, particularly in the HSC population where GSH metabolism and homeostasis is important. Similarly, cyclophosphamide, an agent that targets dividing cells *via* induction of DNA cross-linking, causes a significant amount of reactive species production measured by DCF in HSPCs. This leads to hematopoietic suppression demonstrated by loss of HSPC quiescence and function followed by increases in peripheral blood and splenic progenitor populations (104). This is typified by a significant decrease in the GSH/oxidized glutathione (GSSG) ratio. Interestingly, the application of a glutathione disulfide mimetic significantly decreases the production of ROS and partially rescues the changes in cellular GSH (35). This GSSG mimetic also increases the number of HSPCs depleted by cyclophosphamide treatment and partially rescues HSPC function. Treatment also rescues cyclophosphamide suppression of antioxidant gene expression, particularly SOD3 (copper-zinc extracellular superoxide dismutase) and GPX2 (35).

Perhaps one of the most potent and popular chemotherapeutics approved for treatment of leukemias is the anthracyclines. Of this class, doxorubicin/adriamycin (DOX/ADR) is possibly the most well-documented redox stress inducer. DNA intercalation and inhibition of topoisomerase II is the primary mechanism of DOX-induced cytotoxicity in cancer. However, the ability to act as a redox modifier results from nicotinamide adenine dinucleotide phosphate (NADPH)-mediated formation of a semi-quinone structure, which allows for the generation of oxygen radicals, H_2O_2 , DNA damage, and alterations in bioenergetics (34, 36, 50, 86, 100, 101, 172, 178). Fenton type chemistry may then exacerbate this process, leading to the generation of hydroxyl radicals. It is no surprise then that the use of anthracyclines such as DOX is applied at the detriment of normal hematopoiesis (14, 28, 31). Traditionally, pharmacological doses of DOX induce myelosuppression, cardiac toxicity, and the enrichment of refractory CSCs through the elevated expression of multidrug-resistant proteins such as MDR1 and MRP1/ABCC1 (multidrug resistant protein 1/ATP-binding cassette subfamily C member 1) (14). DOX treatment specifically suppresses the number of ATP binding cassette subfamily G member 2 (ABCG2)⁺ HSPCs and BM sphere formation in comparison with vehicle and paclitaxel controls *in vitro*. Decreases in ABCG2⁺ HSPCs as well as peripheral white blood cells have also been demonstrated *in vivo* at doses of 1–5 mg/kg DOX

(14). More recently, Mahbub *et al.* discovered that DOX and etoposide (ETP) can be combined with several different polyphenol compounds to enhance malignant cell death in both lymphoid and myeloid lineage cancer models while at the same time protect normal CD34⁺ and CD133⁺ HSPC populations (92, 93). With DOX and ETP *in vitro* concentrations ranging from 0.1 to 0.4 μM , combinations of five different polyphenol compounds (quercetin, apigenin, emodin, rhein, and cis-stilbene) all recovered levels of adenosine triphosphate (ATP) production and caspase 3 activity when compared with DOX and ETP treatment alone. In these studies, cellular GSH levels were identified as a key factor in mediation of protection in combination treatments (92, 93). Interestingly, quercetin and apigenin, compounds recognized as mitocans (which specifically target mitochondria of cancerous cells), can bind adenine nucleotide translocase of the mitochondrial permeability transition pore, inhibiting its function and the efflux of mitochondrial ATP (52, 111).

Redox-Based Therapeutic Protection of the HSPC

A precedent for HSPC protection has been established in the past with the use of granulocyte colony-stimulating factor, erythropoietin, and other hematopoietic stimulants such as pegfilgrastim (Nulasta). These have been used to bolster production of peripheral blood cells in the face of small molecule chemotherapeutics, such as cyclophosphamide, as well as antibody-based therapies such as ublituximab (89). Although the use of antioxidants, such as resveratrol and NAC, in cancer therapeutic regimens has been a subject of debate, the prospect of protecting normal HSPC function while killing malignant cells merits investigation and consideration, as the potential impact of accomplishing both simultaneously cannot be overstated. HSPC protection in the face of oxidative insult has been accomplished through the upregulation of antioxidant enzyme function. Furthermore, this protection is actually supported by the induction of redox stress, (*i.e.*, the generation of oxidoreductive reactive species that upregulate endogenous antioxidant machinery). Liposomal delivery of a vector containing an MnSOD construct protected HSPCs of mice receiving a limiting dose of donor BM in the face of a 10 gray (Gy) total body irradiation (TBI) (99). This protective effect is enhanced when the MnSOD vector is delivered before the TBI. Protection of the HSPC is accompanied by a significant decrease in ROS production. Interestingly, HSCs overexpressing CAT and MnSOD performed better in long-term competitive repopulation assays, with CAT outperforming the MnSOD overexpressers. Importantly, the repopulation ability of these subjects was significantly increased after recipients were exposed to a lower 2 Gy dose of radiation. This indicates that upregulation of antioxidant defenses primes HSCs to rebuff subsequent oxidative insult, resulting in an increase in HSC function (99).

The antioxidant protective effect of upregulated MnSOD in HSPCs can also be achieved through the application of manganese-containing porphyrin compounds (8, 10, 147). A significant characteristic of these compounds is the stable Mn³⁺ center and the ability to accumulate in the mitochondrial fraction (132). Manganese and iron-containing porphyrins mimicking endogenous SOD function have been successfully utilized for the protection of BM, among other tissues, against the oxidative challenges of radiation therapy (10, 21, 147, 149, 166). Mn (III) *meso*-tetrakis (N-ethylpyr-

ridinium-2-yl) porphyrin (MnTE-2-PyP⁵⁺ or MnTE) has demonstrated protection of the HSPC in the face of a sublethal TBI dose of 6.5 Gy. Administered at a dose of 6 mg/kg on the day of TBI injury and for 30 days subsequent to TBI injury, MnTE-2-PyP⁵⁺ improved hematopoietic recovery (10, 88, 147). This resulted from improved function of both: hematopoietic stem and progenitor cells compared with control-treated mice. Importantly, MnTE-treated HSPCs demonstrated a decrease in senescence induced by TBI through the downregulation of p16^{Ink4a} expression (114, 127, 163). Pharmacological protection of both the splenic and BM HSPC compartments from TBI of 7.5 Gy has also been achieved through the implementation of the flavonoid, baicalein (113). Both *in vitro* and *in vivo* treatment with baicalein resulted in an inhibition of phosphatase MKP3, ERK activation, nuclear accumulation of NRF2, and an increase in NRF2 target mRNA (113). These studies indicate that redox-active compounds may offer dual targeting of the NRF2 and MAPK signaling pathways in normal HSPCs, a concept that may aid redox-active therapeutic targeting of malignant HSPC populations, over normal HSPCs.

Our studies demonstrated similar effects of improved number of HSPCs and function generated by overexpression of mitochondrial MnSOD. Our more recent work has further explored this concept utilizing the MnSOD mimetic Mn (III) *meso*-tetrakis(N-(n-butoxyethyl) pyridinium-2-yl) porphyrin (MnTnBuOE-2-PyP⁵⁺ or MnTnBuOE). MnTnBuOE treatment *in vitro* as well as chronic treatment *in vivo* results in an increase in the HSPC LSK population, similar to *MnSODtgH* mice (transgenic mice overexpressing MnSOD) (179). Moreover, LSK populations significantly increase through tertiary BM transplants from donor cells treated with MnTnBuOE for 16 h *in vitro* as well as donor BM from mice treated for a total duration 60 days at 2 mg/kg, 3 times/week. Not only does MnTnBuOE increase HSPC function evidenced by long-term transplant studies, but MnTnBuOE treatment also significantly improves the recovery of red blood cells (RBCs) and hemoglobin after induced acute blood loss (179). Although MnTnBuOE is traditionally considered to function as an antioxidant, due to its MnSOD-like activity, we found that MnTnBuOE treatment decreases mitochondrial function and ATP-linked oxygen consumption within the HSPC fraction. Concurrently, MnTnBuOE induces mild ROS production, measured by DCF. Here, an increase in DCF identifies an increase in general reactive species. This is presumably due to an increase in H₂O₂ production resulting from the reduction of superoxide by MnTnBuOE. However, because SOD mimetics are also known to modulate peroxynitrite levels, further study is required to elucidate the precise ROS resulting from MnTnBuOE treatment. Rather than oxidative activation of p38 MAPK signaling resulting in cellular senescence and apoptosis as previously mentioned, MnTnBuOE treatment results in the activation of E26 transformation specific and NRF2 transcriptional machinery. MnTnBuOE treatment results in an increase of NRF2 target transcript levels (CAT, MnSOD, glutamate-cysteine ligase catalytic subunit [GCLC], GCLC, NAD(P)H dehydrogenase quinone 1 [Nq01], uncoupling proteins 1 and 3 [UPC1/3], and PRDXR1) (179). Western blot analysis of the LSK population confirms the increase of MnSOD, CAT, GSTp1, and UPC3 protein levels after MnTnBuOE treatment. These results indicate that MnTnBuOE initially acts as a transient pro-oxidant, ultimately resulting in activation of an antioxidant phenotype that improves and protects HSPC

function. In fact, we found that the HSPC/LSC population has a significantly higher 2GSH/GSSG ratio as well as an increased ability to remove H_2O_2 in comparison with differentiated myeloid progenitor cells (179).

Protecting or supplementing HSPC function through the upregulation of antioxidant activity *via* gene delivery or chemotherapies is demonstrated by the application of limiting doses of sublethal and lethal TBI (10, 55, 88, 99, 114, 127, 163, 179). The management of NADPH/NADP⁺ levels has emerged as a critical factor for the protection of HSPC function. HSPCs express multiple isoforms of the NADPH oxidases (NOXs) that constantly produce ROS (118). TBI causes DNA damage and double strand breaks leading to the upregulation of the INK4A cell cycle regulators and HSPC senescence (127, 163). Inhibition of NOX by treatment with diphenylene iodonium (DPI) resulted in HSPC protection from ROS-induced DNA damage (114). This is similar to the protection achieved by HSPC treatment with MnTE (88). As previously noted, MnTE treatment protects irradiated HSPC function by downregulation of p16^{INK4A}, a key player in p38 MAPK-induced HSPC senescence. MnTnBuOE treatment of nonirradiated HSPCs significantly improves HSPC function through the transient production of ROS and activation of NRF2. This results in upregulation of not only MnSOD and CAT protein levels but also results in an increase of NQO1 transcript levels (179). Together, these results indicate that the redox management of the NADPH/NADP⁺ system lies squarely at the center of HSPC regulation. This also presents the NOX and NADPH dehydrogenases as potential targets for redox-active compounds that include the Mn porphyrins (MnPs). Other natural extract compounds such as apocynin and pumbagin also target the NOX system as well as modify the cellular redox environment through changing the cellular GSH concentrations (95). Thus, emerging redox-active compounds with the ability to act not only as a mild antioxidant but also as a mild pro-oxidant may

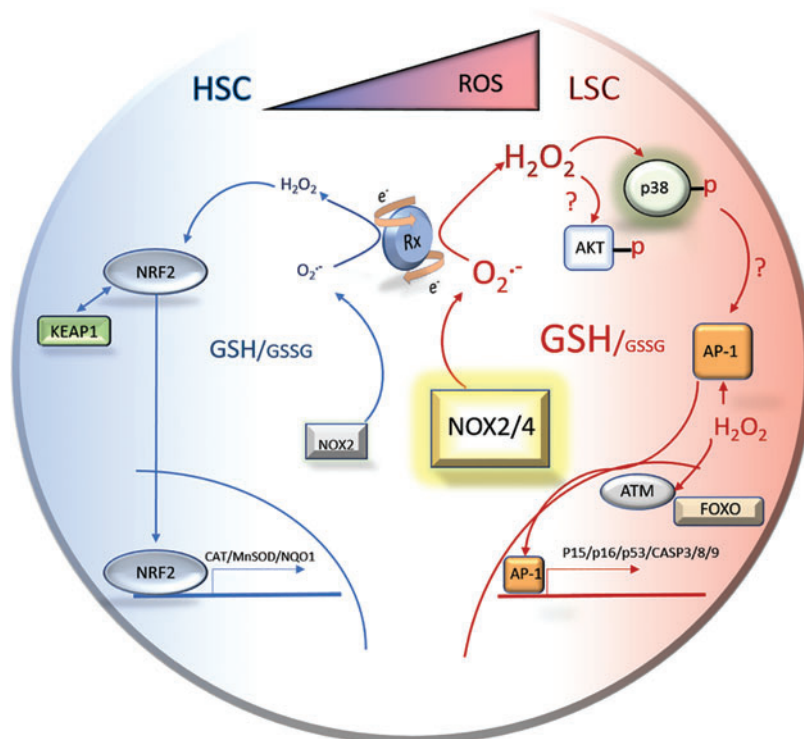
not only be successful in eradicating malignant cells but also may protect normal HSPC function.

These studies further suggest that, contrary to the currently accepted dogma of ROS effects on long-term HSC function, modulation of oxidative challenge to illicit a low-to-moderate level of redox stress can actually offer protection by upregulating HSPC function through NRF2 activation. This is accomplished through functional duality of redox-cycling compounds (Fig. 4). For example, MnTnBuOE has the ability to oxidize superoxide to molecular oxygen in one half reaction while maintaining the same proclivity for the reduction of superoxide to H_2O_2 in another half reaction (8–10, 147, 149). Redox-active compounds can accomplish this by closely mimicking structure–function characteristics of endogenous enzymes. In the case of MnTnBuOE, functional mimic is characterized by the half-wave reduction potential of the manganese center, which is very close to the potential of MnSOD itself (8–10, 147, 149). Other compounds such as polyphenols and flavonoids can have differential effects on normal and malignant cells also by targeting antioxidant machinery such as components of GSH metabolism and NRF2 activation discussed later in this review. Mixed oxidoreductase function of compounds such as MnTnBuOE are contrary to conventional chemotherapeutics, such as DOX, that can act as oxidizing agents resulting from metabolic/bioenergetic alteration or interaction with the cellular redox milieu. With this principal in mind, differences in the basal redox environment between normal and malignant cells now expose cancer cells to redox-based therapies that offer little harm to normal HSPCs (Fig. 5).

Redox-Based Therapies Targeting the LSC Redox Environment

The reactive species generated by malignant HSPCs is closely linked to metabolism. Thus, as malignant HSPCs or

FIG. 5. The prosurvival response of normal HSCs to mild pro-oxidants has led to the concept of differential response to redox cycling compounds between normal and malignant HSCs. Redox-active compounds will use the normal levels of ROS produced by healthy HSCs to activate an antioxidant response leading to increased proliferation and HSC function. Conversely, LSCs perpetually produce elevated levels of superoxide *via* aberrant constitutive enzyme activity that can be used by redox-active compounds to produce second messenger ROS such as hydrogen peroxide. This may lead to LSC senescence and death. The theoretical result is the protection of normal HSC function with simultaneous cytotoxic, anticancer affects in the LSC population. AP-1, activating protein-1. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars



LSCs undergo clonal expansion at an unchecked rate, in comparison with their normal counterparts, the HSPC redox environment has emerged as an identifying trait between normal and malignant stem cells (1, 2, 54, 66, 67, 117, 119, 129, 137, 143). Although most cancer drugs were developed to interfere with DNA synthesis, a number of common therapeutic compound classes already discussed do in fact induce ROS generation. Because the compounds such as DOX, MTX, ETP, cyclophosphamide, and cytarabine are all administered to the peril of the normal HSPC, new methods of employment must be considered. Transient, low-dose regimens and/or combination treatments present viable options for safer usage, and several studies within the past 5 years have begun to explore these options and are reviewed here.

As previously discussed, the GSH system has emerged as a key regulator of HSPC function and as such may be targeted by redox-active compounds for the treatment of hematopoietic malignancies. BSO is a potent inhibitor of GSH synthesis and as previously discussed has been used to demonstrate stem cell exhaustion. For this reason, clinical utilization of BSO has some potential downfalls. Recently, however, Tagde *et al.* have shown the effective combination of melphalan (L-PAM) and BSO in preclinical models of multiple myeloma (MM). High GSH levels are indicative of an aggressive MM relapse phenotype that may be resistant to L-PAM, depletion of GSH with BSO sensitizes nine MM cell lines and primary MM samples to L-PAM treatment (142). This potentiation is blocked by treatment with NAC, which does not change cellular GSH concentrations. The combination of BSO and L-PAM results in DNA strand breaks, mitochondrial dysfunction, and activation of apoptosis. This combination was also successful in depleting GSH and inducing engraftment suppression within three human MM xenograft models (142).

The regulation of antioxidant enzyme expression within LSCs differs *versus* their normal counterparts, but these defense enzymes also differ between types of hematopoietic malignancies. For example, CD34⁺ cells isolated from acute myeloid leukemia (AML) patients express low levels of manganese superoxide dismutase (SOD2) and CAT and higher levels of GCLC, glutamate-cysteine ligase regulatory subunit (GCLM), GPX1, and TXN than their normal CD34⁺ counterparts (115). SOD2 and CAT levels vary among other cancers within the myeloid lineage (151, 177). However, elevated expression of GSH metabolism enzymes highlights, just as in normal HSPCs, the importance of GSH regulation within malignant HSPC populations. Indeed the GSH/GSSG system has successfully been targeted for therapy using several compounds that contain α,β -unsaturated- γ -lactone groups or have strong electrophilic character and have the potential to react with free thiol groups: PTL and piperlongumine (PLM) (115). Both of these compounds preferentially deplete cellular GSH levels in CD34⁺ malignant cells compared with normal counterparts. In addition, PTL and PLM-treated normal HSPCs demonstrate some degree of GSH/GSSG recovery, whereas the malignant cells do not, offering protection of normal HSPCs and cytotoxic effects in malignant HSPCs. The result of this GSH depletion is an increase in cleaved caspase 3 and cleaved poly (ADP-ribose) polymerase (PARP), leading to a significant induction of LSC cell death compared with normal CD34⁺ HSPCs. Ultimately, PTL was found to actually bind several enzymes involved with GSH synthesis, including GCLC and GCLM

(115). PTL also exhibited a synergistic effect when paired with low doses of cytarabine and idarubicin (59).

PTL and several other natural compounds, including polyphenol and flavonoids, have demonstrated potential therapeutic efficacy in chronic myelogenous leukemia (CML) and lymphocytic leukemia. For example, Wu *et al.* evaluated the effects of 27 different compounds that include α,β -unsaturated carbonyls, sulfhydryl reactive metals, isothiocyanates, polyphenols, and flavones on NRF2 activation and cytotoxicity in peripheral blood mononuclear cells (PBMCs) isolated from normal and chronic lymphocytic leukemia (CLL) patients (168). Through the analysis of the NRF2 target heme oxygenase-1 (HO-1) and NRF2 protein levels, they first found that NRF2 activity was higher in PBMCs from CLL patients than in their normal controls. In all, six α,β unsaturated carbonyls, three arsenic-containing sulfhydryl reactive metals, two isothiocyanates, two flavones, and five polyphenol compounds were able to activate NRF2 in a FRET β -lactamase-based reporter assay in HepG2 cells (168). Cytotoxicity evaluation *via* MTT analysis showed that several α,β -unsaturated carbonyls, sulfhydryl reactive metals, isothiocyanates, and flavones were selectively cytotoxic to primary CLL PBMCs compared with their normal counterparts. In the case of PTL and ethacrynic acid, loss of α,β unsaturated carbonyl groups diminished NRF2 activation and CLL-selective cytotoxicity (168).

As discussed previously, Tsai *et al.* were able to utilize the *Nrf2*^{-/-} mouse model to demonstrate a downregulation of homing and chemotaxis associated with the loss of CXCR4 (153). Similarly, an increase in CXCR4 expression resulting from an upregulation of CXCL12 results in ADR-resistant K562 CML cells (164). This is characterized by an increase in PI3K activation and nuclear factor- κ B (NF- κ B) translocation to the nucleus, whereas CXCR4 siRNA downregulates PI3K/AKT, and IKK α activation sensitizing K562/ADM cells to ADR treatment demonstrated by an inhibition of cell growth and increase in apoptosis (164). Both *in vitro* and *in vivo*, treatment with oroxylin A, a monoflavonoid, targets leukemic/ADM cells with low off target toxicity by increasing apoptosis *via* downregulation of CXCR4 expression (164). Taken together, these results suggest that compounds such as polyphenols or flavonoids may be able to target the malignant cell redox environment through the manipulation of the NRF2 pathway. As discussed earlier, the flavone baicalein offers protection of splenic and BM HSPC from TBI through ERK and NRF2 activation (113, 176). However, within malignant K562 CML cells, treatment with both baicalein and baicalin results in an increase in NOTCH-1 activation followed by an increase in Hes-1, cyclin D1, Hey-1, and Hey-2 mRNA levels (161). In addition, treatment of K562 cells with baicalin resulted in an increase of cells in G₀/G₁ phase as well as a decrease of cells entering S phase and G₂/M phase, whereas treatment with baicalein only resulted in a decrease of cells in G₂/M phase (161). Interestingly, both compounds inhibited K562 colony formation in soft agar assays (161). Again, this highlights the ability of redox-active compounds to selectively target the malignant cellular redox environment, while, at the same time, offering protection to normal HSPC populations.

GSH homeostasis is also targeted by synergistic combination treatments of lymphoid and myeloid cell lines with polyphenol compounds and DOX or ETP (92, 93, 176).

Quercetin, apigenin, emodin, rhein, and cis-stilbene all had synergistic effects in cytotoxic induction of DOX and ETP within CCRF-CEM and Jurkat malignant lymphoid cell lines. Quercetin and apigenin, however, were the only polyphenols that demonstrated synergistic effects with DOX and ETP in THP-1 and KG-1a malignant myeloid cell lines. All synergistic effects were closely associated with a decrease of cellular GSH, a decrease of ATP production, and activation of the apoptosis cascade (92, 93). Of note, the lymphoid lineage cell lines being most sensitive to this redox-based combination treatment actually demonstrated the lowest basal levels of GSH, indicating that lymphoid cancers may be excellent targets for redox-based therapies. Other examples of polyphenol induction of LSC cytotoxicity include the use of Nispex, a polyphenol mixture that mainly comprises quercetin, quercitroside, avicularin, and oleandrin. This mixture has been proven effective against the AML/HL-60 cell line both *in vivo* and in a xenograft *in vivo* model (56, 57, 169).

The HL-60 cell line is one of the earliest malignant promyelocytic leukemia cell lines used for the elucidation of chemotherapeutic mechanisms. DOX treatment of HL-60 cells results in the activation of caspases 3, 8, and 9 as well as DNA fragmentation (140). The development of the HL-60/ADR (DOX resistant) cell line has led to the implementation of alternative therapies that include DOX. For example, pretreatment with a sublethal dose of DOX and subsequent combination with TRAIL, a member of the tumor necrosis factor (TNF) family, induces cytotoxicity in the parental and HL-60/ADR cell lines, indicating DOX induced redox signaling even in drug-resistant tumor types (78). This occurs through the depolarization of the mitochondrial membrane and increased levels of caspase 9 and Apaf-1 from the activation of mitochondrial type II apoptosis pathway (78). Importantly, HL-60 cells have demonstrated sensitivity to other more basic redox-active compounds such as exogenous H_2O_2 and the nitroxide compound, tempol (109). HL-60 cells demonstrate significant sensitivity to H_2O_2 -induced cell death measured by apoptotic markers at concentrations as low as $5 \mu M$, whereas normal BM and mononuclear cells isolated from cord blood remain resistant to cell death. Primitive populations isolated from normal control were not affected by H_2O_2 treatment, yet $CD34^+$ populations isolated from the HL-60 pool were significantly reduced without differentiation at all H_2O_2 concentrations evaluated. The authors continued to identify AKT as a potential mediator of H_2O_2 -induced cell death within progenitor populations of HL-60 cells (109). Tempol treatment of HL-60 cells results in a p53-independent upregulation of p21, indicating that peroxide generating and nitroxide compounds may be promising alternative therapies for chemo-resistant tumor types (52, 109).

MnSOD has been widely discussed as a tumor suppressor for many years, and just as the roles of GSH homeostasis and CAT activity play important parts of normal and malignant HSPC treatment, MnSOD has also been recently demonstrated as a potential therapeutic specific to malignant HSCs (110, 180). A phenotypic trait that separates HSPCs from LSCs is the differential expression of various surface markers such as CD33 and CD123 (119). For example, some LSC populations exhibit high levels of CD123 or interleukin-3 receptor (IL-3R) expression, whereas normal HSC populations do not (119). This has led to a targeted approach for the

delivery of MnSOD as a tumor suppressor. Much like the liposomal delivery of MnSOD previously discussed in normal HSPCs, Li *et al.* utilized CD123 for the targeted delivery of adenoviral particles carrying the MnSOD gene. Adenoviral delivery of MnSOD induced dose-dependent cytotoxicity in HL-60 and KG-1 malignant myeloid cell lines (87). Upon infection, cytotoxicity is accompanied by increases in cleaved caspases 7 and 9 as well as increases in cleaved PARP, indicating mitochondrial activation of apoptosis. In addition, delivery of MnSOD to xenografted HL-60 cells prolonged survival in mice (87). Similar results were seen *via* treatment of murine thymic lymphoma cells. Here, Jaramillo *et al.* found that elevated expression of MnSOD increased cellular H_2O_2 , sensitizing WEHI7.2 cells to glucocorticoid and H_2O_2 -induced apoptosis. This result was recapitulated with the application of MnTE. Although application of MnTE can in some cases produce peroxide, sensitization of WEHI7.2 cells may occur through another mechanism, such as the oxidation of NF- κ B (71). In addition, MnTE combination treatment with cyclophosphamide inhibited cell growth. No sensitization of WEHI7.2 cells was demonstrated in a combination of MnTE and DOX; however, MnTE addition did protect cardiomyocytes from DOX-induced injury (71). These findings have large implications for the future of leukemia drug development: delivery of MnSOD to normal HSPC populations either by genetic overexpression or by treatment with an SOD2 mimetic promotes HSPC function, whereas ectopic MnSOD expression or MnP treatment in leukemia cell populations clearly induces cell death (71, 87, 88, 99, 179). Furthermore, MnP function within malignant populations may be enhanced by the presence of other biologically relevant redox-active compounds and/or proteins. For example, Ferrer-Sueta *et al.* have shown that MnPs may be reduced by enzymes such as xanthine oxidase and complexes I and II of the mitochondrial electron transport chain (42). This reduction shown in submitochondrial particles allows for catalytic redox cycling and reduction of peroxynitrite, offering mitochondrial protection against peroxynitrite formation and damage (42, 158). In addition, the combination of MnPs with vitamin C has a promising outlook. Vitamin C itself has demonstrated a protective effect against oxidative insult within HL-60 cells (53). However, various concentrations of vitamin C in combination with MnPs may be effective. Although this combination has not been evaluated in HSPC or LSC populations, vitamin C has been shown to sensitize pancreatic and breast cancer cell populations through metabolic alterations to the cellular redox environment and production of species such as peroxides (37, 148, 154). Furthermore, combination of MnP with vitamin C has demonstrated significant effects on the malignant cell redox environment, primarily through the generation of H_2O_2 , within prostate and breast cancer, as well as glioblastoma populations (38, 148, 174, 181). This strategy is supported by the finding that exogenous H_2O_2 kills primitive LSCs while leaving normal HSPC populations unharmed (109). This indicates that redox-active compounds such as MnSOD mimetics, themselves or in combination with other redox-active drugs, may be able to modulate redox levels that support healthy HSPC function, leading to the safe eradication of LSCs from the hematopoietic compartment.

Perhaps one of the most primitive and aggressive of all hematopoietic cancers are CMLs. CML patients in blast crisis

undergo rapid clonal expansion of progenitor cell compartments and are often resistant to tyrosine kinase inhibitor (TKI) therapies due to altered expression of the breakpoint cluster region-Abl kinase fusion protein (BCR-Abl) fusion gene (30, 63). Redox-based therapies, however, offer potential solutions to refractory disease. Treatment of the KBM5- and the KBM5-T3151 TKI-resistant CML cell line with ROS inducers such as adaphostin or β -phenylethyl isothiocyanate (PEITC) results in complete inhibition of CML cell growth within cell lines and primary samples (24, 112, 151, 177). The cytotoxicity is based on these ROS inducers' ability to modify the redox environment of the CML cell that results in the oxidative modification and subsequent cleavage of the BCR-ABL protein. The modification of the redox milieu is caused by a sharp depletion of cellular GSH. Decreases in GSH, BCR-ABL cleavage, and cytotoxic effects are reversed by the pretreatment of CML cells with NAC. Similar effects of BCR-ABL degradation are caused by treatment with BSO. PEITC-induced cytotoxicity is also observed in primary CML patient samples drawn from individuals who display both imatinib sensitivity and resistance (177).

Similar studies have been conducted in the CD34⁺ K562 CML cell line. The K562 line is a BCR-ABL⁺ cell line that demonstrates resistance to imatinib. However, K562 cells are sensitized to imatinib by cotreatment with simvastatin, a known nitric oxide producer (25). The sensitization of K562 cells to this combination treatment is rescued by pretreatment with NAC, implying that the production of reactive species allows for successful treatment. This may very well proceed through the depletion of cellular GSH subsequent to the production of peroxynitrite (25). More recently, a combination treatment of DOX and polyphenol extract from *Viscum album* rescued K562 cells from G2/M arrest by expression of cyclins B1 and D1. This resulted in the activation of apoptosis and sensitization of K562 cells to DOX treatment (135). Elucidation of redox modifiers that can impact primitive and resistant malignant populations is clearing the way for emerging combination treatments, allowing for the use of subtoxic doses of classic chemotherapeutics that, originally, were detrimental to the patient hematopoietic compartment. These findings highlight the important ability of redox modifiers to provide new solutions for refractory diseases, ultimately improving outcomes for patients with a poor prognosis.

Redox-Based Therapies Targeting LSC Metabolism

Expertly reviewed by Pollyea and Jordan, the LSC redox environment can be specifically targeted through differential metabolic profiles between HSPCs and LSCs (119). Potential targets of LSC mitochondrial-induced apoptosis are IKK2 (IKB kinase 2) and NF- κ B (47). Inhibition of NF- κ B activation results in mitochondrial dysfunction, rupture, and cell death. Importantly, NF- κ B demonstrates constitutive activation in AML and has been used to characterize LSC populations (15, 103). As demonstrated in the combination treatment of TNF-related apoptosis-inducing ligand (TRAIL) and DOX for refractory AML cells, inhibition of NF- κ B with small molecules such as JSH-23 or AS602868 also sensitizes LSCs to treatment with TRAIL (39, 47, 146). As mentioned throughout this review, targeting GSH metabolism may also occur in the mitochondria, separating the LSC population apart from normal HSPC populations. Recently, however, more exciting discov-

eries have come to light. Several recent studies have identified the isocitrate dehydrogenases as redox-active targets for cancer therapies. For example, downregulation of IDH1 may sensitize cells to H₂O₂ production. Conversely, over production of the oxidative metabolite 2-hydroxyglutarate sensitizes LSCs to B cell lymphoma 2 downregulation (23, 44, 83, 119). The overproduction of 2-hydroxyglutarate, stemming from mutations in the isocitrate dehydrogenase enzymes, may cause epigenetic changes that push leukemogenesis (23, 44, 83, 84, 119, 130). Thus, from these studies, we may learn of redox-driven epigenetic events that initialize HSPC transformation in the BM niche. In addition, we have found that the MnTnBuOE porphyrins are excellent candidates for the alteration of HSPC and LSC mitochondrial function. Treatment of HSPCs with MnTnBuOE results in a decrease of mitochondrial function, an increase in antioxidant defense enzyme levels, and an increase in uncoupling protein expression, eventually offering protection of HSPCs (179). Moreover, as previous evidence has suggested, these same changes may have cytotoxic effects in LSC populations.

Targeting the NADPH Oxidase System

As previously mentioned, normal HSPC irradiation produces a phenotype of HSPC exhaustion resulting from DNA damage and the upregulation of cell cycle inhibitor proteins, leading to HSPC senescence (127, 163). This exhaustion can be protected against by treatment with redox-active compounds (71, 88, 99, 179). The principal mediator of these effects is the ROS produced by the NOX enzymes, implicating NOX enzymes as potential redox-based therapeutic target for leukemias (62, 72, 94, 118, 136). Aggressive subpopulations of AML can be identified by internal tandem duplication of the FMS-like tyrosine kinase 3-internal tandem repeat (FLT3-ITD) (122, 136). This population has elevated levels of DNA damage and ROS production. However, recent studies, particularly those demonstrating that *Nox4*^{-/-} mice are resistant to a FLT3-ITD-mediated induction of an MPD phenotype, elucidate NOX as a driving factor in the generation or propagation of LSC (72). These studies further suggest that redox-based therapies targeting NOX-produced ROS may simultaneously be effective in the treatment of LSCs. Inhibition of the FLT3-ITD signaling *via* PCK412 treatment results in decreased STAT5 signaling and the downregulation of the NOX subunit p22phox (167). This and the inhibition of NOX *via* DPI or VAS2870 treatment led to a decrease in DNA damage as well as a decrease in H₂O₂ production in the cytosol and nucleus of AML blast cells. Both culture models and primary patient HSCs that demonstrate elevated ROS production by NOX have depleted GSH levels and diminished antioxidant capabilities (136, 167). These facets promote the growth of LSC population over their normal CD34⁺ counterparts, and this growth advantage is also mediated through loss of response to the p38 MAPK signaling pathway. Taken together, these results show that just as normal HSPCs undergo exhaustion in response to ROS-mediated MAPK signaling, malignant LSC populations are propagated through NOX-induced ROS and loss of their normal p38 MAPK response that limits HSPC pools. In addition to NOX inhibitors, this presents another therapeutic window for the MnPs in leukemia therapeutic regimens, for the redox-active MnTnBuOE and MnTE are able to modulate

the cellular redox environment offering control over the p38 MAPK, NRF2, and NOX/NQO1 systems that may protect normal HSPCs while driving malignant cells to activate cell senescence mechanisms (Fig. 5).

Epigenetic Considerations in LSC Redox Treatments

Targeting epigenetic characteristics have also become a successful avenue of treatment for many hematopoietic disorders, including AML, myelodysplastic syndrome (MDS), and CML. Several cytogenetic abnormalities have become very prominent across the leukemias, particularly those involving fusion of mixed lineage leukemia/eleven nineteen leukemia and HOXA and nuclear pore complex protein 98 (NUP98) genes within AML, BCR-ABL in CML and acute lymphoblastic leukemia (ALL), and myelodysplastic 5q deletion syndrome in MDS (12, 13, 17, 25, 77, 85, 126, 130, 135, 145). Aberrant DNA methylation patterns have widely been recognized for the characterization of hematopoietic disorders within both the myeloid and lymphoid lineage (45, 48, 51, 68, 74). As patient populations grow increasingly resistant to therapies, methyl transferase or demethylase inhibitors such as the azacytidines and histone deacetylase inhibitors have been developed (128, 130). Even still, fractions of patient populations demonstrate resistance to epigenetic therapies. As with resistant CMLs however, there does seem to be a role for redox-active compounds in combination treatments that result in sensitization to epigenetic treatments. Recently, inhibition of lysine specific histone demethylase 1A (LSD1) demethylase with *trans*-2-phenylcyclopropylamine combined with all-*trans* retinoic acid (ATRA) treatment induced sensitivity in several AML models, demonstrated by decreased AML engraftment *in vivo* as well as an increase in the expression of differentiation markers such as CD11b (124). This has potential redox implications as ATRA has been known to induce ROS-mediated embryonic stem cell death (20). In addition, ATRA has been identified as an NRF2 inhibitor and has potentiated arsenic trioxide toxicity in human AML cells (157, 162). Combination treatment in MM with the histone deacetylase inhibitor PXD101 and bortezomib induces cell death in primary CD138⁺ MM cells but not in normal mononuclear populations (41).

These effects are driven by activation of caspases 3, 8, and 9 with coordinate activation of p53 and the p38 MAPK pathway, all of which can be blocked with NAC treatment (33, 41, 139, 175). Finally, the methyltransferase inhibitor 3-deazaneplanocin (DZNep) inhibits the polycomb-repressive complex-2 (PRC2) through the upregulation of thioredoxin interacting protein (TXNIP). TXNIP activity then results in TXN inhibition and a subsequent increase in oxidative stress that may not be a direct result of TXNIP-mediated inhibition of TXN itself. This ultimately results in apoptotic signaling within AML culture lines as well as primary samples (182). This also identifies DZNep as a potential therapy for CML (125, 170). The discovery of TXNIP downregulation in AMLs and the identification of PRC2/EZH2 as a target for redox-based therapies are an exciting advancement (125, 170). Recently the *Txnip*^{-/-} model has identified TXNIP as a crucial regulator of normal LT-HSC function (73, 76). *Txnip*^{-/-} mice undergo HSPC exhaustion that is characterized by a loss of repopulation capacity, increased Wnt signaling, a decrease in p21 expression, and a loss of CXCL12 activity

within the BM niche (73). Loss of TXNIP, as has been demonstrated in AMLs, results in an advanced aging phenotype in which HSC exhaustion and BM failure occurs through activation of the p53 and p38 MAPK pathway and induction of cell cycle regulators (182). Together, these results demonstrate that the *Txnip*^{-/-} phenotype closely resembles hematopoietic phenotypes characterized by pharmacological induction of redox stress or those generated by the selection of ROS high populations. These studies highlight the potential redox targeting of pathways of normal HSPCs use for maintenance within malignant populations. This supports the employment of ROS and redox modulation for the successful development of cancer therapies that are highlighted by the protection of normal tissues (Fig. 5). We have summarized the redox-active compounds as they have been cited and discussed throughout this review to highlight potential areas of therapeutic development that may safely and successfully target malignant populations in the future (Fig. 6).

Summary and Future Directions

In the past, the limited, primitive, and quiescent state of HSPCs has made it difficult to meaningfully evaluate the impacts of redox modification in normal and malignant HSPCs. In the past 10 years, we have begun to significantly evaluate and realize the important impact that management of the HSPC redox environment has on normal hematopoiesis and overall patient outcome. We now know that the HSPC redox environment can be manipulated by redox-active compounds to protect and improve HSPC function while still creating stress conditions that may differentially target malignant LSCs. This may be accomplished through combination therapies utilizing low-dose chemotherapeutics and the implementation of a single redox cyclers. To this purpose, several crucial pathways, such as FOXO, Atm, NRF2, NOX, NF- κ B, and p38 MAPKs, have emerged as critical HSPC redox regulators.

Through the years, there has been some debate over the importance of an impact of GSH homeostasis in the management of cellular redox environments and the treatment of cancers (46). However, within the HSPC compartment, the management of GSH/GSSG levels appears to be indispensable. Accurate measurement of cellular GSH and GSSG concentrations obviously provides insight into the mechanistic underpinnings of drug activity as previously discussed, and new methods of quantitation have emerged allowing for GSH measurement in HSPC populations (19, 134). These studies may also aid in the identification of therapeutics not previously thought to be redox active. For example, lenalidomide (LEN) has been used for the treatment of several hematopoietic diseases (96, 126, 145). This compound, previously thought to only act as an immunosuppressive agent, has demonstrated the redox-mediated activation of AP-1 signaling in MM treatment (32). Subsequently, we found that LEN can deplete cellular GSH levels in an MDS model at a level and rate similar to DOX (19). This now opens both LEN and MDS to consideration for redox-based therapies. In addition, our previous work has demonstrated that modulation of MRP1 expression dictates cellular GSH/GSSG concentrations. Most notably, an increase in HSPC GSH concentrations is demonstrated in the *Mrp1*^{-/-} mouse model, in which loss of MRP1 results in an increase in HSPC function.

Compound	Indication/Redox Target/Outcome	Redox Effect on Normal HSPCs	Reference
N-acetyl-L-cysteine (NAC)	AML/CML, CLL/ Protects against cytotoxicity	Protection of Glutathione pools and HSPC function	64, 69, 70, 91, 142
Buthionine Sulfoximine (BSO)	MM, CML, AML/ Glutathione synthesis/ apoptosis	GSH depletion, ROS increase, loss of HSPC function and HSC exhaustion	69, 142, 171
Methotrexate	AML, CML/ ROS increase/ apoptosis	Inhibition of MnSOD, GPx, GR, GCL, superoxide increase, GSH depletion, loss of HSPC function	6, 141
Cyclophosphamide	AML, CML/ ROS increase/ apoptosis	Decreased SOD3 and GPX2 expression, ROS increase, loss of HSPC quiescence and function	35, 104
Doxorubicin (DOX)	AML, MDS, CML, ALL/ ROS increase/ apoptosis	Increase oxygen radicals and hydrogen peroxide, myelosuppression and HSPC exhaustion	34, 36, 50, 86, 100, 101, 172, 178
Etoposide (ETP)	AML, CML/ ROS increase/ apoptosis		92, 93
Quercetin + DOX/ETP	ALL, CML, AML/ decreased GSH and ATP/ apoptosis	Protect ATP levels and caspase 3 activation, HSPC protection	52, 92, 93, 111, 169
Apigenin + DOX/ETP	ALL, CML, AML/ decreased GSH and ATP/ apoptosis	Protect ATP levels and caspase 3 activation, HSPC protection	52, 92, 93, 111
Emodin + DOX/ETP	ALL/ decreased GSH and ATP/ apoptosis	Protect ATP levels and caspase 3 activation, HSPC protection	92, 93
Rhein + DOX/ETP	ALL/ decreased GSH and ATP/ apoptosis	Protect ATP levels and caspase 3 activation, HSPC protection	92, 93
Cis-stilbene + DOX/ETP	ALL/ decreased GSH and ATP/ apoptosis	Protect ATP levels and caspase 3 activation, HSPC protection	92, 93
Baicalin	CML/ NOTCH-1 activation/ G2M decrease	Inhibition of MKP3, NF2 and ERK activation, HSPC protection form TBI	113, 161, 176
Baicalin	AML, CML/ mitochondria, PARP cleavage/ apoptosis	Protect HSPC function after TBI	161
Liposomal/adenoviral MnSOD	AML, CML/ mitochondria, PARP cleavage/ apoptosis	Protect HSPC function after TBI	99
Liposomal CAT	Lymphoid/ peroxide generation, NF- κ B/ apoptosis	downregulation of p16, protection of HSPC function after TBI	99
MintE-2-PyP ⁺ (MintE)		downregulation of p16, protection of HSPC function after TBI	8, 71, 88, 147, 164, 180
MnTnBuOE-2-PyP ⁺ (MnTnBuOE)		Mild ROS increase, NF2 and ETS activation, increase in MnSOD, CAT, GSTp1, UPC3 expression, decrease in mitochondrial function, increase in HSPC function	180
Diphenylene iodonium (DPI)	AML/ decrease in peroxide production and GSH depletion	NOX inhibition, decreased ROS	114, 136
VAS2870	AML/ decrease in peroxide production and GSH depletion		167
PCK412	AML/ FLT3-ITD, STAT5 and p22phox downregulation/ apoptosis		167
L-PAM + BSO	MM/ DNA damage, mitochondrial function, GSH synthesis/ apoptosis	GSH depletion, ROS increase, loss of HSPC function and HSC exhaustion	142
Parthenolide (PTL)	AML/ GCLC, GCLM, GSH depletion, caspase and PARP activation/ apoptosis		59, 115, 168
Piperlongumine (PLM)	AML/ GSH depletion, caspase and PARP activation/ apoptosis	Mild GSH depletion	115
α - β unsaturated carbonyls	CLL/ NRF2 activation/ cytotoxicity	Mild GSH depletion	168
Arsenic containing sulfhydryl reactive metal isothiocyanates	CLL/ NRF2 activation/ cytotoxicity	PBMC protection	168
Polyphenol	CLL/ NRF2 activation/ cytotoxicity	PBMC protection	24, 112, 150, 168, 177
Flavones	CLL/ NRF2 activation/ cytotoxicity	PBMC protection	52, 92, 93, 111, 168, 169
Oroxylin-A	CLL/ NRF2 activation/ cytotoxicity	PBMC protection	113, 163, 168, 176
Tempol	CML/ CXCR4 downregulation/ apoptosis	PBMC protection	163, 168
Adaphostin	AML/ peroxide generation, p21 upregulation/ cell death		52, 109
β -phenylethyl isothiocyanate (PEITC)	CML/ ROS increase, GSH depletion/ cytotoxicity		24, 112, 150, 177
ISH-23	CML, CLL/ ROS increase, GSH depletion/ cytotoxicity		24, 112, 150, 168, 177
AS602868	AML/ NF- κ B, ROS increase/ apoptosis		39, 47, 146
TRAIL	AML/ NF- κ B, ROS increase/ apoptosis		39, 47, 146
all-trans-retinoic acid (ATRA)	AML/ ROS increase, mitochondrial depolarization/ apoptosis		39, 47, 78, 146
trans-2-phenylcyclopropylamine (TCP)	AML/ inhibition of LSD1 and NRF2, increased ROS/ differentiation, apoptosis		20, 41, 124, 157, 162
3-Deazaplanocin (DZNeP)	AML/ inhibition of LSD1, increased ROS/ differentiation, apoptosis		124
Lenalidomide (LEN)	AML/ inhibition of PRC2, upregulation of TXNIP/ apoptosis		125, 170, 182
	MDS, MM/ Increase in ROS, AP-1 signaling/ apoptosis	Hematopoietic suppression	19, 32, 96, 126, 145

FIG. 6. The table summarizes the redox-active compounds that have been discussed throughout this review as well as their effects within their indicated disease states and normal HSPC populations where applicable. This dual application highlights the potential for selective targeting of malignant populations while offering protection to normal HSPCs for many redox-active compounds discussed and cited within this review. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; FLT3-ITD, FMS-like tyrosine kinase 3-internal tandem repeat; GCLC, glutamate-cysteine ligase catalytic subunit; GCLM, glutamate-cysteine ligase regulatory subunit; LSD1, lysine specific histone demethylase 1A; MDS, myelodysplastic syndrome; MM, multiple myeloma; NF- κ B, nuclear factor- κ B; PARP, poly (ADP-ribose) polymerase; PBMC, peripheral blood mononuclear cell; TRAIL, TNF-related apoptosis-inducing ligand; TXNIP, thioredoxin-interacting protein.

These results are demonstrated by colony formation assays as well as by *in vivo* serial transplantation experiments. Improvements in WT HSPC function, compared with the *Mrp1*^{-/-}, can also be recapitulated with the addition of NAC (120). This indicates that ABC transporter inhibitors may have a role in the development of leukemia therapies that protect the HSPC compartment. Moreover, GSH and GSSG levels appear to be a potential biomarker for the redox-based therapy candidacy. We have previously found that peripheral leukocytes isolated from AML patients contain significantly higher cellular GSH levels, resulting in a significant difference in the 2GSH/GSSG ratio between patients and healthy donors (19). These elevated GSH levels may be the result of an increase in reactive species produced by malignant cells, relative to healthy cells. Redox-active therapies must then be able to overcome the increased redox buffer capacity demonstrated in malignant cells to elicit a desired therapeutic effect. Thus, the 2GSH-GSSG ratio may not only be a marker for disease but also serve as an indicator for redox-based therapies. Because the cellular redox potential can be calculated based on the 2GSH/GSSG ratio, we may be able to establish limits of HSPC/LSC redox potentials in which we know that certain redox-based therapies will be efficacious (81, 123). In the future, evaluation of patient cellular redox potentials across diseases (CML, AML, ALL *etc.*) may allow for the prediction of response of disease states to specific redox-based therapies.

The focus of these principles should expand to include a better understanding of other factors affecting the HSPC redox environment, such as thioredoxin activity and the function of TXNIP. TXNIP has recently been identified as an important regulator of the HSPC, wherein loss results in p38 activation and LT-HSC exhaustion (73). If this occurs through NRF2 regulation, TXNIP may emerge as a druggable target for the treatment of hematopoietic malignancies. In addition, cytogenetic abnormalities, such as chromosomal rearrangements and deletions, have been well documented throughout the hematopoietic diseases, yet major changes in epigenetic programming remain unclear. For example, TXNIP studies involving DNA methylation status indicate that future efforts must focus on the impact that redox-active compounds have on the epigenetic regulation of the normal HSPC as well as the malignant LSC. Redox evaluation of epigenetic changes in hematopoietic malignancies, such as those demonstrated by the isocitrate dehydrogenase enzymes (IDH1/2), may provide valuable insight into potential initiating events that drive LSC propagation (23, 44, 80, 84, 130). This may provide avenues for new genetic-based therapies that utilize the manipulation of the LSC redox environment.

Finally, redox modifiers and redox cycling drugs should be used to elucidate the impact of signaling pathways contributing to malignant HSPC expansion. For example, subpopulations of aggressive hematopoietic malignancies express several isoforms of the NOX family, as compared with limited NOX expression in normal HSCs (62, 72, 94). However, in normal HSPCs, increased NOX expression and activity results in overproduction of ROS ($O_2^{\cdot-}$, H_2O_2) leading to the activation p38 MAPKs, which results in the upregulation of cell cycle regulators. It is unclear whether constitutive NOX activity in malignant populations leads to the loss of AKT and p38 MAPK regulation over LSC expansion or whether components within, such as the AP-1 family of transcription

factors, may be targeted for redox regulation by redox-active compounds to regain control of progenitor compartments undergoing clonal expansion (Fig. 5). These aspects have the potential to improve redox-based therapies, providing new directions for safe, effective treatments.

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Abbreviations Used

53BP1 = p53 binding protein
 ABCG2 = ATP binding cassette subfamily G member 2
 ALL = acute lymphoblastic leukemia
 AML = acute myeloid leukemia
 AP-1 = activating protein-1
 Atm = ataxia-telangiectasia mutated
 ATP = adenosine tri-phosphate
 ATRA = all-*trans* retinoic acid
 BCL-2 = B cell lymphoma 2
 BCR-Abl = breakpoint cluster region-Abl kinase fusion protein
 BM = bone marrow
 BSO = buthionine sulfoximine
 CAT = catalase
 CD = cluster of differentiation protein
 CD45RA (PTPRC) = cluster of differentiation protein 45 isoform A (protein tyrosine phosphatase, receptor type C isoform A)
 CLL = chronic lymphocytic leukemia
 CLP = common lymphoid progenitor
 CML = chronic myelogenous leukemia
 CMP = common myeloid progenitors
 CSC = cancer stem cell
 CXCR4 = chemokine receptor type 4
 DCF = 2'-7'-dichlorofluorescence
 DOX = doxorubicin
 DPI = diphenylene iodonium
 DZNep = 3-deazaneplanocin
 ETP = etoposide
 FLT3-ITD = FMS-like tyrosine kinase 3-internal tandem repeat
 Foxo3a = forkhead box O3a
 GCLC = glutamate-cysteine ligase catalytic subunit
 GCLM = glutamate-cysteine ligase regulatory subunit
 GPx = glutathione peroxidase
 GR = glutathione reductase
 GSH = reduced glutathione
 GSSG = oxidized glutathione
 GST = glutathione-S-transferase
 Gy = gray
 H₂O₂ = hydrogen peroxide
 HSC = hematopoietic stem cell
 HSPC = hematopoietic stem/progenitor cell
 IDH1 = isocitrate dehydrogenase 1
 LEN = lenalidomide
 Lin = lineage
 L-PAM = melphalan
 LSC = leukemic stem cell
 LSD1 = lysine specific histone demethylase 1A
 LSK = Lin⁻/Sca-1⁺/c-Kit⁺
 LTC-IC = long-term culture-initiating cell

LT-HSC = long-term hematopoietic stem cell
 MDS = myelodysplastic syndrome
 MM = multiple myeloma
 MnPs = Mn porphyrins
 MnTE/MnTE-2-PyP⁵⁺ = Mn (III) *meso*-tetrakis (N-ethylpyridinium-2-yl) porphyrin
 MnTnBuOE/MnTnBuOE-2-PyP⁵⁺ = Mn (III) *meso*-tetrakis(N-(n-butoxyethyl)pyridinium-2-yl) porphyrin
 MPD = myeloproliferative disorder
 MPP = multipotent progenitor cell
 MRP1/ABCC1 = multidrug-resistant protein 1/ATP-binding cassette subfamily C member 1
 MTX = methotrexate
 NAC = N-acetyl-L-cysteine
 NADPH = nicotinamide adenine dinucleotide phosphate
 NF- κ B = nuclear factor- κ B
 NOX = NADPH oxidase
 Nq01 = NAD(P)H dehydrogenase quinone 1
 Nrf2 = nuclear factor (erythroid-derived 2)-like 2
 NUP98 = nuclear pore complex protein 98
 O₂ = molecular oxygen
 O₂⁻ = superoxide
 p15^{INK4B} = cyclin-dependent kinase 4 inhibitor B
 p16^{INK4A} = cyclin-dependent kinase inhibitor 2A
 p18^{INK4C} = cyclin-dependent kinase 4 inhibitor C
 p19^{ARF} = cyclin-dependent kinase 4/6 inhibitor
 p21^{CIP} = cyclin-dependent kinase inhibitor 1
 p38 MAPK = p38 mitogen-activated protein kinase
 PARP = poly (ADP-ribose) polymerase
 PBMC = peripheral blood mononuclear cell
 PEITC = β -phenylethyl isothiocyanate
 PLM = piperlongumine
 PRC2 = polycomb-repressive complex-2
 PTL = parthenolide
 ROS = reactive oxygen species
 Sca-1 = stem cell antigen 1
 SOD2 = manganese superoxide dismutase
 ST-HSC = short-term hematopoietic stem cell
 TBI = total body irradiation
 TKI = tyrosine kinase inhibitor
 TNF = tumor necrosis factor
 TRAIL = TNF-related apoptosis-inducing ligand
 TXN = thioredoxin
 TXNIP = thioredoxin-interacting protein
 UPC1/3 = uncoupling protein 1/3