Oxidative stress and apoptosis: a new treatment paradigm in cancer

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1. ABSTRACT

Redox regulation has been shown to be an important component of malignant cell survival. Tipping the cellular redox balance through pharmacologic regulation in favor of increasing intracellular reactive oxygen species (ROS) and/or depleting protective reducing metabolites (such as glutathione and nicotinamide adenine dinucleotide phosphate) may lead to oxidative stress and resultant induction of apoptosis for the treatment of cancer. We review the biology and importance of ROS with regard to malignant and normal cells. Moreover, we discuss preclinical and clinical data regarding novel therapeutic agents that modulate the cellular redox system including buthionine sulfoximine, ascorbic acid, arsenic trioxide, imexon, and motexafin gadolinium as single-agents and in combination. Continued research is needed to better understand the mechanisms and specific apoptotic pathways involved in ROS-induced cell death, as well as, to determine the most rationale and effective combination of redox-active agents.

2. INTRODUCTION

Redox regulation and oxidative stress has been shown to be an important component of malignant cell survival. We will discuss the biology and importance of reactive oxygen species (ROS) with regard to malignant cell survival, as well as discuss specific pharmacologic agents that are able to modulate the cellular redox system such as buthionine sulfoximine (BSO), ascorbic acid, arsenic trioxide (As$_2$O$_3$), imexon, and motexafin gadolinium (MGd). The mechanism of these agents is in part through depletion of reducing metabolites such as glutathione (GSH) with formation of ROS with resultant lowering of the apoptotic threshold for cell cytotoxicity. We will review the various preclinical and clinical studies of these redox-active agents and discuss their potential role in the treatment of cancer.

2.1. The Biology

Mitochondria are critical to cell survival and are involved in various cell growth pathways (1,2). Mitochondria play an important role as one of the primary mediators of programmed cell death (apoptosis) in solid and hematologic malignancies (1-5). Mitochondria are organelles that function to create energy in the form of adenosine triphosphate (ATP) via cellular respiration, but also serve as the site of detoxification of
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Figure 1. Proposed mechanism of ROS-induced apoptosis. This schematic depicts the varied caspase pathways involved in apoptosis. The intrinsic or mitochondrial-mediating pathway involves loss of mitochondrial membrane potential and cytochrome c release leading to activation of caspase 9 followed by downstream effector caspase 3 activation and resultant cell death. The extrinsic pathway involves stimulation of pathways such as Fas (CD95) leading to activation of upstream caspase 8 with resultant stimulation of effector cascades. Reactive oxygen species (ROS) may act as an extracellular intermediate directly stimulating the mitochondria and/or Fas cell death pathways.

any endogenous or exogenous toxins (6). ATP may be created either inside the mitochondria through aerobic respiration or anaerobic respiration through glycolysis. Inside the mitochondria, ATP is created by oxidative phosphorylation of adenosine diphosphate through the electron transport system from the reduction of oxygen. The electron transport system is highly dependent on the maintenance of a membrane potential; loss of the potential leads to cellular death. Through oxidative phosphorylation, large amounts of oxygen are consumed and byproducts such as hydrogen peroxide ($\text{H}_2\text{O}_2$) and superoxide anion radicals ($\text{O}_2^-$) are formed. These intermediate products are called ROS and are toxic to the cell.

2.2. Reactive Oxygen Species (ROS) in Oncology

ROS are free radicals and other molecules with unpaired electrons (such as $\text{O}_2^-$ and $\text{H}_2\text{O}_2$) that are highly reactive and can react with biologic macromolecules, modify the structure and function of proteins, and cause oxidative damage to DNA (7). ROS damage cellular DNA through oxidative stress-induced destruction of pyrimidine and purine bases and single strand breaks and oxidation of protein thiols and lipids (8-10). ROS have been demonstrated to act directly on the mitochondria to trigger the initiation of apoptosis (11,12). This cell death process may be initiated through ROS acting directly on the mitochondria and/or through activation of cell surface death receptors to initiate a pro-apoptotic signal (see Figure 1). ROS has also been shown to be important for VEGF signaling in vitro and angiogenesis in vivo (13).

It has been known for over 40 years that an essential mechanism of action of varied chemotherapeutic agents is the generation of ROS (14-17). In the early 1960’s, Berenice and colleagues reported that procarbazine is oxidized in solution to form ROS such as $\text{H}_2\text{O}_2$ with resultant damage to DNA (14), and they showed that procarbazine is synergistic with radiation
Figure 2. Glutathione and Thioredoxin Systems. Reducing metabolites such as nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione are able to donate electrons to detoxify reactive oxygen species (ROS) such as hydrogen peroxide (H₂O₂) to oxygen (O₂) and water. Thioredoxin is able to maintain redox balance among various cellular proteins such as ribonucleotide reductase.

2.3. Cellular Defense of ROS

Important components of cellular response to oxidative stress include reducing metabolites such as the GSH, nicotinamide adenine dinucleotide phosphate (NADPH), and redox regulatory proteins such as thioredoxin reductase and thioredoxin (See Figure 2) (21). GSH is a nonprotein cellular thiol that is responsible for many cellular functions including the protection of cells from toxic oxidant damage (including radiation) (22,23). GSH production is through the gamma-glutamyl cycle and is dependent on several key enzymes (24). GSH has great reducing power, with the ability to donate electrons to free radicals thereby acting as antioxidant. Thioredoxin in its reduced form is largely responsible for the maintenance of proper redox balance among many cellular proteins including ribonucleotide reductase, an enzyme essential for DNA synthesis (8,25). If an imbalance exists between the formation of free radicals and radical-scavenging systems in excess of the former, a condition known as oxidative stress results. Tumor mitochondria are known to contain large amounts of GSH (26). Modulation of the GSH system has been well studied in vitro. Depletion of GSH has been demonstrated to sensitize tumor cells to oxidative cytolysis (26-28). Tipping the cellular redox balance through pharmacologic manipulation in favor of increasing intracellular ROS and/or depleting protective reducing metabolites (such as GSH) may lead to oxidative stress and subsequent induction of apoptosis within cancer cells.

3. PRE-CLINICAL DATA

3.1. Buthionine sulfoximine (BSO)

An agent that had been used for many years to modulate the GSH system through depletion of intracellular levels of GSH is BSO, a selective inhibitor of gamma glutamylcysteine synthetase (29,30). An early study examined the effect of BSO alone on human myeloma cells in vitro where sulfhydryl reducing metabolites were depleted with associated cytotoxicity at drug concentrations achievable in patients (31). Gartenhaus and colleagues demonstrated that growth inhibition of chemotherapy and steroid-resistant multiple myeloma cell lines by As₂O₃ were significantly potentiated by BSO indicating that these resistant cell lines can be converted to a sensitive phenotype by manipulation of the cellular redox state (32). They also documented that As₂O₃ and BSO-induced cytotoxicity was in part due to induction of apoptosis with activation of caspases 3, 8 and 9. It is not surprising that the
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caspase system, which is associated with redox-regulated apoptosis mechanisms, is activated following As₂O₃ exposure and GSH depletion in these cell lines. A recent report has shown that caspase 8 is activated in the NB4 cell line in a GSH concentration dependent-manner after exposure to As₂O₃ (33). Furthermore, reduction of the GSH level by BSO pretreatment prior to As₂O₃ converted a resistant subline, NB4/As, to a sensitive phenotype. Interestingly, in that study, caspase 8 activation appeared to be independent of Fas ligand-receptor interaction. The role that Fas-signaling plays in As₂O₃-mediated killing of multiple myeloma cells is unknown and is an active area of research. BSO used concurrently with cis-dichlorodiammineplatinum (CDDP) decreased intracellular GSH in KU7 bladder cancer cells and improved sensitivity to CDDP (34). A similar study showed that cisplatin-refractory MCF-7 breast cancer cells were converted to a sensitive phenotype if pretreated with BSO (35). Finally, in vivo studies showed that reduced GSH levels were associated with increased sensitivity to alkylating agents in BSO-treated MCF-7 breast cancer cells (36).

3.2. Ascorbic acid (AA)

AA, known for its antioxidant activity, also acts as a prooxidant (37). The cycle of prooxidant activity starts in the plasma as AA is initially oxidized to monohydroascorbic acid and then to dehydroascorbic acid. Once inside the cell, dehydroascorbic acid is reduced through glutaredoxin and transformed back to AA. However, during this process, GSH is converted into GSH disulfide, depleting intracellular stores of GSH and increasing ROS. AA has been shown to synergize with As₂O₃ for effective growth-inhibition and apoptosis (37,39). Grad and colleagues showed that ascorbic acid potentiated the cytotoxic effect of As₂O₃ in multiple myeloma cell lines (40). They also demonstrated that ascorbic acid suppressed GSH, increased ROS and enhanced apoptotic changes.

3.3. Arsenic trioxide

Arsenic is a naturally occurring substance that has been used as a medicinal agent for more than 2,400 years (41). More recently, As₂O₃ has been demonstrated to be an effective therapeutic agent for the treatment of acute promyelocytic leukemia (APL) and As₂O₃ has provided clinical responses in heavily pretreated MM patients (42-44). Studies have demonstrated GSH content to be closely associated to the effectiveness of As₂O₃ cytotoxicity (45,46). As₂O₃ causes depletion of GSH through the conjugation of GSH and exportation of GSH out of the cell through multi-drug resistance efflux pumps (37). As₂O₃ can lead to membrane potential changes and increased membrane permeability with resultant degradation phase of apoptosis. The ability of As₂O₃ to induce apoptosis is dependent in part on the generation of ROS (47). ROS is generated by As₂O₃ through inhibition of glutathione transferase, an enzyme used to detoxify As₂O₃, as well as inhibition of glutathione peroxidase, an enzyme responsible for the conversion of H₂O₂ to water (48). The GSH content is closely associated to the effectiveness of As₂O₃ as malignant cells with lower GSH levels are often more sensitive to As₂O₃ cytotoxicity (45,46). Clearly, the ability to diminish GSH levels prior to exposure with As₂O₃ should improve its therapeutic effect.

Induction of apoptosis has been demonstrated to be an integral component of As₂O₃ cytotoxicity in several preclinical multiple myeloma studies (50,51). Park and colleagues demonstrated arsenic-induced G1 and/or G2M phase arrest in myeloma cell lines (52). There was simultaneous induction of cyclin-dependent kinase inhibitor, p21. There is also evidence of an immune mechanism with As₂O₃ in myeloma cells with elevated lymphokine activated cells and other immune cells (53). In APL studies, similar apoptotic mechanisms have been documented. As₂O₃ induced a differential effect that was shown to be dose dependent in APL: preferentially induced partial differentiation at low concentrations (0.1 to 0.5 micromol/L) and induced apoptosis at relatively high concentrations (0.5 to 2.0 micromol/L) (49,50). The effective in vivo therapeutic dose studied thus far of As₂O₃ for multiple myeloma and APL is 1 to 2 micromol/L (44,54). In leukemia cell lines, there is also evidence that As₂O₃-induced Bcl-2 family mitochondrial apoptosis (increased Bax activation) is dependent on the intracellular production of ROS (55,56).

3.4. Imexon

Imexon (4-imino-1,3-diazabicyclo-[3.1.0] hexan-one) is a 2-cyanoaziridine that has been shown to deplete cellular stores of GSH with resultant increase in oxidative stress. Imexon has been studied in hematologic and solid cancers and it has demonstrated antineoplastic activity as a single agent (4,57). Imexon has also previously been shown to be an immune stimulant (58,59). Hersh and colleagues documented that imexon was active in several tumor cell lines, including multiple myeloma (60). Dvorakova and colleagues have demonstrated that imexon can alkylate cellular thiols by binding to sulfhydryl groups (57). Imexon depleted cellular stores of cysteine and GSH by forming a conjugate, and apoptosis was induced with enhancement of cellular oxidative stress (57,61,62). They subsequently established that with N-acetyl-L-cysteine (NAC) pretreatment to prevent depletion of thiols, myeloma cell lines were protected from the effects of imexon. Studies have shown that a key event in the effector phase of apoptosis is the progressive permeabilization of the mitochondrial membrane secondary to the action of permeability transition pore complex (63,64). Dvorakova and colleagues have subsequently demonstrated in myeloma cell lines that sensitivity to imexon correlated with mitochondrial changes such as: loss of mitochondrial membrane potential, mitochondrial enlargement/swelling, and release of cytochrome c from the mitochondria into the cytosol (4). They also documented that treatment with imexon was associated with the generation of ROS. Dorr and colleagues collected imexon pharmacokinetic data with animal studies showing a short half-life and that 20% of an oral dose was absorbed (65).

We studied imexon in dexamethasone-sensitive and resistant and chemotherapy-sensitive and resistant myeloma cell lines and documented significant cytotoxicity following incubation with modest imexon concentrations in a time- and dose-dependent manner.
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Figure 3. Imexon dose-dependent cytotoxicity in dexamethasone- and chemotherapy-sensitive and -resistant myeloma cell lines with minimal effect on normal lymphocytes. Cell viability was measured as a percentage of cells alive after 48-hour exposure with increasing concentrations of imexon in the dexamethasone-sensitive cell line C2E3, dexamethasone-resistant line 1-310, and highly dexamethasone-resistant cell line 1-414 (A); the chemotherapy-sensitive cell line RPMI-8226, chemotherapy-resistant line DOX-1V, and highly chemotherapy-resistant line DOX-10V (B); and in normal human lymphocytes and lymphocytes after 48-hour phytohemagglutinin (PHA) stimulation (C). Results shown (means of the SD) were averaged from three or more independent experiments done in triplicate for each time point (P < 0.01 all cell lines for imexon cytotoxicity compared with control cells). Reproduced with permission from American Association for Cancer Research, Inc. (67).

We showed that the cytotoxicity of imexon in these cell lines was related to induction of apoptosis, which appeared to be regulated in part by alteration of bcl-2:bax (decreased ratio) and activation of caspase-3. However, at the concentrations of imexon we studied, the cytotoxicity was not explained by an increased prooxidant state.

3.5. Motexafin gadolinium (MGd)

MGd is a member of a class of rationally designed porphyrin-like molecules called texaphyrins. The mechanism of action of MGd is related in part to its electron affinity, as it is easily reduced (70,71). MGd is redox active as it catalyzes the oxidation of critical protein thiols and several intracellular reducing metabolites such as GSH, AA and NADPH (See Figure 4) (71-73). Through a process known as futile redox cycling, MGd transfers electrons directly to molecular oxygen (O₂) to produce ROS. The combination of protein and metabolite oxidation and generation of ROS are inducers of apoptosis that alter the threshold for cytotoxicity of many commonly used chemotherapy agents. Nuclear magnetic resonance measurements have shown alterations in high-energy phosphates on subcutaneously implanted MCa tumors (mouse mammary carcinoma), consistent with disruption in tumor cell metabolism (72). Furthermore, using gene expression profiling, various stress related genes are upregulated in response to MGd including genes encoding metallothioneins, heat shock proteins and heme oxygenase (personal communication, Dr. Richard A. Miller, Pharmacyclics, Inc.).

Using fluorescence microscopy, MGd has been shown to localize in tumor cell lysosomes, endoplasmic reticulum and mitochondria (74). These findings suggest that MGd alters cancer cell responses to ionizing radiation and to cytotoxic agents by disrupting their metabolic state. Murine studies using radiolabeled MGd injected into tumor-bearing animals showed rapid clearance of the drug from blood and normal tissues and delayed clearance from tumors, resulting in up to 8-fold greater concentrations in tumors than in surrounding tissues (75). Magnetic resonance imaging (MRI) studies of EMT-6 tumor-bearing BALB/c mice demonstrated selective accumulation of MGd in tumors (76). In vitro studies in single- and multi-fraction experiments combining MGd and radiation in a variety of tumor models demonstrate a dose-dependent improvement in survival of tumor-bearing animals (72). Mice treated with MGd combined with radiation showed improved survival compared to mice treated with radiation alone. MGd has also been shown to be an effective modulator of tumor oxygen tension, which has important implications for radiation enhancement. Tumor oxygenation in EMT6 mouse mammary tumors in Balb/c Rw mice was shifted toward higher oxygen tensions 6 to 8 hours after MGd, thereby reducing the percentage of severely hypoxic readings (77).

In pre-clinical models, the combination of MGd and chemotherapy agents was more effective against tumor cells than chemotherapeutic agents alone (78). In vitro studies of MGd with bleomycin demonstrated increased MGd dose-dependent cytotoxicity to both MES-SA and Rif-1 cells. In vivo studies of the combined treatment of MGd with doxorubicin or bleomycin showed significant delay in tumor growth compared with control animals treated with either chemotherapy alone. The combination of carboplatin and MGd showed a delay in tumor re-growth in Lewis lung carcinoma-implanted mice compared with mice receiving carboplatin alone (79). An in vivo study performed on A549 human lung cancer showed that MGd enhanced the antitumor activity of the combination.
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Figure 4. Motexafin Gadolinium Catalyzes Oxidation of Key Reducing Metabolites and Thiols. Motexafin gadolinium (MGd) catalyzes the oxidation of proteins and reducing metabolites such as glutathione, ascorbate and NADPH. MGd transfers electrons directly to oxygen to produce reactive oxygen species (ROS) such as hydrogen peroxide. The oxidation of proteins and reducing metabolites with generation of ROS leads to cytotoxicity often occurring through induction of apoptosis.

4. CLINICAL STUDIES

4.1. Buthionine sulfoximine and Melphalan

A phase 1 trial by O’Dwyer and colleagues combined BSO with melphalan (82). They compared pre- to post-treatment GSH levels in peripheral mononuclear cells (PMN), and from tumor biopsies. The mean GSH nadir levels in PMN’s were depleted to 10% of control values, while GSH levels from tumor biopsies were more variable, but decreased. It was a well-tolerated regimen with the main toxicity being grade 1/2 nausea or vomiting occurring in 50% of patients, another phase I study by Bailey and colleagues to evaluate GSH depletion and toxicity (83). They concluded the combination was safe with toxicity consisting of infrequent myelosuppression, and as observed by O’Dwyer, grade 1/2 nausea and vomiting. Furthermore, intracellular GSH levels in PMN’S were reduced to 40% of pretreatment levels with BSO therapy.

4.2. Imexon

Imexon was first studied in the 1970’s and documented to be a safe agent (67,68). It was shown to have a short half-life and poor oral bioavailability, but that clinically achievable and effective intravenous in vivo dosing were feasible (65). Single-agent antitumor activity (solid tumors) and safety of imexon in MM and other tumor types was confirmed in subsequent preclinical and phase I/II studies (68,69). Dorr and colleagues have also recently documented the maximum tolerated dose (MTD) of imexon through a phase I study (84). They found that imexon is non-myelosuppressive and safe. Further clinical trials of imexon in cancer are ongoing.

4.3. Arsenic trioxide in Acute Promyelocytic Leukemia

Acute promyelocytic leukemia (APL) has a specific chromosomal translocation in almost all cases, t(15;17), that produces a hybrid protein, promyelocytic leukaemia-retinoic acid receptor α (PML-RARα) (85-87,95). All-trans retinoic acid (ATRA) and anthracycline therapy with or without cytarabine produces remission rates of 70-95% (85). ATRA and chemotherapy has increased the survival rate when compared to chemotherapy alone (86). However, relapse occurs in nearly 15 to 20% of APL patients with ATRA-based therapy, therefore, other effective therapeutic agents are needed. As₂O₃ has a tolerable risk profile and is effective as a single-agent in relapsed patients with APL (43,85).

Soignet and colleagues confirmed previous studies in the treatment of relapsed APL with As₂O₃ (86). In 12 heavily treated APL patients, they showed As₂O₃ induced complete remission in 11 of 12 patients; one died early from an intracranial hemorrhage. Eight of 11 patients achieved negative molecular status (based on RT-PCR studies). The United States Multicenter Trial was a 40-patient trial of relapsed APL treated with single-agent As₂O₃ (87). As₂O₃ was used for induction and patients who obtained a complete remission could receive As₂O₃ consolidation and maintenance treatment. Complete remission was achieved in 34 of 40 patients (85%) and disappearance of t(15;17) was seen in all who obtained complete remission. Moreover, 10 of 21 patients who were treated with As₂O₃ alone (i.e., no autologous or allogeneic stem cell transplant) were alive at publication. In general, As₂O₃ has been a well-tolerated agent, although side effects such as cardiac toxicity (ventricular arrhythmias) and leukocytosis with retinoic acid differentiation syndrome occur (41,54,86-
96). Vigilant management of electrolytes, especially magnesium and potassium, is mandatory.

The combination of As$_2$O$_3$ with ATRA has been used in the treatment of newly diagnosed APL. Shen and colleagues compared the combination of ATRA with As$_2$O$_3$ or separately for the induction and maintenance of 61 newly diagnosed APL patients (97). ATRA and As$_2$O$_3$ combination therapy showed superiority in shortening the time for complete remission without increasing toxicity. Moreover, the disease free survival was significantly prolonged in the combination group. The most common toxicity was grade 1 liver dysfunction, and all liver abnormalities recovered 1 to 2 weeks following treatment. Studies incorporating As$_2$O$_3$ earlier into the treatment plan for APL (induction and/or consolidation) in combination with ATRA and chemotherapy are ongoing (Eastern Cooperative Oncology Group C9710).

4.4. Arsenic trioxide in multiple myeloma

Munshi and colleagues evaluated As$_2$O$_3$ in a phase II trial of 14 heavily treated patients with relapsed multiple myeloma (98). Responses were seen in three patients and another patient had stable disease over six months. This study showed activity in this population of patients, although significant cytopenias were experienced. The myelosuppression may have been related to the heavy pretreatment of these patients. The main side effect profile related to As$_2$O$_3$ therapy has come from recent clinical trials in APL as this arsenic-treated patient population has been studied extensively as mentioned above (41,42,54,85). Another phase II study of 24 multiple myeloma patients (8 studied extensively as mentioned above (41,42,54,85). Another phase II study of 24 multiple myeloma patients (8 relapsed, 16 refractory to prior treatment) showed As$_2$O$_3$ was active and well tolerated (99). Objective responses were seen in 33% of patients, while another 25% of patients had stable disease. Similar to the prior study, neutropenia was common (67% of patients had grade 3 or grade 4 neutropenia). Unlike the As$_2$O$_3$-related cardiac toxicity in APL, QT prolongation was rare. Clinical experience is more limited with multiple myeloma and somewhat different toxicities such as less leukocytosis and more cytopenias have been reported (44,100). A recent report by Wang and colleagues suggested that these effects might be dose related (101). These early studies by Munshi and Hussein have demonstrated that As$_2$O$_3$ has clinical activity in poor prognosis relapsed/refractory multiple myeloma as well as apparent overall low toxicity (98,100). As discussed above, modulation of the cellular reduct system with agents that deplete GSH and/or increase ROS may be used in combination with As$_2$O$_3$ to increase efficacy.

4.5. Arsenic trioxide and Asorbic acid

AA may sensitize multiple myeloma cells to As$_2$O$_3$ through the depletion of GSH as discussed before. Bahlis and colleagues initiated a Phase I/II clinical trial of combination As$_2$O$_3$ and AA in relapsed/refractory multiple myeloma (37). They showed that AA did not significantly alter the pharmacokinetics of As$_2$O$_3$, but depleted intracellular levels of GSH. In this cohort of heavily treated patients, two of six patients achieved a partial response and four patients had stable disease. Three of the four patients were able to maintain a lower M protein level as long as they continued treatment. Other myeloma trials are combining As$_2$O$_3$ and AA with conventional chemotherapy agents (102).

4.6. Motexafin gadolinium

MGd is being developed as a broad-spectrum anti-cancer agent and is now in clinical trials as a single-agent and in combination regimens with chemotherapy and/or radiation therapy. The initial cancer clinical trials with MGd were in combination with external beam radiation. The safety profile of MGd in combination with radiation was investigated in a single-dose phase I clinical trial (103). Adults with incurable cancers of any histology requiring radiation therapy were eligible. A single intravenous MGd dose (0.6 to 29.6 mg/kg) was followed at least 2 hours later by external beam radiation therapy. The MTD was 22.3 mg/kg, as assessed by the dose limiting toxicity (DLT), reversible acute tubular necrosis, which occurred at 29.6 mg/kg. The median half-life of a single dose of MGd was 7.4 hours. As demonstrated by Barocas and colleagues, MGd selectively accumulated in primary and metastatic tumors, without increase in radiation toxicity or MGd uptake to normal brain tissue.

Carda and colleagues reported a phase Ib/II trial where they examined concurrent daily MGd therapy and whole-brain radiation therapy (WBRT) for a total of 10 daily infusions and fractions, respectively in patients with brain metastases (104). Thirty-five of the 61 patients enrolled had primary non-small cell lung cancer (NSCLC). The MTD in the phase Ib segment (39 patients at 10 dose levels) was 6.3 mg/kg with reversible grade 3 increase in liver function tests representing the DLT. The most frequent adverse events (16% grade 3 or 4) recognized were dose-dependent transient greenish discoloration of skin (56% of patients), urine (43%) and sclera (18%). The olive-green skin and other discoloration is due to the dark-green color of MGd. Discoloration develops gradually after repeated dosing and clears completely in 3 to 4 days following the last dose of MGd. Three patients in either the 5.5 mg/kg or 6.3 mg/kg cohort developed paresthesias at the fingertips followed by a vesicular rash around the fingernails consistent with pseudo-porphyrria. No hematologic toxicity was documented. In the phase II segment, 22 patients received 3 doses of 5.0 mg/kg, 5.5 mg/kg and 6.3 mg/kg with a response rate of 72%. There were no recognized differences in area under the curve or $C_{\text{max}}$ within this dose range between 5.0 mg/kg once daily and 6.3 mg/kg once daily. Plasma pharmacokinetics showed similar short half-life with <11% of the maximum observed plasma concentration at 24 hours.

A phase III international study for patients with brain metastases arising from solid tumors compared survival and neurologic progression in patients treated with WBRT alone (control) versus WBRT subsequent to MGd dosing (105-107). This study began with a 25-patient lead-in phase to confirm the safety of MGd and radiation therapy observed in the phase Ib/II trial (105). The randomized phase comprised 401 patients (251 with NSCLC, 75 breast cancer, and 75 other cancers). Control
patients received WBRT (10 fractions of 3 Gy), and patients in the MGd arm received WBRT with MGd (5.0 mg/kg) preceded 2 to 5 hours before each of the 10 fractions. No significant survival difference was seen, although in the NSCLC subgroup, a significant difference was observed in time to neurologic progression between the two study arms (control arm, median time to progression 7.4 months versus MGd arm, median exceed 1 year, p = 0.048 unadjusted). Moreover, lung cancer patients treated with MGd and WBRT (as compared to radiation alone) had improved memory and executive function (P = 0.062) and improved neurologic function as assessed by a blinded events review committee (P = 0.048) (107).

A phase III trial, known as the Study of neurologic progression with MGd and Radiation Therapy (SMART) trial, has been initiated in the US, Europe, Canada and Australia that will enroll 550 patients with brain metastases from NSCLC comparing WBRT versus WBRT plus MGd. Several ongoing phase I and II solid tumor trials are studying MGd as a single agent and in combination with radiation and/or chemotherapy.

MGd has recently begun to be studied in hematologic malignancy clinical trials. Lin and colleagues reported a pilot phase II trial which enrolled 13 patients with relapsed/refractory chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) (108). Patients had a median age of 66 years (range 54-80), median of 4 prior therapies (range 2-9), and 12 of 13 patients had fludarabine-refractory disease. MGd was administered 5mg/kg/day IV for 5 days every 3 weeks. Median white blood count (WBC) was 26.9 x 10^9/L (range 5.4-152.6 x 10^9/L), median platelet count was 95,000 x 10^9/L (range 33,000-214,000 x 10^9/L) with 7 patients < 100,000 x 10^9/L and 3 patients < 50,000 x 10^9/L. No grade 4 hematologic toxicity was seen. Evidence of tumor response was seen in three patients and included decrease in WBC, nodes and/or chemotherapy-refractory disease. MGd was administered prior therapies of 2.8, and all refractory to single-agent rituximab and/or chemotherapy-rituximab combination. No grade 3 or 4 non-hematologic toxicities or grade 4 hematologic toxicities have been seen. Overall response rate is 86% with responses according to histology: follicular 100% (4 complete remission, 1 partial remission), large-cell with partial remission, mantle-cell with progressive disease. Patient 1 had a local scalp relapse, but systemic disease remains in remission at 20 months. Follicular lymphoma patients 2 and 3 continue in remission at 12 and 11 months.

The first patient had a day 4 non-contrast MRI of a large scalp mass showing 30% increase in MRI signal intensity (compared to a pre-MGd scan) indicating lymphoma-selective uptake of MGd. MGd is now in numerous anti-cancer clinical trials for use as a single-agent and in combination with chemotherapy and/or radiation therapy, including multicenter clinical trials examining the efficacy and safety of single-agent MGd for the treatment of relapsed/refractory low-grade NHL, CLL, and multiple myeloma.

5. CONCLUSION

The cellular redox system is an important component of malignant cell response to cytotoxic treatment. Novel therapeutic agents exist to modulate the cellular redox environment within malignant cells in favor of oxidative stress and resultant apoptosis for the treatment of cancer. Pre-clinical study and clinical trials are ongoing examining redox-regulating therapies such as imexon, MGd and As2O3 alone and in combination with other redox-active agents/modalities such as AA, radiation and/or chemotherapy in solid tumor and hematologic malignancies. Furthermore, recent studies have shown that anti-cancer treatments such as rituximab antibody therapy (110), the proteasome inhibitor bortezomib (111,112), histone deacetylase inhibitors such as suberoylanilide hydroxamic acid (SAHA) (113) and the anti-leukemia agent, adaphostin (114) appear to work in part through ROS-related mechanisms. Further research is necessary to better understand the mechanisms of apoptosis and precise cell death pathways involved in ROS-related cytotoxicity, as well as, to determine the most rationale and effective combinations of redox-active therapies for the treatment of cancer.

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Abbreviations: ROS, reactive oxygen species; BSO, buthionine sulfoximine; As\textsubscript{2}O\textsubscript{3}, arsenic trioxide; MGd, motexafin gadolinium; GSH, glutathione; ATP, adenosine triphosphate; DNA, deoxyribonucleic acid; H\textsubscript{2}O\textsubscript{2}, hydrogen peroxide; O\textsubscript{2}\textsuperscript{-}, superoxide anion; NADPH, nicotinamide adenine dinucleotide phosphate; CDDP, cis-dichlorodiammineplatinum; AA, ascorbic acid; APL, acute promyelocytic leukemia; NAC, N-acetyl-L-cysteine; O\textsubscript{2}, molecular oxygen; MCa, mouse mammary carcinoma; MRI, magnetic resonance imaging; PMN, peripheral mononuclear cells; MTD, maximum tolerated dose; ATRA, all-trans retinoic acid; DLT, dose limiting toxicity; WBRT, whole brain radiation therapy; NSCLC, non-small cell lung cancer; CLL, chronic lymphocytic leukemia; SLL, small lymphocytic leukemia; WBC, white blood cells; NCI, National Cancer Institute; NHL, non-hodgkin’s lymphoma.

Key Words: Oxidative Stress, Reactive Oxygen Species, Apoptosis, Glutathione, Cancer, Review

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