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Clinically Evaluated Cancer Drugs Inhibiting Redox Signaling

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Abstract

Significance: There are a number of redox-active anticancer agents currently in development based on the premise that altered redox homeostasis is necessary for cancer cell's survival.
Recent Advances: This review focuses on the relatively few agents that target cellular redox homeostasis to have entered clinical trial as anticancer drugs.
Critical Issues: The success rate of redox anticancer drugs has been disappointing compared to other classes of anticancer agents. This is due, in part, to our incomplete understanding of the functions of the redox targets in normal and cancer tissues, leading to off-target toxicities and low therapeutic indexes of the drugs. The field also lags behind in the use biomarkers and other means to select patients who are most likely to respond to redox-targeted therapy.
Future Directions: If we wish to derive clinical benefit from agents that attack redox targets, then the future will require a more sophisticated understanding of the role of redox targets in cancer and the increased application of personalized medicine principles for their use. *Antioxid. Redox Signal.* 26, 262–273.

Keywords: redox, signaling, cancer, therapy, clinical

Introduction

A BALANCE BETWEEN oxidants and antioxidants exists under physiological conditions even though reactive oxygen species (ROS) are continuously generated. The maintenance of redox homeostasis is accomplished by elimination of ROS by scavenging systems. The two main sources of ROS are the mitochondria and NADPH oxidases (76). Other ROS producers are the cytochrome P450 system, xanthine oxidase, and nitric oxide (NO) synthase. ROS are eliminated by intracellular enzymes, including superoxide dismutase (SOD), glutathione (GSH) peroxidase, catalase, thioredoxin (Trx) reductase, and glutathione S-transferase (GST). Cells maintain a redox balance, and under normal conditions, low levels of locally produced ROS act as mitogens driving cell survival and proliferation. Oxidative stress and aberrant redox signaling result from a loss of this redox balance and can lead to cancer development because of resulting DNA mutations, genomic instability, and protumorigenic signaling (1). Cancer cells can also become dependent on high levels of ROS formation required for an altered redox environment that maintains the procancerous state, thus presenting an ''Achilles Heel'' for developing treatment options based on the loss of normal redox homeostatsis (76). The clinical outcome of approaches that attempt to exploit this cancer redox vulnerability will be discussed in the review in the context of mechanisms of redox regulation described below.

Mechanisms of Redox Regulation

Mitochondria drive a number of cellular functions from metabolism to apoptosis, while providing the cell's energy needs (52). Redox signaling molecules emanating from ROS generated by the mitochondria are H_2O_2, NO, and superoxide, or other less significant naturally occurring redox species such as peroxynitrite, prostaglandin-like molecules, and 4-hydroxynonenal. To generate redox signals, they must bring about reversible change in the activity of a protein. Generally, this involves modification of cysteine residues (17).

S-oxidation (cysteine oxidation)

S-glutathionylation and S-nitrosylation are processes that regulate key mitochondrial functions, including nutrient oxidation, oxidative phosphorylation, ROS production, the mitochondrial permeability transition, apoptosis, and mitochondrial fission and fusion (61). All modifications act as redox switches, altering function and enabling proteins to respond to the reduction potential of a particular redox couple.

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The Trx system

The cytoplasmic Trx system comprising thioredoxin 1 (Trx1), thioredoxin reductase 1 (TrxR-1), and NADPH, one of the major antioxidant systems, maintains intracellular redox homeostasis through reversible cysteine modification (81). Trx1 regulates redox-sensitive transcription factors by acting as a cofactor to reduce specific cysteine residues (58, 53) and acts as a growth factor that stimulates cancer cell proliferation (8, 77). The Trx system maintains cellular redox homeostasis by scavenging ROS and regulating redox enzymes. There is also a mitochondrial Trx system comprising Trx-2 and TrxR-2 that attenuates some of the effects of Trx-1 and increases in mitochondrial ROS (116). The absence of Trx-1 in homozygous null mice leads to embryonic lethality and of Trx-2 to embryonic lethality at the time of maturation of the mitochondria, both associated with a massive increase in apoptosis (68). Expression of Trx1 and TrxR and activity of TrxR is upregulated in many human cancers resulting in proliferation, survival, chemoresistance, and inhibition of apoptosis (7).

The glutathione system

Glutathione/Glutaredoxin (GRX) maintain cellular redox through S-glutathionylation and reduction, although this is dependent on Trx redox regulation (63). GRX, a small dithiol protein, regulates certain enzyme activities *via* a disulfide exchange reaction and when overexpressed protects cells from H_2O_2-induced apoptosis. Elevated GSH levels are observed in various types of tumors, making them more resistant to chemotherapy (14, 107). Removing this molecular shield has been an approach to reinstate death signals driving cancer cells to apoptosis.

NO metabolism

NO metabolism modifies protein thiol groups to S-nitrosothiols, which may then be further modified to disulfide, sulfenic acid, or S-GSH-modified protein all referred to as redox switches (67). These posttranslational modifications are reversible and able to react sensitively to levels of ROS (17).

Redox transcription factors

There are a number of reported redox-sensitive transcription factors (Table 1) (13, 23).

Drugs Targeting Redox Systems and Redox Regulators

The drugs that we describe in this review have been explored clinically for targeting redox processes and are summarized in Table 2.

Trx-Trx reductase inhibitors

Auranofin. Since the selenoenzyme thioredoxin reductase (TrxR) is a putative target for cytotoxic gold complexes, a group of structurally diverse gold(III) compounds, including the clinically used antiarthritic gold(I) drugs auranofin, approved for the treatment of rheumatoid arthritis in 1985, and aurothiomalate, were investigated for the mechanism of TrxR inhibition (33). All gold(III) or gold(I) compounds were found to produce potent enzyme inhibition but only after prereduction of TrxR with NADPH. TrxR inhibition was attributed to structural modification occurring, upon cofactor binding, with auranofin's gold(I) coordination to the active site selenocysteine (29, 31, 62, 75). An alternate mechanism has been described for gold(III) compounds through oxidative protein damage and inhibition of the ubiquitin–proteasome system by targeting deubiquitinases involved in cell cycle regulation, protein degradation, gene expression, and DNA repair (15, 57). Both these mechanisms of action result in apoptosis. Although the use of auranofin for the treatment of rheumatoid arthritis is declining because of adverse effects associated with its long-term use (90), recent clinical trials have been initiated exploring its use as an anticancer agent. A pharmacokinetic Phase I, open-label study in healthy adult subjects was initiated in 2014 to determine terminal phase pharmacokinetic parameters in blood and gold measurements in stool samples (NCT02089048).

Auranofin has also been evaluated in a number of Phase II studies as a single agent or in combination with chemotherapy. A Phase I and II study to evaluate the safety and effectiveness of auranofin to treat patients with chronic lymphocytic leukemia, small lymphocytic lymphoma, or prolymphocytic lymphoma is awaiting study results (NCT01419691). An ongoing Phase I/II clinical trial initiated in 2012 is examining the combination of sirolimus (rapamycin) and auranofin in patients with advanced or recurrent nonsmall-cell lung cancer or small-cell lung cancer (NCT01737502).

Texaphyrins. Motexafin gadolinium (MGd, Xcytrin) an inhibitor of TrxR and ribonucleotide reductase, was developed

Table 1. Mammalian Redox-Regulated Transcription Factors

Transcription Factor	*Redox Regulator*	*Consequence*
AP-1	Trx-1, nucleoredoxin	Enhanced DNA binding
β-catenin	Trx-1,nucleoredoxin, Ref-1/APE	Enhanced DNA binding
Egr-1	Ref-1/APE	Enhanced DNA binding
FOXO	Cys oxidation PTP1b	Block nuclear export
GR	Trx-1	Enhanced DNA binding
HIF-1	Trx-1, APE/Ref-1	HIF-1 stability, HIF-1 activation
HSF1	H_2O_2	Altered DNA binding
TP53	Trx-1, APE/Ref-1	Enhanced DNA binding
NF-κB	Trx-1, APE/Ref-1	Enhanced DNA binding
NRF2	Trx-1	Prevent degradation
Sp1	Trx-1	Enhanced DNA binding
TTF	APE/Ref-1	Enhanced DNA binding

Table 2. Drugs Targeting Redox Systems for the Treatment of Cancer

Drug	*Target*	*Clinical activity*	*Status*[a]
Aurofin	Thioredoxin reductase	Ongoing trials	NCT02089048 NCT01419691 I NCT01737502 I
Motexafin gadolinium	Thioredoxin reductase, ribonucleotide reductase	Radio/chemosensitizer	Not approved by FDA
Motexafin lutetium	Apoptosis	Photosensitization	No longer being developed
PX-12	Thioredoxin, HIF-1	Stable disease	Iv use no longer pursued
Arsenic trioxide	Mitochondria, Trx-1/-2,GSH	FDA approved for APL	NCT00275067 NCT00661544 NCT00258245
Buthionine sulfoximine	γ-glutamylcysteine ligase (GSH)	Chemosensitizer no clinical advantage	No longer being developed
Telcyta (TLK-286)	Glutathione S-transferase -ρi	Chemosensitizer no clinical advantage	Development status unknown
Telintra (TLK199)	Glutathione S-transferase -pi	Chemosensitizer	Development status unknown
Disulfiram	Acetaldehyde dehydrogenase, GSH/GSSG	Chemosensitizer no clinical advantage	No longer being developed
NOV-002	γ-glutamyl-transpeptidase, GSH/GSSG	Chemosensitizer no clinical advantage	No longer being developed
N-acetylcysteine	GSH prodrug	Decrease side effects, no clinical advantage	NCT02241876,
PX-478	HIF-1	Phase I trial only	Not currently being developed
EZN-2968	HIF-1	Lowers HIF-1	NCT02564614
BAY 87-2243	HIF-1/2, mitochondrial complex-1	Toxic at doses used	development status unknown
Topotecan	topoisomerase I (HIF-1)	Trend to lower HIF-1, a partial tumor response	FDA-approved drug, development status as HIF-1 inhibitor unknown
EZN-2208	Topoisomerase I (HIF-1)	HIF-1 activity unknown, no clinical survival advantage	Company not developing clinical products
Bortezomib	Proteasome (NF-κB)	Significant clinical activity, not known if NF-κB related	FDA-approved drug
Fenretinide	Retinoic acid receptor agonist, increases ROS	Induces apoptosis, not known if activity related to ROS	NCT01535157 NCT00646230
α-Tocopherol	Free radical scavenger	Large-scale clinical cancer prevention trials show no clinical benefit	NCT02397486
Vitamin C	Free radical scavenger, H_2O_2 prodrug	No convincing evidence of clinical benefit	Development of high-dose iv vitamin C continues
Curcumin	Free radical scavenger	No convincing evidence of clinical advantage	Development of more bioavailable forms continues

[a]Refers to an ongoing clinical trial from the NIH Clinical Trials database at ClinicalTrials.gov.

APL, acute promyelocytic leukemia; FDA, Food and Drug Administration; GSH, glutathione; GSSG, oxidized glutathione; ROS, reactive oxygen species.

by Pharmacyclics as a radiosensitizing agent initially for use in brain metastases (60). Although the mechanism of sensitization remains unclear, it has been proposed that MGd is endocytosed by the clathrin-dependent pathway producing high concentrations in malignant cells, which along with the synergistic effect of irradiation causes apoptosis (5, 10). MGd was evaluated in a number of Phase II trials for lymphomas, multiple myeloma, renal cell cancer, glioblastoma multiforme with radiation, and in NSCLC with chemotherapy (65). Pharmacyclics completed a multicenter US Phase III clinical trial of MGd in NSCLC patients with metastatic tumors of the brain who require whole brain radiotherapy (NCT00054795), but it did not receive Food and Drug Administration (FDA) approval.

Antrin® (motexafin lutetium), another texaphyrin by Pharmacyclics (94), was developed for photodynamic therapy (photoangioplasty) to promote a redox-sensitive apoptotic cell death pathway in vascular cells for the reduction of atherosclerosis involving peripheral arteries. It has been reported to preferentially accumulate in tumor cells and absorb light, forming an extended high-energy conformational state producing high quantum yields of singlet oxygen and a local cytotoxic effect. A number of clinical trials were initiated and terminated (NCT00005067, NCT00005808, NCT00087191) and this agent is no longer being developed.

PX-12. The thioredoxin inhibitor, PX-12 (1-methylpropyl 2-imidazolyl disulfide), has shown both an *in vitro* and promising *in vivo* antitumor activity (54). Irreversible thioalkylation of the Cys73 thioredoxin residue, by PX-12, was shown to result in inhibition of Trx redox activity and subsequent inhibition of the hypoxia-induced increase in HIF-1α protein and vascular endothelial growth factor (VEGF) secretion (110, 111). This inhibition is accompanied by the rapid metabolism of PX-12 producing two inactive metabolites, volatile 2-butanethiol and 2-mercaptoimidazole. PX-12, the first Trx-1 inhibitor to enter clinical trials, was administered as a 1-h intravenous (IV) infusion (79). The infusion time was subsequently increased to 3, 24, and 72 h (9, 78, 80) to overcome lung irritation that was observed at the higher dose levels due to expulsion of the volatile metabolite (9). Although there were no objective responses based on response evaluation criteria in solid tumor criteria in the first Phase I trial with 1- and 3-h infusions, seven (18%) patients achieved durable stable disease, receiving at least six cycles of therapy (range, 6–14 cycles). These included two patients with sarcoma and one patient each with colorectal, appendiceal, and renal cancer. A patient with appendiceal adenocarcinoma had a minor response of 322 days (79).

PX-12 was also investigated in a Phase II trial as a 3-h infusion at two dose levels in patients with previously treated advanced pancreatic cancer (78). Plasma Trx-1 levels were found elevated in only 3 of 28 (11%) patients screened for study. Although the therapy was well tolerated, the best response was stable disease in two patients with unexpectedly low baseline Trx-1. The development of P-12 as an IV agent has been terminated.

Arsenic trioxide (ATO). The traditional Chinese medicine Pi Shuang or arsenic trioxide [As_2O_3 (ATO)] was and is still used to treat cancer and other conditions (11, 36). Work in the 1970s to investigate the potential of ATO to treat acute promyelocytic leukemia (82, 114) led to the approval of ATO (TRISENOX) by the FDA for the treatment of leukemia in 2000 (82). Several mechanisms have been proposed to explain its anticancer activity, which occurs mainly through mitochondrial dysfunction, as it was determined that ATO could lower both reduced and oxidized forms of thioredoxin 1 (Trx1) with no effect on Trx1 redox potential (115). The result is altered cellular redox homeostasis through hydrogen peroxide generation, GSH depletion, and Trx-1 downregulation (59). Lu *et al.* determined that ATO irreversibly inhibited mammalian TrxR, with both the N-terminal redox-active dithiol and the C-terminal selenothiol-active participating in the reaction (43). Numerous clinical studies have investigated ATO use as a single agent in the treatment of solid tumors, however, therapeutic efficacy has been limited (100). Because of this, the combination of ATO with other clinically used chemotherapeutic drugs has been evaluated to improve its therapeutic efficacy in treating human solid tumors. There are currently a number of ongoing clinical trials of ATO in combination with chemotherapy in solid tumors, including in malignant glioma (NCT00275067) and in multiple myeloma (NCT00661544, NCT00258245).

GSH inhibitors

Buthionine sulfoximine. Elevated GSH has been associated with cancer cell resistance to various cytotoxic agents (14, 15). Thus, lowering GSH has been sought by specific inhibition of γ-glutamylcysteine ligase, a key enzyme of GSH biosynthesis. Such an inhibitor, L-*S,R* buthionine sulfoximine (BSO) (38), was shown to enhance the cytotoxicity to cancer cells of chemotherapeutic agents experimentally both *in vitro* and *in vivo* (42, 95). Early clinical trials of BSO evaluated its ability to enhance the cytotoxicity of chemotherapeutic agents (6). A Phase I dose escalation trial of IV BSO administered as a short infusion every 12 h, with and without the alkylating drug L-PAM, showed that BSO, alone and in combination with L-PAM, could be safely given to patients, but that a schedule of short infusions every 12 h did not result in GSH depletion <30% of baseline values (7). In subsequent clinical studies, BSO was evaluated in combination with melphalan in children with neuroblastoma (NCT00005835) (2, 3) and patients with persistent or recurrent malignant melanoma (NCT00661336), which was not completed due to lack of patients. Ultimately, BSOs short half-life (96) led to focused attempts to find alternatives for modification of GSH levels.

Telcyta. Telcyta (TLK-286), a GSH analogue that inhibits glutathione S-transferase pi isoform (GST P1-1), has been tested clinically in combination with platinum, taxanes, and anthracyclines in tumors with very high GST-PI-1 levels (102). It is a prodrug that is activated by GST P1-1 to produce an anticancer alkylating agent and releases a glutathione derivative (25). Telcyta has been tested in Phase II and III clinical trials for the treatment of ovarian, nonsmall cell lung and breast cancer, as a single agent and in combination with other chemotherapeutic agents. NCT00350948, a Phase III randomized study of Telcyta and doxorubicin in platinum refractory ovarian cancer (ASSIST-5), was terminated after data provided showed that women treated with Telcyta had poorer outcomes than those treated with standard of care.

Telintra (TLK199), an inhibitor of GST-P1-1, was granted orphan status and approved to be evaluated in a Phase III study for the treatment of myelodisplastic syndrome (MDS). Other exploratory studies have been discontinued. Telintra, whose inhibition of GST-PI-1 was shown to lead to activation of Jun kinase (91), was shown to cause clinically significant and sustained reduction in red blood cell transfusion responses in MDS patients (87, 88). Telintra has been evaluated in a number of Phase 1 and 2 trials as both a liposomal preparation for injection (NCT00035867) and an oral tablet (NCT00701870), although a number of trials have been terminated due to lack of enrollment. Hematologic improvement was observed in patients who had failed or progressed following a range of prior therapies and supportive care regimens. In 2014, Telik, the company developing Telintra, merged into MabVax and the current development status of Telintra is unknown.

Disulfiram. Disulfiram (1,1′,1″,1‴-[disulfanediylbis-(carbonothioylnitrilo)]tetraethane, Antabuse), discovered almost 100 years ago as an agent that inhibits acetaldehyde dehydrogenase resulting in an immediate hangover when alcohol is ingested and used as a support for the treatment of chronic alcoholism, also shifts the ratio of GSH:oxidized glutathione (GSSG) to the oxidized state and induces apoptosis (12). It has been evaluated in a clinical trial for metastatic melanoma (NCT00256230) in a study completed in 2011. Another study with disulfiram in combination with ATO (NCT00571116) was halted due to slow accrual and lack of study findings.

NOV-002. A unique antitumor agent NOV-002, a stabilized formulation of GSSG and cisplatin at a ratio of 1000:1, (developed by Novelos and now Cellectar Biosciences, Inc.), has been reported to alter the GSH:GSSG ratio by acting as a competitive substrate for gamma-glutamyl-transpeptidase (GGT) (105). GGT catalyzes the transfer of the gamma-glutamyl moiety of GSH to an acceptor protein to give S-glutathionylation proteins. NOV-002 has been reported to inhibit tumor cell proliferation, survival, and invasion, and in some settings can ameliorate cytotoxic chemotherapy-induced hematopoietic and immune suppression through its ability to modulate cellular redox by upregulation of the expression of SOD-3 and glutathione peroxidase-2 (21). NOV-002 has been studied in combination with standard chemotherapy for Stage IIIb/IV NSCLC in Phase III (NCT00347412) and in a neoadjuvant breast cancer setting in Phase II (NCT00499122). In a neoadjuvant trail of women with newly diagnosed Stage IIB or IIIC breast cancer, NOV-002 in combination with doxorubicin and cyclophosphamide followed by docetaxel met the primary endpoint of a pathologic complete response of 38%. Unfortunately, in a study of 903 NSCLC patients, NOV-002 and carboplatin failed to improve overall survival compared to the paclitaxel and carboplatin alone. In another study of NOV-002 in combination with carboplatin in chemotherapy-resistant ovarian cancer (NCT00345540) failed to reach its endpoint of ≥ 2 responses and its development has been discontinued.

N-acetylcysteine. *N*-acetylcysteine (NAC; Mucomyst) is a prodrug of L-cysteine, a precursor of GSH, and hence, particularly during times of oxidative stress (20), has been shown to support the body's antioxidant and NO systems (64). Because *de novo* synthesis is the primary mechanism by which glutathione is replenished (22), NAC has been tested in a number of clinical trials with the objective of improving cancer outcomes (64). NAC have been evaluated for ability to suppress colon polyps (28), as an adjunct to standard therapy in the eradication of *Helicobacter pylori* (NCT01109381; the study was terminated due to poor efficacy) (41), and in Phase II and III studies to explore if NAC could decrease the risk of ototoxicity in patients on hemodialysis who are receiving gentamicin (NCT01271088, NCT01131468) (30). In addition, it is currently being explored for its ability to attenuate cisplatin-induced toxicities (NCT02241876) and its ability to reduce mucositis in head and neck patients receiving radiation therapy (NCT02123511). However, a 2-year supplementation of NAC, with or without retinyl palmitate in more than 2500 patients, resulted in no benefit in terms of survival, event-free survival, or second primary tumors for patients with head and neck cancer or with lung cancer, most of whom were previous or current smokers (108).

HIF inhibitors

PX-478. A number of agents have been studied clinically that are reported to inhibit HIF-1 activity directly or indirectly (51). PX-478, a small molecule that downregulates hypoxia-induced increase as well as constitutive expression of HIF-1α and HIF-1 transcription factor activity (101, 112), was evaluated in an open-label, oral dose escalation Phase I trial in patients with advanced cancers to examine safety, tolerability, pharmacokinetics, pharmacodynamic, and antitumor activity (NCT00522652) (104). Pharmocodynamic studies revealed dose-proportional inhibition of HIF-1α levels. Adverse events included gastrointestinal (GI) nausea, diarrhea, and vomiting, as well as fatigue. One patient experienced prolonged Grade 3 thrombocytopenia at the highest dose level. Pharmacokinetic analyses showed evidence of conversion of PX-478 to melphalan consistent with its anticipated mechanism of HIF-1α inhibition.

EZN-2968, a locked nucleic acid antisense oligonucleotide inhibitor of HIF-1α that causes downregulation of HIF-1α mRNA and protein, was studied as a 2-h iv infusion in a pilot trial in patients with refractory solid tumors (NCT00466583) (49). The purpose of the study was to evaluate modulation of HIF-1 mRNA and protein levels as well as antitumor response. Of 10 patients enrolled, one patient with duodenal neuroendocrine tumor had prolonged stabilization of disease (24 weeks). Four of six patients with paired tumor biopsies showed reduction in HIF-1 mRNA levels compared to baseline, while reduction in HIF-1 protein and mRNA levels of some target genes was observed in two patients. The trial provided preliminary proof-of-concept for modulation of HIF-1α mRNA and protein expression. EZN-2968 was acquired by Santaris and subsequently by ROCHE (RO7070179) and is now being evaluated in a Phase Ib proof-of-mechanism study in adults with hepatocellular carcinoma (HCC) (NCT02564614). The primary outcome of this study that began enrolling patients in November 2015 is a change from baseline of HIF-1α mRNA levels in tumor tissue.

BAY 87-2243, a small molecule that inhibits HIF-1α and HIF-2α protein accumulation under hypoxic conditions *in vitro,* inhibits mitochondrial complex I activity but has no effect on complex III activity. Under conditions of glucose depletion that favor mitochondrial ATP generation in cancer cells as energy source, BAY 87-2243 inhibited cell proliferation in the nanomolar range (27). BAY 87-2243 interference with mitochondrial function resulted in reduced hypoxia-induced HIF-1 activity in tumors and prompted exploration of its use with radiation as a therapeutic approach to overcome chemo- and radiotherapy resistance of hypoxic tumors (45). It was found that BAY 87-2243 markedly decreased nuclear HIF-1α expression and pimonidazole hypoxic fraction (a measure of tumor hypoxia); however, its application before, after, or during RT did not improve local tumor control demonstrating that its radiosensitizing effect depends on treatment schedule. An open-label, Phase I study of BAY 87-2243 given orally once daily in subjects with advanced malignancies treated five subjects but was prematurely discontinued due to adverse GI events and it was determined that BAY 87-2243 was not tolerated at the doses used.

Topotecan. Topotecan (TPT), an FDA-approved camptothecin analogue that poisons topoisomerase I generating double-strand DNA breaks currently used as second-line therapy for patients with small-cell lung cancer or ovarian cancer, was found in a high-throughput screen using a cell-based assay for HIF-1 transcriptional activity (69, 84). It was found to block HIF-1α translation by a Top1-dependent but DNA damage-independent mechanism (85, 86). In xenograft models, it was shown that administration of daily TPT in combination with the anti-VEGF antibody bevacizumab exerted a synergistic antitumor activity, providing a rationale for clinical development of this combination strategy (83). A pilot study examined daily oral TPT administered to patients with advanced solid tumors expressing HIF-1α in at least 10% of tumor cells (26, 56) (NCT00182676). In 4 of 15 patients, HIF-1α nuclear staining became undetectable after treatment and decreased levels of VEGF and glucose transporter-1 mRNA were seen in three patients. Decreased tumor blood flow and permeability were observed by dynamic contrast magnetic resonance imaging (DCE-MRI) in seven of ten patients after one cycle. One patient had a partial response accompanied by inhibition of HIF-1α in tumor and reduction in tumor blood flow on DCE-MRI. While trends in both HIF-1α modulation and radiologic correlates were seen, these were not statistically significant due to low patient numbers.

EZN-2208, a PEGylated form of the small-molecule topoisomerase 1 inhibitor SN38 (the active metabolite irinotecan; CPT-11), with improved pharmacokinetics showed good antitumor activity in preclinical models of solid tumors and lymphomas, including CPT-11-resistant tumors (73). Its antitumor activity is possibly explained by the ability of this agent to inhibit HIF-1α accumulation (93). EZN-2208 was evaluated in a Phase I clinical trial and found to have an acceptable safety profile as a 1-h infusion (74) with stable disease as the best response (NCT00520390). A Phase II trial in metastatic breast cancer demonstrated activity in patients with triple negative breast cancer and in platinum-resistant patients (70) (NCT00520390). EZN-2208 was also evaluated in combination with bevacizumab in solid tumors (NCT01251926) (50) as well as with cetuximab in metastatic colorectal cancer (NCT00931840) where it was found to be well tolerated but provided no survival advantage (34). Enzon is no longer developing any clinical products.

Nuclear factor kappa B inhibitors

Signaling by the nuclear factor kappa B (NF-kB) pathway controls the expression of many genes involved in critical physiological responses, including oxidative stress responses and apoptosis (72). NF-κB signaling can be targeted at multiple points, for example, IKK activation, IκB degradation, and NF-κB DNA binding. Most development efforts have focused on inhibitors of IKKβ, to block the phosphorylation of IκBα, preventing its degradation and maintaining NF-κB in an inactive state in the cytoplasm. In 2006, the list of NF-κB inhibitors of this pathway totaled 785 although as yet no specific NF-kB inhibitors have reached clinical studies (37). Indirect inhibitors include the proteasome inhibitor, bortezomib, and the promiscuous curcumin (below). Bortezomib (VELCADE, previously known as PS-341), a proteasome inhibitor, was one of the first compounds used to inhibit the function of NF-κB (90). Bortezomib has shown significant efficacy in hematologic and solid tumors, including multiple myeloma as well as lung, breast, prostate, pancreatic, and head-neck carcinomas, yet it is unclear if its effect are in fact mediated through the inhibition of NF-kB (47). Bortezomib in combination with temozolomide in a Phase I/II study in advance refractory solid tumors or melanoma was terminated due to lack of efficacy (NCT00512798). Other histone deacetylase inhibitors, vorinostat and romidepsin, which block the posttranslational acetylation of NF-kB to regulate its activity, are approved for treating T-cell lymphoma.

ROS generators

Fenretinide. Fenretinide (N-4-hydroxyphenyl-retinamide or 4-HPR), a semisynthetic retinoid, was initially developed as a low-dose chemopreventative agent (18). Apoptosis by fenretinide has been linked to generation of ROS. It has been shown to bind to and activate retinoic acid receptors resulting in the induction of cell differentiation and apoptosis (16). The mechanism of its induced apoptosis has been hypothesized to operate through coenzyme Q of the mitochondrial respiratory chain (101, 113). Fenretinide has also been reported to cause the *de novo* synthesis of ceramides associated with increase of ROS resulting in cell death through apoptosis and/or necrosis (39). A study of 14 women with breast cancer, where fenretinide was administered before scheduled biopsy, lumpectomy, or mastectomy, found that fenretinide and its major metabolite accumulated preferentially in fatty tissue of the breast *versus* plasma (4, 101a). The data supported its study in premenopausal/ER-negative breast cancer prevention (NCT00001378) (32). A Phase III clinical trial of fenretinide suggested that it reduces breast cancer relapse in premenopausal women and provided a significant risk reduction of second breast cancer (NCT00002646) (109). Fenretinide has also been evaluated in a number of other cancer indications and is currently being evaluated in a trial in combination with ketoconazole in recurrent ovarian cancer (NCT01535157) and in Phase I safety trial children with recurrent/resistant neuroblastoma (NCT00646230) or ALL, AML, and NHL (NCT01187810).

Dietary antioxidants

Tocopherol. Nutrition supplements and nutraceuticals with vague claims of health benefit are widely used by the general public, probably more so than antioxidants proposed to reduce levels of harmful oxidation product. Only a few have undergone rigorous clinical evaluation. Because of its ability to neutralize free radicals, the fat soluble antioxidant vitamin E has been suggested to possess anticancer activity by protecting cells against oxidative damage; yet, clinical support has not been forthcoming (99). One component of vitamin E, α-tocopherol, has been evaluated in a number of clinical settings to determine its ability to reduce oxidative stress relating to cancer (35). Through the process of neutralizing a free radical, α-tocopherol is oxidized, its antioxidant capacity is lost, and the presence of other antioxidants, such as vitamin C, is required to regenerate the antioxidant capacity of α-tocopherol (106). The consumption of 400 mg per day α-tocopherol and 400 mg ascorbate has been shown to dramatically reduce the formation of lipid-soluble fecal mutagens (24). A double-blind placebo-controlled clinical cancer prevention trial of high-dose α-tocopherol and beta-carotene for lung cancer prevention in smokers found no benefit (43, 103), but a secondary finding was a 32% reduction in the incidence of prostate cancer in men given daily supplements of synthetic α-tocopherol (46). This finding was further evaluated in a subsequent Phase III randomized controlled clinical trial funded primarily by NCI to determine if selenium and vitamin E (dl-alpha tocopheryl acetate) taken as dietary supplements alone or together could help prevent prostate cancer (NCT00006392) (44). Initially planned for a minimum of 7 years, the trial was terminated early because at a 3-year time point, more cases of prostate cancer in men taking only vitamin E were appearing. By the planned end of the study, the incidence of prostate cancer in this group was found to be significantly higher at 17% compared to placebo (55).

Vitamin E has also been tested for its ability to lessen the harmful effects of medical treatments such as dialysis and radiation and to reduce unwanted side effects of drugs such as hair loss with doxorubicin and the lung damage with amiodarone (99). A number of randomized placebo-controlled studies in adults and children found that topical vitamin E might reduce oral mucositis induced by chemotherapy or radiotherapy (NCT00311116). In addition, the incidence and severity of peripheral neurotoxicity induced by taxanes or platinum-based chemotherapy by alpha-tocopherol were found reduced in five randomized trials, although this finding was not confirmed in two subsequent randomized placebo-controlled studies (35). Hence, despite several large prospective cohort studies, each have failed to find significant associations between vitamin E intake and the risk of cancer (55) or clinical benefit; yet, studies to evaluate its ability to reduce toxicities are still continuing (NCT02397486).

Vitamin C. High-dose vitamin C given by oral and IV routs has been studied as a treatment for patients with cancer since the 1970s. However, carefully controlled clinical trials in the late 1970s failed to show that oral vitamin C had any therapeutic benefit against cancer (19). More recently, interest has revived in the use of IV vitamin C that can reach peak plasma concentrations greater that 25-fold those of oral doses, with the suggestion that vitamin C may act as a pro-drug for hydrogen peroxide delivery to tumors, without the presence of hydrogen peroxide in the blood (71). A recent systematic review of the literature found no credible evidence for an antitumor effect of vitamin C in combination with chemotherapy in however form of administration (48).

Curcumin. Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], the spice turmeric derived from the rhizome of *Curcuma longa L*, has both antioxidant and anti-inflammatory properties (66) by being able to scavenge reactive oxygen and nitrogen species *in vitro* (97, 98). It also has numerous other reported activities, including the ability to modulate signaling molecules such as proinflammatory cytokines, apoptotic proteins, NF-κB, cyclooxygenase-2, 5-LOX, STAT3, C-reactive protein, and prostaglandin E(2), among others. Curcumin has undergone clinical trials in patients with colorectal, pancreatic, breast, prostate, and lung cancer, and multiple myeloma [see review by Gupta *et al.* (40)]. Most studies involved relatively small numbers of patients and limited tumor shrinkages were seen although some biomarkers changed and some patient symptoms improved. Given the pleiotropic effects of curcumin, it is difficult to ascribe any of its effects in cancer to its redox activity (92).

Conclusions

This review covers 21 redox modifying agents that have been tested in clinical trials for anticancer activity. What are the lessons that can be learned from these agents? Three of these were natural products (α-tocopherol, vitamin C, and curcumin), widely expected to be clinically active based on epidemiological and anecdotal evidence, yet none showed convincing antitumor activity. Three other agents are FDA approved, but only one (ATO) for a mechanism that involves potential redox activity. Many agents are no longer being developed, or their development status is unknown. It is noteworthy that very few of the agents were tested specifically in patients known to express the redox drug target, who might be expected to respond.

The approval number for redox agents is about what would be expected for chemotherapy drugs that enter cancer clinical trials (around 1:20), but much less than for molecularly targeted drugs (1:5). Most of the redox agents were developed against specific targets but were not tested clinically in this way. There are four agents still in clinical trials so that the number approved could be higher, but not significantly so. The reason for the disappointing success rate for redox anticancer drugs probably also relates to the complexity and our incomplete knowledge of the role of redox mechanisms in cancer, and the difficulty of identifying good molecular targets that are critical for tumor growth and progression. There are also the practical difficulties of making redox-active agents that can survive the journey to the tumor through the homeostatically redox-regulated body environment. However, recent advance in chemistry for designing irreversibly acting drugs that often have fine-tuned redox reactive groups may help improve the success of developing these agents in the future.

It is also increasingly clear that redox signaling is a critical process for normal cells and only when redox signaling is deranged becomes important for cancer cells. Drugs often fail because of unanticipated toxic side effects on normal tissues and this appears to be the case for many of the redox drugs tested so far. With increasing knowledge of the role of normal redox signaling, and not just pathological processes where redox defenses are overwhelmed, which has been the major focus until now, it can be hoped that untoward effects on normal tissues will become less of an issue for new generations of redox drugs. This is also the need for more rigorous validation of future redox target so that we can be sure that they are molecularly and clinically relevant to cancer. With this in mind, our improving knowledge of physiological redox mechanisms and the application of advances being made in biomarker development for patient selection for personalized cancer medicine, it can be hoped that the next generation of redox cancer drugs will show more target selectivity, less off target effects, and overall, increased success.

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Abbreviations Used

ATO = arsenic trioxide
BSO = buthionine sulfoximine
DCE-MRI = dynamic contrast magnetic resonance imaging
FDA = Food and Drug Administration
GGT = gamma-glutamyl-transpeptidase
GI = gastrointestinal
GRX = glutaredoxin
GSH = glutathione
GSSG = oxidized glutathione
GST = glutathione S-transferase
IV = intravenous
MDS = myelodisplastic syndrome
MGd = motexafin gadolinium
NAC = N-acetylcysteine
NF-kB = nuclear factor kappa B
NO = nitric oxide
ROS = reactive oxygen species
SOD = superoxide dismutase
TPT = topotecan
Trx = thioredoxin
TrxR = thioredoxin reductase
VEGF = vascular endothelial growth factor