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# 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 pre-clinical 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 discussthe 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 ($As_2O_3$), 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

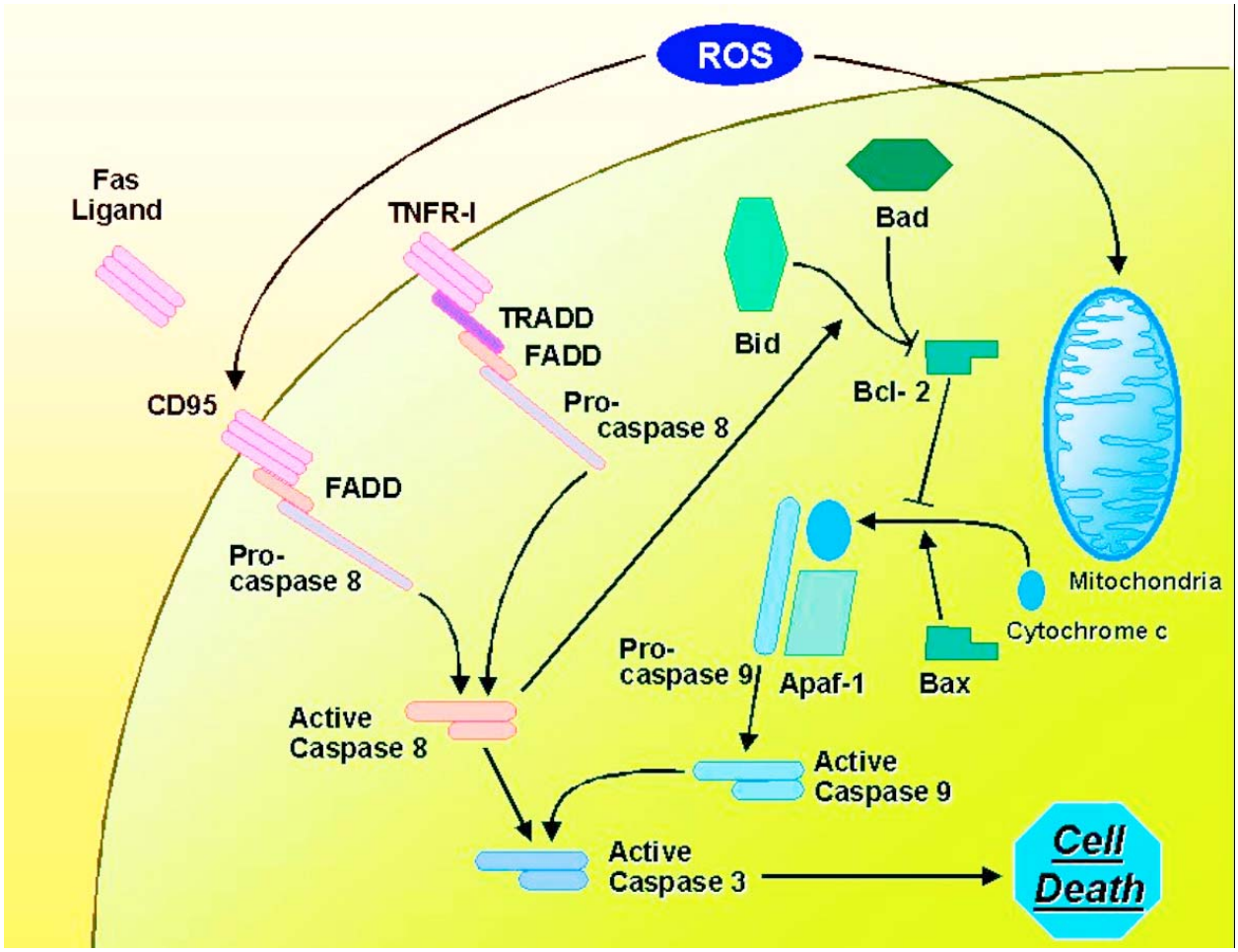


**Figure 1.** Proposed mechanism of ROS-induced apoptosis. This schematic depicts the varied caspase pathways involved in apoptosis. The intrinsic or mitochondrial-mediating pathway involves loss of mitochondrial membrane potential and cytochrome c release leading to activation of caspase 9 follwed by 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 caspases. Reactive oxygen species (ROS) may act as an extracellular intermediate directly stimulating the mithochondria 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 ($H_2O_2$) and superoxide anion radicals ($O_2^-$) 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 $O_2^-$ and $H_2O_2$) 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 though 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, Bereneis and colleagues reported that procarbazine is oxidized in solution to form ROS such as $H_2O_2$ with resultant damage to DNA (14), and they showed that procarbazine is synergistic with radiation

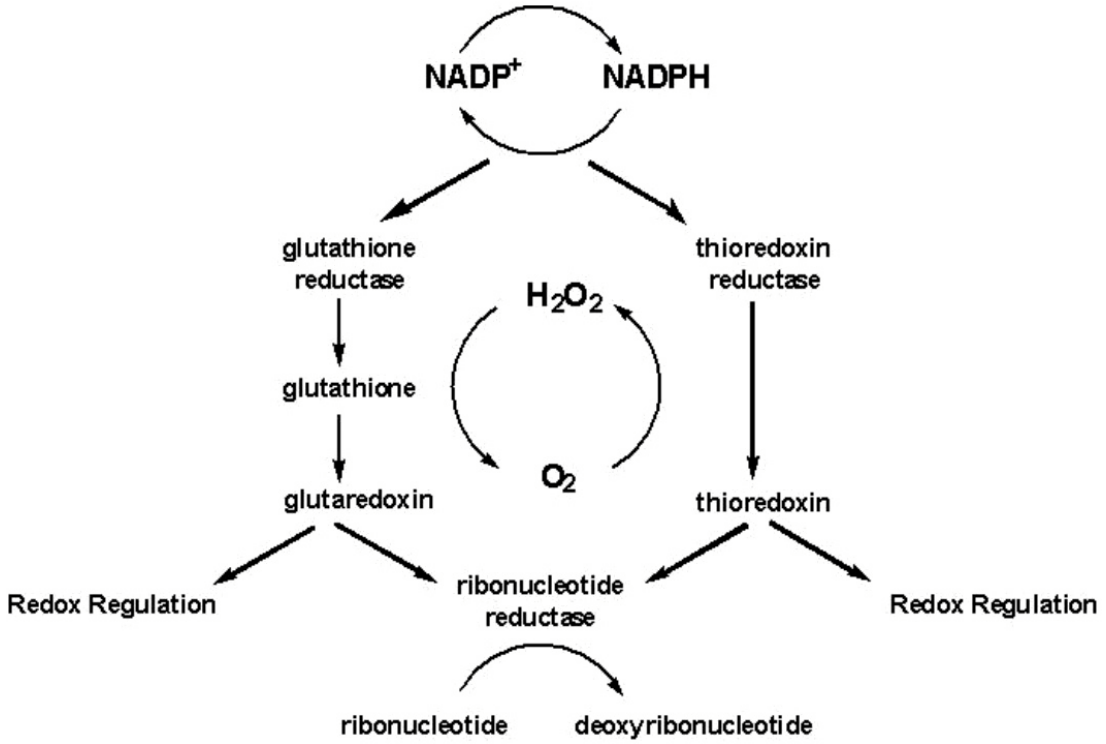


**Figure 2 .** Glutathione and Thioredoxin Systems. Reducing metabolites such as nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione are able to donate electrons to detoxify reactive oxygen species (ROS) such as hydrogen peroxide ($H_2O_2$) to oxygen ($O_2$) and water. Thioredoxin is able to maintain redox balance among various cellular proteins such as ribonucleotide reductase.

through production of free radicals (15). Moreover, pre-clinical studies have shown that the formation of ROS is important for induction of apoptosis for other commonly used chemotherapy agents such as cisplatin, bleomycin, and etoposide (18-20). Within normal and tumor cells, ROS must be neutralized by anti-oxidants in order to avoid potential cellular injury and induction of the apoptotic cascade.

### 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 $As_2O_3$ 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 $As_2O_3$ 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

caspase system, which is associated with redox-regulated apoptosis mechanisms, is activated following $As_2O_3$ 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 $As_2O_3$ (33). Furthermore, reduction of the GSH level by BSO pretreatment prior to $As_2O_3$ 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 $As_2O_3$ -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 monohydroascorbyl and then to dehydroascorbic acid. Once inside the cell, dehydoascorbic acid is reduced though 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 $As_2O_3$ for effective growth-inhibition and apoptosis (37,39). Grad and colleagues showed that ascorbic acid potentiated the cytotoxic effect of $As_2O_3$ 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, $As_2O_3$ has been demonstrated to be an effective therapeutic agent for the treatment of acute promyelocytic leukemia (APL) and $As_2O_3$ has provided clinical responses in heavily pretreated MM patients (42-44). Studies have demonstrated GSH content to be closely associated to the effectiveness of $As_2O_3$ cytotoxicity (45,46). $As_2O_3$ causes depletion of GSH through the conjugation of GSH and exportation of GSH out of the cell through multi-drug resistance efflux pumps (37). $As_2O_3$ can lead to membrane potential changes and increased membrane permeability with resultant degradation phase of apoptosis. The ability of $As_2O_3$ to induce apoptosis is dependent in part on the generation of ROS (47). ROS is generated by $As_2O_3$ through inhibition of glutathione transferase, an enzyme used to detoxify $As_2O_3$, as well as inhibition of glutathione peroxidase, an enzyme responsible for the conversion of $H_2O_2$ to water (48). The GSH content is closely associated to the effectiveness of $As_2O_3$ as malignant cells with lower GSH levels are often more sensitive to $As_2O_3$ cytotoxicity (45,46). Clearly, the ability to diminish GSH levels prior to exposure with $As_2O_3$ should improve its therapeutic effect.

Induction of apoptosis has been demonstrated to be an integral component of $As_2O_3$ 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 $As_2O_3$ in myeloma cells with elevated lymphokine activated cells and other immune cells (53). In APL studies, similar apoptotic mechanisms have been documented. $As_2O_3$ 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 $As_2O_3$ for multiple myeloma and APL is 1 to 2 micromol/L (44,54). In leukemia cell lines, there is also evidence that $As_2O_3$-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

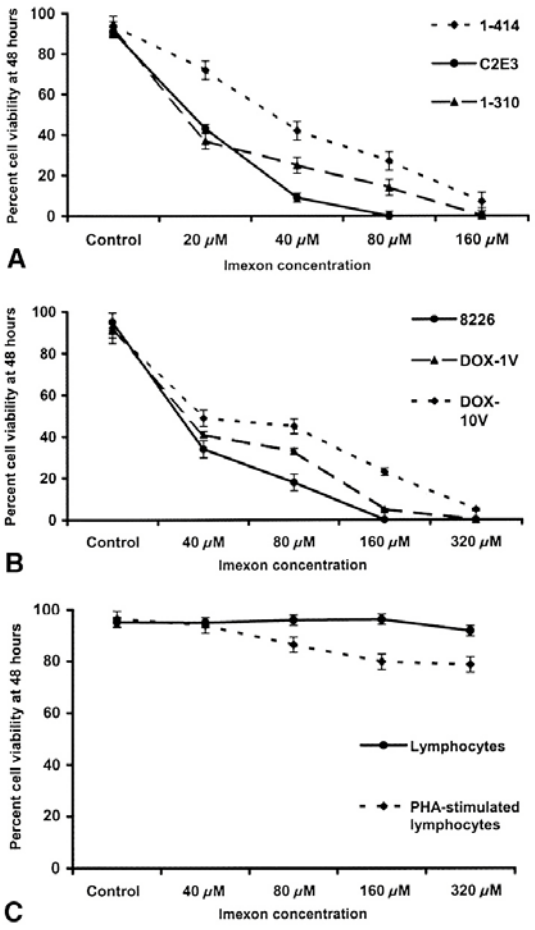


**Figure 3.** Imexon dose-dependent cytotoxicity in dexamethasone- and chemotherapy-sensitive and -resistant myeloma cell lines with minimal effect on normal lymphocytes.Cell viability was measured as a percentage of cells alive after 48-hour exposure with increasing concentrations of imexon in the dexamethasone-sensitive 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).

(See Figure 3) (66). 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 ($O_2$) 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 vivo* 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 delayed tumor re-growth and 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

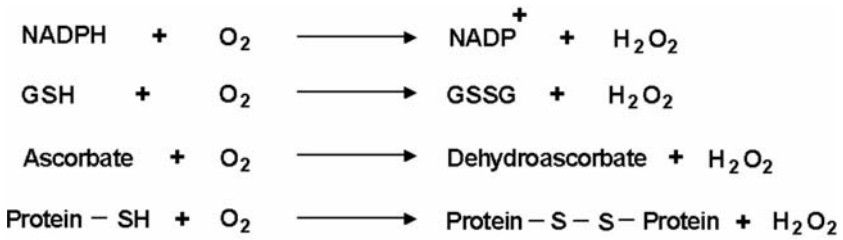


**Figure 4.** Motexafin Gadolinium Catalyzes Oxidation of Key Reducing Metabolites and Thiols. Motexafin gadolinium (MGd) catalyzes the oxidation of proteins and reducing metabolites such as glutathione, ascorbate and NADPH. MGd transfers electrons directly to oxygen to produce reactive oxygen species (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.

of docetaxel and showed a significant tumor growth delay compared with docetaxel alone (tumor growth delay of 22 days, p=0.0416) (80).

We studied the cell viability and mechanism of cell death with MGd in sensitive and highly resistant-steroid and chemotherapy multiple myeloma cell lines (81). We found complete inhibition of proliferation in every cell line within 24 hours of exposure to 50μM of MGd and 50μM to 100μM of ascorbate (the latter mimics physiologic *in vivo* levels), which was required for the effect**.** The cytotoxic effect of MGd was shown in our experiments to be mediated through apoptosis with elevated annexin-V expression and loss of mitochondrial membrane potential at clinically achievable concentrations of MGd. This was accompanied by depletion of intracellular GSH and increased ROS production. Using fluorescence microscopy and flow cytometry, we found intracellular uptake of MGd into all myeloma cell lines and demonstrated that intracellular accumulation of MGd was associated with intracellular ROS production (as detected by 2'7' dichlorofluorescein). Catalase reversed the growth inhibitory effect of MGd supporting the conclusion that the cytotoxic effects are mediated in part through the extracellular production of hydrogen peroxide.

## 4. CLINICAL STUDIES

### 4.1. Buthionine sulfoximine and Melphalan

A phase I 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 of grade 1/2 nausea or vomiting occurring in 50% of patients. Another phase I study by Bailey and colleagues used infusional BSO followed by melphalan treatment 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 leukemia-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. $As_2O_3$ 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 $As_2O_3$ (86). In 12 heavily treated APL patients, they showed $As_2O_3$ 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 $As_2O_3$ (87). $As_2O_3$ was used for induction and patients who obtained a complete remission could receive $As_2O_3$ 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 $As_2O_3$ alone (i.e., no autologous or allogeneic stem cell transplant) were alive at publication.. In general, $As_2O_3$ 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 $As_2O_3$ with ATRA has been used in the treatment of newly diagnosed APL. Shen and colleagues compared the combination of ATRA with $As_2O_3$ or separately for the induction and maintenance of 61 newly diagnosed APL patients (97). ATRA and $As_2O_3$ 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 $As_2O_3$ 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 $As_2O_3$ 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 $As_2O_3$ 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 relapsed, 16 refractory to prior treatment) showed $As_2O_3$ 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 $As_2O_3$-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 $As_2O_3$ 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 redox system with agents that deplete GSH and/or increase ROS may be used in combination with $As_2O_3$ to increase efficacy.

### 4.5. Arsenic trioxide and Asorbic acid

AA may sensitize multiple myeloma cells to $As_2O_3$ through the depletion of GSH as discussed before. Bahlis and colleagues initiated a Phase I/II clinical trial of combination $As_2O_3$ and AA in relapsed/refractory multiple myeloma (37). They showed that AA did not significantly alter the pharmacokinetics of $As_2O_3$, 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 $As_2O_3$ 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 MRI, MGd selectively accumulated in primary and metastatic tumors, without increase in radiation toxicity or MGd uptake to normal brain tissue.

Carde 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 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-porphyria. 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 $C_{max}$ 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 exceeded 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 splenomegaly, although no patients met NCI 96 response criteria. One responding patient during cycle 2 of therapy developed a bowel perforation and was found to have massive tumor necrosis of large cell transformation at the perforation site at surgery.

We have recently initiated a phase I/II trial that has thus far enrolled 6 patients with relapsed/refractory NHL to receive combination $^{90}$Yttrium-Zevalin with escalating dose MGd (109). Patients receive MGd days 1 through 4 and days 8 through 11 with standard $^{90}$Yttrium-Zevalin given day 8. MRI is used to visualize tumor uptake of MGd. There are 7 evaluable patients (5 follicular lymphoma, 1 diffuse large B-cell, 1 mantle-cell) with a mean age 66 years, mean 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 $As_2O_3$ 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.

## 6. REFERENCES

1. Dalton WS: Targeting the mitochondria: an exciting new approach to myeloma therapy. *Clin Cancer Res* 8,3643-3645 (2002)

2. Brookes PS, A.L. Levonen , S. Shiva , P. Sarti , V.M. Darley-Usmar: Mitochondria: regulators of signal transduction by reactive oxygen and nitrogen species. *Free Radic Biol Med* 33,755-764 (2002)

3. Smith PG, F. Wang , K.N .Wilkinson, K. Savage, U. Klein, D. Neuberg, G. Bollag, M. Shipp, R.C Aguiar,: The phosphodiesterase PDE4B limits cAMP-associated PI3K/AKT-dependent apoptosis in diffuse large B-cell lymphoma. Blood 105,308-16 (2005)

4. Dvorakova K, C.N. Waltmire, C.M. Payne, M.E. Tome, M.M. Briehl, R.T. Dorr. Induction of mitochondrial changes in myeloma cells by imexon. Blood 97, 3544-3551 (2001)

5. Byrd JC, S. Kitada , I.W. Flinn , J.L. Aron, M. Pearson, D. Lucas, J. Reed:. The mechanism of tumor cell clearance by rituximab *in vivo* in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Blood 99, 1038-1043 (2002)

6. Kumar V, A.K. Abbas, N. Fausto: Cellular Adaptations, Cell Injury, and Cell Death. In: Robbins and

Cotran Pathologic Basis of Disease, Editor Gruliow, R Elsevier Saunders Philadelphia, Pennsylvania 7th ed, 14-20 (2005)

7. Toyokuni S: Reactive oxygen species-induced molecular damage and its application in pathology. *Pathol Iny* 49, 91-102. (1999)

8. Gromer S, S. Urig, K. Becker: The thioredoxin system--from science to clinic. *Med Res Rev* 24,40-89 (2004)

9. Cerutti PA: Prooxidant states and tumor promotion. Science 227,375-381(1985)

10. Jing Y, J. Dai, R.M. Chalmers-Redman, W.G. Tatton , S. Waxman:Arsenic trioxide selectively induces acute promyelocytic leukemia cell apoptosis via a hydrogen peroxide-dependent pathway. Blood 94,2102-2111 (1999)

11. Chen Q, Y.C. Chai, S. Mazumder , C. Jiang, R.M. Macklis, G.M. Chisolm, A. Almasan*:* The late increase in intracellular free radical oxygen species during apoptosis is associated with cytochrome c release, caspase activation, and mitochondrial dysfunction. *Cell Death Differ* 10,323-334 (2003)

12. Li PF, R. Dietz, R. Von Harsdorf: p53 regulates mitochondrial membrane potential through reactive oxygen species and induces cytochrome c-independent apoptosis blocked by Bcl-2. *Embo J* 18,6027-6036 ( 1999)

13. Usgui-Fukai M, R.W. Alexander:. Reactive oxygen species as mediators of angiogenesis signaling: role of NAD(P)H oxidase. *Mol Cell Biochem* 264 (1-2),85-87 (Sep 2004)

14. Berneis K, M. Kofler, W. Bollag, A. Kaiser, A. Langeman: The degradation of deoxyribonucleic acid by new tumour inhibiting compounds: the intermediate formation of hydrogen peroxide. *Experientia* 19,132–133 (1963)

15. Berneis K, W.Bollag, M.Kofler and H.Luthy: The enhancement of the after effect of ionizing radiation by a cytotoxic methylhydrazine derivative. *Eur J Cancer* 21,43–49 (1966)

16. Martz G, A. D'Alessandri, H.J. Keel, W. Bollag: Preliminary clinical results with a new antitumor agent RO 4-6467 (NSC 77213). *Cancer Chemother Rep* 33,5–14 (1963)

17. Mathe G, O. Schweisguth, M. Schneider, J.L Amiel, L.Berumen, G. Brule, A. Cattan, L. Schwarzenberg: Methyl-hydrazine in treatment of Hodgkin's disease and various forms of hematosarcoma and leukemia. Lancet 12,1077–1080 (1963)

18. Kurosu T, T. Fukuda, T. Miki,O. Miura: BCL6 overexpression prevents increase in reactive oxygen species and inhibits apoptosis induced by chemotherapeutic reagents in B-cell lymphoma cells. Oncogene 22(29),4459-68 (2003)

19. Muller I, D. Niethammer, G. Bruchelt: Anthracycline-derived chemotherapeutics in apoptosis and free radical cytotoxicity. *Int J Mol Med* 1(2), 491-4 (1998 Feb)

20. Mahmutoglu I, H. Kappus: Oxy radical formation during redox cycling of the bleomycin-iron (III) complex by NADPH-cytochrome P-450 reductase. *Biochem Pharmacol* 34(17), 3091-4 (1985 Sep)

21. Williamson JM, B. Boettcher, A. Meister:.Intracellular cysteine delivery system that protects against toxicity by promoting glutathione synthesis. *Proc Natl Acad Sci U S A* 79(20), 6246-9 (1982)

22. Meister A: Glutathione metabolism and its selective modification. *J Biol Chem* 263, 17205-17208 (1988)

23. Sun J, Y. Chen, M. Li, Z. Ge: Role of antioxidant enzymes on ionizing radiation resistance. *Free Radic Biol Med* 24,586-593 (1998)

24. Kidd, P: Glutathione: Systemic Protectant Against Oxidative and Free Radical Damage. *Alt Med Review* 2 (3), 155-176 (1997)

25. Powis G, W.R. Montfort: Properties and biological activities of thioredoxins. *Annu Rev Pharmacol Toxicol* 41,261-295 (2001)

26. Nathan CF, B.A. Arrick,H.W.Murray, N.M. DeSantis, Z.A. Cohn*:* Tumor cell anti-oxidant defenses.Inhibition of the glutathione redox cycle enhances macrophage-mediated cytolysis. *J Exp Med* 153,766-82 (1981)

27. Arrick BA, C.F. Nathan, O.W. Griffith, Z.A. Cohn: Glutathione depletion sensitizes tumor cells to oxidative cytolysis. *J Biol Chem* 257,1231-37 (1982)

28. Arrick BA, C.F. Nathan: Glutathione as a determinant of therapeutic efficacy: a review. *Cancer Res* 44,4224-32 (1984)

29. Griffith OW, A. Meister: Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine. *J Biol Chem* 254,7558-60 (1979)

30. Bailey HH: L-S, R-buthionine sulfoximine: historical development and clinical issues. *Chem Biol Interact* 111-112,239-54 (1998)

31. Dorr RT, J.D. Liddil, M.J. Soble: Cytotoxic effects of glutathione synthesis inhibition by L-buthionine-(SR)-sulfoximine on human and murine tumor cells. *Invest New Drugs* 4, 305-13(1986)

32. Gartenhaus RB, S.N. Prachand, M. Paniaqua, Y. Li, L.I. Gordon: Arsenic trioxide cytotoxicity in steroid and chemotherapy-resistant myeloma cell lines. *Clin Cancer Res* 8(2), 566-72 (2002 Feb)

33. Kitamura K, Y. Minami, K.Yamamoto, Y. Akao, H. Kiyoi, H. Saito, T. Naoe: Involvement of CD95-independent caspases 8 activation in arsenic trioxide-induced apoptosis. Leukemia 14,1743-50 (2000)

34. Miyajima A, J. Nakashima, K. Yoshioka, M. Tachibana, H. Tazaki, M .Murai: Role of reactive oxygen species in cis-dichlorodiammineplatinum-induced cytotoxicity on bladder cancer cells. *Br J Cancer* 76(2),206-210 (1997)

35. Rudin CM,Z. Yang, L.M. Schumaker. D.J. VanderWeele, K. Newkirk, M.J. Egorin, E.G. Zuhowski, K.J. Cullen*:* Inhibition of glutathione synthesis reverses Bcl-2-mediated cisplatin resistance. *Cancer Res* 63(2), 312–318 (2003)

36. Skapek SX , O.M. Colvin, O.W. Griffith, G.B. Elion, D.D. Bigner, H.S. Friedman: Enhanced melphalan cytotoxicity following buthionine sulfoximine-mediated glutathione depletion in a human medulloblastoma xenograft in athymic mice. *Cancer Res* 48 (10),2764–2767 (1988)

37. Bahlis, N: Feasibility and Correlates of Arsenic Trioxide Combined with Ascorbic Acid-mediated Depletion of Intracellular Glutathione for the Treatment of Relapsed/Refractory Multiple Myeloma. Clinical Cancer Research, Vol 8 3658-3668 ( Dec 2002)

38. Dai J, R.S. Weinberg, S. Waxman,Y. Jing: Malignant cells can be sensitized to undergo growth inhibition and apoptosis by arsenic trioxide through modulation of the glutathione redox system. Blood 93,268-77 (1999)

39. Chen GQ, J. Zhu, X.G. Shi, J.H. Ni, H.J. Zhong, G.Y. Si, X.L. Jin, W. Tang, X.S. Li, S.M. Xong, Z.X. Shen, G.L. Sun, J. Ma, P. Zhang, T.D. Zhang, C. Gazin, T. Naoe, S.J. Chen, Z.Y.Wang, Z. Chen: *In vitro* studies on cellular and molecular mechanisms of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia: As2O3 induces NB4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins. Blood 88,1052-61 (1996)

40. Grad JM, N.J. Bahlis, I. Reis, M.M. Oshiro, W.S. Dalton, L.H. Boise: Ascorbic acid enhances arsenic trioxide-induced cytotoxicity in multiple myeloma cells. Blood 98,805-13 (2001)

41. Klassen, CD: Heavy metals and heavy-metal antagonists. In: Goodman &Gilman's The Pharmacological Basis of Therapeutics. Eds: JG Hardman LE Limbird McGraw Hill, NY 1649-72 (1996)

42. Soignet SL,S. Frankel , M. Tallman *:* Arsenic trioxide (ATO) in relapsed acute promyelocytic leukemia (APL): the combined results and follow-up from the U.S. pilot and multicenter trials. Blood 96 (suppl, pt 1 of 2), 827a (2000)

43. Niu C, H. Yan , T. Yu H.P. Sun, J.X. Liu, X.S. Li, W. Wu, F.Q. Zhang, Y. Chen, L. Zhou, J.M. Li, X.Y. Zeng, R.Yang, M.M. Yuan, M.Y. Ren, F.Y. Gu, Q. Cao, B.W. Gu, X.Y. Su, G.Q. Chen, S.M. Xiong, T.D. Zhang, S. Waxman, Z.Y. Wang, Z. Chen, J. Hu, Z.X. Shen, S.J. Chen: Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients. Blood 94,3315-24 (1999)

44. Munshi N, R. Desikan, M. Zangari: Marked antitumor effect of arsenic trioxide (As2O3) in high- risk refractory multiple myeloma. Blood 94 (suppl 1),123a (1999)

45. Ochi T, T. Kaise, Y. Oya-Ohta: Glutathione plays different roles in the induction of the cytotoxic effects of inorganic and organic arsenic compounds in cultured BALB/c 3T3 cells. Experientia 50,115-20 (1994)

46. Scott N, K.M. Hatlelid, N.E. MacKenzie, D.E. Carter: Reactions of arsenic (III) and arsenic (V) species and glutathione. *Chem Res Toxicol* 6,102-6 (1993)

47. Jing Y, J. Dai, R.M. Chalmers-Redman, W.G. Tatton, S. Waxman: Arsenic trioxide selectively induces acute promyelocytic leukemia cell apoptosis via a hydrogen peroxide-dependent pathway. Blood 94,2102-11 (1999)

48. Schuliga M, S. Chouchane , E.T. Snow: Upregulation of glutathione-related genes and enzyme activities in cultured human cells by sublethal concentrations of inorganic arsenic. *Toxicol Sci* 70,183-92 (2002)

49. Karasavvas N, J.M. Carcamo, G. Stratis, D.W. Golde: Vitamin C protects HL60 and U266 cells from arsenic toxicity. Blood 105(10), 4004-12 (2005)

50. Zhu XH, Y.L Shen ,Y.K Jing, X. Cai, P. M. Jia, Y. Huang, W. Tang, G. Y. Shi, Y. P. Sun, J. Dai, Z. Y.Wang, S. J.Chen, T. D. Zhang: Apoptosis and growth inhibition in malignant lymphocytes after treatment with arsenic trioxide in clinically achievable concentrations. *J Natl Cancer Inst* 91,772-8 (1999)

51. Rousselot P, S. Labaume, J.P. Marolleau J. Larghero, M. H. Noguera, J. C. Brouet, J. P.
Fermand: Arsenic trioxide and melarsoprol induce apoptosis in plasma cell lines and in plasma cells from myeloma patients. *Cancer Res* 59,1041-48 (1999)

52. Park WH, J.G. Seol, E.S. Kim, J. M. Hyun, C. W. Jung, C. C .Lee, B. K. Kim, Y. Y. Lee: Arsenic trioxide-mediated growth inhibition in MC/CAR myeloma cells via cell cycle arrest in association with induction of cyclin-dependent kinase inhibitor, p21, and apoptosis. *Cancer Res* 60, 3065-71 (2000)

53. Deaglio S, D. Cannell , G. Baj G, A. Arnulfo, S.Waxman, F. Malavasi*:* Evidence of an immunologic mechanism behind the therapeutical effects of arsenic trioxide (As2O3) on myeloma cells*.* *Leuk Res* 25,227-35 (2001)

54. Shen ZX, G.Q. Chen, J.H. Ni, X. S. Li, S. M. Xiong, Q. Y. Qiu, J.Zhu, W.Tang, G. L. Sun, K. Q.Yang, Y. Chen, L. Zhou, Z. W.Fang, Y. T. Wang, J.Ma, P.Zhang, T. D.Zhang, S. J. Chen, Z.Chen, Z. Y. Wang: Use of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in elapsed patients. Blood 89,3354-60 (1997)

55. Zheng Y, H. Yamaguchi, C Tian,M. W.Lee, H. Tang, H.G.Wang, Q.Chen: Arsenic trioxide induces apoptosis through acitivation of Bax in hematopoietic cells. Oncogene 24(20), 3339-47 (2005)

56. Perkins C, C.N. Kim, G. Fang, K.N. Bhalla*:* Arsenic induces apoptosis of multidrug –resistant human myeloid leukemia cells that express Bcr-Abl or overexpress MDR,MRP, Bcl-2 or Bcl-c(L). Blood 95(3),1014-22 (2000)

57. Dvorakova K, C. Payne, M.E. Tome, M. M. Briehl, T. McClure, R. T. Dorr: Induction of oxidative stress and poptosis in myeloma cells by the aziridine-containing agent imexon. *Biochem Pharmacol* 60,749-58 (2000)

58. Mead JR, R.A. Burger, J.D. Morrey, R. P. Warren, K. M. Okleberry, R. W. Sidwell: Effect of immunomodulators in the hu-PBL-SCID mouse model. *Biotechnol Ther* 4,133-43 (1993)

59. Funk CY, J. Eisman, E.M. Hersh: Treatment of the murine, retrovirus-induced lymphoproliferative immunodeficiency disease (LP-BM5) in C57BL/10 mice with the immunomodulator Imexon. *AIDS Res Hum Retroviruses* 8,633-8 (1992)

60. Hersh EM, C.R. Gschwind, C.W. Taylor, R. T.Dorr, R. Taetle, S. E. Salmon: Antiproliferative and antitumor activity of the 2-cyanoaziridine compound Imexon on tumor cell lines and fresh tumor cells *in vitro. J Natl Cancer Inst* 84,1238-44 (1992)

61. Marchetti P,D. Decaudin , A. Macho, N. Zamzami, T.Hirsch, S. A. Susin, G. KroemeT: Redox regulation of apoptosis: Impact of thiol oxidation status on mitochondrial function. *Eur J Immunol* 27,289-96 (1997)

62. Sato M, M. Sasaki, T. Oguro T, Y.Kuroiwa, T. Yoshida: Induction of metallothionein synthesis by glutathione depletion after trans- and cis-stilbene oxide administration in rats. *Chem Biol Interact* 98,15-25 (1995)

63. Cai X, Y.L. Shen, X. Zhu, P. M. Jia, Y. Yu, L. Zhou, Y.Huang, J. W. Zhang, S. M. Xiong, S. J.Chen, Z. Y. Wang, Z. Chen, G. Q. Chen: Arsenic trioxide-induced apoptosis and differentiation are associated respectively with mitochondrial transmembrane potential collapse and retinoic acid signaling pathways

in acute promyelocytic leukemia. Leukemia 14,262-70 (2000)
64. Sordet O, C. Rebe, I. Leroy I, J. M. Bruey, C. Garrido, C. Miguet, G.Lizard, S. Plenchette, L. Corcos, E.Solary: Mithochondria-targeting drugs arsenic trioxide and lonidamine bypass the resistance of TPA-differentiated leukemic cells to apoptosis. Blood 97,3931-40 (2001)
65. Dorr RT, J.D Liddil, M.K. Klein, E.M. Hersh: Preclinical pharmacokinetics and antitumor activity of Imexon. *Invest New Drugs* 13,113-6 (1995)
66. Evens AM, S. Prachand, B. Shi B, M. Paniaqua, L.I. Gordon, R.B. Gartenhaus: Imexon-induced apoptosis in multiple myeloma tumor cells is caspase-8 dependent. *Clin Cancer Res* 10,1481-1491 (2004)
67. M Micksche,EM Kokoschka , P Sagaster, U Bicker:Phase I study for a new immunostimulating drug, BM 06 002, in man. In: Immune Modulation and Control of Neoplasia by Adjuvant Therapy. Ed. Chirigos MA, Raven Press,,NY, 403-13(1978).
68. Salmon SE, E.M. Hersh: Sensitivity of multiple myeloma to imexon in the human in the human tumor cloning assay. *J Natl Cancer Inst* 86,228-30 (1994)
69. Sagaster P, E.M. Kokoschka, O. Kokran, M, Micksche: Antitumor activity of Imexon. *J Natl Cancer Inst* 87,935-36 (1995)
70. Sessler JL, R.A. Miller: Texaphyrins: new drugs with diverse clinical applications in radiation and photodynamic therapy. *Biochem Pharmacol* 59,733-739(2000)
71. Magda D, N. Gerasimchuk, P. Lecane,R.A. Miller, J.E. Biaglow, J.L. Sessler: Motexafin gadolinium reacts with ascorbate to produce reactive oxygen species. *Chem Commun (Camb)* 22, 2730-2731 (2002)
72. Xu S, K. Zakian, H. Thaler, C. Matei, A. Alfieri, Y. Chen, J. A. Koutcher: Effects of Motexafin gadolinium on tumor metabolism and radiation sensitivity. *Int J Radiat Oncol Biol Phys* 49,1381-1390 (2001)
73. Magda D,C. Lepp, N. Gerasimchuk , I. Lee, J. L. Sessler, A. Lin, J. E. Biaglow, R. A. Miller: Redox cycling by motexafin gadolinium enhances cellular response to ionizing radiation by forming reactive oxygen species. *Int J Radiat Oncol Biol Phys* 51, 1025-1036 (2001)
74. Woodburn KW. Intracellular localization of the radiation enhancer motexafin gadolinium using interferometric Fourier fluorescence microscopy. *J Pharmacol Exp Ther* 297, 888-894 (2001)
75. Miller RA, K. Woodburn, Q. Fan, M.F .Renschler, J.L. Sessler, J.A. Koutcher: *In vivo* animal studies with gadolinium (III) texaphyrin as a radiation enhancer. *Int J Radiat Oncol Biol Phys* 45,981-989 (1999)
76. Young SW, F. Qing, A. Harriman , J. L. Sessler, W. C. Dow, T. D. Mody, G. W. Hemmi, Y. Hao,
R. A. Miller: Gadolinium(III) texaphyrin: a tumor selective radiation sensitizer that is detectable by MRI. *Proc Natl Acad Sci*
*US A* 93, 6610-6615 (1996)
77. Donnelly ET, Y. Liu, Y.O. Fatunmbi, I. Lee, D. Magda, S. Rockwell: Effects of texaphyrins on the oxygenation of EMT6 mouse mammary tumors. *Int J Radiat Oncol Biol Phys* 58,1570-1576 (2004)
78. Miller RA, K.W. Woodburn, Q. Fan , I. Lee, D. Miles, G. Duran, B. Sikic, D. Magda: Motexafin gadolinium: a redox active drug that enhances the efficacy of bleomycin and doxorubicin. *Clin Cancer Res* 7,215-3221 (2001)
79. Miller RA, I. Lee. D. Magda: Motexafin gadolinium (MGd) increases tumor response to chemotherapy in Lewis lung cancer (LLC) animal model. in American Society of Clinicical Onclolgy proceedings (ASCO) 21:117a (2002)
80. Lepp C QF, P. Lecane , D. Magda , R. Miller: Motexafin gadolinium (MGd) enhances the activity of cisplatin, carboplatin, and docetaxel. Conf Proc of AACR (2004)
81. Evens AM, P. Lecane, D. Magda, S. Prachand, S. Singhal: Motexafin gadolinium generates reactive oxygen species and induces apoptosis in sensitive and highly resistant multiple myeloma cells. Blood 105,1265-1273 (2005)
82. O'Dwyer PJ, T.C. Hamilton, F.P. LaCreta, J. M. Gallo, D. Kilpatrick, T. Halbherr,J. Brennan,M. A. Bookman,J. Hoffman,R. C. Young,R. L. Comis,R. F. Ozols: Phase I trial of buthionine sulfoximine in combination with melphalan in patients with cancer. *J Clin Oncol* 14(1),249-56 (1996 Jan)
83. Bailey HH, G. Ripple, K.D. Tutsch, R. Z. Arzoomanian,D. Alberti,C. Feierabend,D. Mahvi,J. Schink,M. Pomplun,R. T. Mulcahy,G. Wilding: Phase I study of continuous-infusion L-S,R-buthionine sulfoximine with intravenous melphalan. *J Natl Cancer Inst* 89(23),1789-96 (1997 Dec)
84. Dorr, R: Phase I clinical trial of imexon. JCO, ASCO Annual Meeting Proccedings, Vol 22 (14S) 3181 (2004 July 15
85. Lazo G: Use of arsenic trioxide (As2O3) in the treatment of patients with acute promyelocytic leukemia: the M.D. Anderson experience. Cancer 97(9),2218-2224 (2003 May)
86. Soignet SL, P. Maslak, Z.G. Wang,S. Jhanwar, S.E. Calleja, L. J. Dardashti, D. Corso,A. DeBlasio,
J. Gabrilove,D. A. Scheinberg,P. P. Pandolfi, R. P., Jr. Warrell: Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. *N Engl J Med* 339,1341-8 (1998)
87. Soignet SL, S.R Frankel, D. Douer, Tallman, M. S. H. Kantarjian,E. Calleja,R. M. Stone,M. Kalaycio,D. A. Scheinberg,P. Steinherz,E. L. Sievers,S. Coutre,S. Dahlberg,R. Ellison,R. P., Jr. Warrell: United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. *J Clin Oncol* 19,3852-3860 (2001)
88. Soignet S, S.R. Frankel, M.S. Tallman: U.S. multicenter trial of arsenic trioxide (AT) in acute promyelocytic leukemia (APL). Blood 94(suppl 1), 698a (1999)
89. Huang CH, W.J. Chen, C.C. Wu, Y. C. Chen,Y. T. Lee: Complete atrioventricular block after arsenic trioxide treatment in an acute promyelocytic leukemia patent. *Pacing Clin Electrophysiol* 22,965-7 (1999)
90. Ohnishi K, H. Yoshida, K. Shigeno,S. Nakamura, S.S. Fujisawa,K. Naito,K. Shinjo,Y. Fujita,H. Matsui,A. Takeshita,S. Sugiyama,H. Satoh, H. Terada,R. Ohno: Prolongation of the QT interval and ventricular tachycardia in patients treated with arsenic trioxide for

acute promyelocytic leukemia. *Ann Intern Med* 133,881-5 (2000)
91. Unnikrishnan D, J.P. Dutcher, N. Varxhneya, R. Lucariello,M. Api, S. Garl,P. H. Wiernik,S. Chiaramida: Torsades de pointes in 3 patients with leukemia treated with arsenic trioxide. Blood 97,1514-6 (2001)
92. Beckman KJ, J.L. Bauman ,P.A. Pimental, C. Garrard, R. J. Hariman: Arsenic-induced torsade de pointes. *Crit Care Med* 19,290-2 (1991)
93. Goldsmith S, A.H. From: Arsenic-induced atypical ventricular tachycardia. *N Engl J Med* 303,1096-8 (1980)
94. Westervelt P, R.A. Brown, D.R. Adkins, H. Khoury,P. Curtin,D. Hurd,S. M. Luger,M. K. Ma,T. J. Ley,J. F. DiPersio: Sudden death among patients with acute promyelocytic leukemia treated with arsenic trioxide. Blood 98,266-71 (2001)
95. Borrow J, A.D. Goddard ,D. Sheer,E. Solomon: Molecular analysis of acute promyelocytic leukemia breakpoint cluster region on chromosome 17. Science 249,1577-80 (1990)
96. Camacho LH, S.L. Soignet, S. Chanel, R. Ho,G. Heller, D. A. Scheinberg,R. Ellison,Warrell, R. P., Jr.: Leukocytosis and the retinoic acid syndrome in patients with acute promyelocytic leukemia treated with arsenic trioxide. *J Clin Oncol* 18,262-5 (2000)
97. Shen ZX, Z.S. Zhan: All-trans retinoic acid/As203 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. *Proc Natl Acad Sci U S A* 101(15),5328-35 (2004 April 13)
98. Munshi, N: Clinical activity of arsenic trioxide for the treatment of multiple myeloma. Leukemia 16, 1835-1837 (2002)
99. Hussein, M. M.Saleh, F. Ravandi, J.Mason, R.M. Rifkin, R. Ellison: Phase 2 study of arsenic trioxide in patients with relapsed of refractory multiple myeloma. British Journal of Haematology 125, 470-476 (2004)
100. Evens AM, M.S. Tallman, R.B. Gartenhaus: The potential of arsenic trioxide in the treatment of malignant disease: past, present, and future. *Leuk Res* (9),891-900 (2004 Sep 28)
101. Wang TS, Y.F. Shu, Y.C. Liu, K. Y. Jan,H. Huang: Glutathione peroxidase and catalase modulate the genotoxicity of arsenite. Toxicology 121,229-37 (1997)
102. Berenson JR, R.A. Swift, D. Ferretti,M. B. Purner: A prospective, open-label safety and efficacy study of combination treatment with melphalan, arsenic trioxide, and ascorbic acid in patients with relapsed or refractory multiple myeloma. *Clin Lymphoma* 5(2),130-4 (2004 Sep)
103. Rosenthal DI,P. Nurenberg, C.R. Becerra, E. P. Frenkel,D. P. Carbone, B. L. Lum,R. Miller,J. Engel,S. Young,D. Miles: A phase I single-dose trial of gadolinium texaphyrin (Gd-Tex), a tumor selective radiation sensitizer detectable by magnetic resonance imaging. *Clin Cancer Res* 5,739-745 (1999)
104. Carde P, R. Timmerman, M.P. Mehta, C. D. Koprowski,J. Ford,R. B. Tishler,D. Miles,R. A. Miller,M. F. Renschler: Multicenter phase Ib/II trial of the radiation enhancer motexafin gadolinium in patients with brain metastases. *J Clin Oncol* 19,2074-2083 (2004)
105. Mehta MP, W.R. Shapiro, M.J. Glantz, R. A. Patchell, M. A. Weitzner,C. A. Meyers,C. J. Schultz,W. H. Roa,M. Leibenhaut,J. Ford,W. Curran,S. Phan,J. A. Smith,R. A. Miller,M. F. Renschler: Lead-in phase to randomized trial of motexafin gadolinium and whole-brain radiation for patients with brain metastases: centralized assessment of magnetic resonance imaging, neurocognitive, and neurologic end points. *J Clin Oncol* 20, 3445-3453 (2002)
106 Mehta MP, P. Rodrigus. C.H. Terhaard, A. Rao, J. Suh,W. Roa,L. Souhami,A. Bezjak,M. Leibenhaut,R. Komaki,C. Schultz,R. Timmerman,W. Curran,J. Smith, S. C. Phan,R. A. Miller,M. F. Renschler: Survival and neurologic outcomes in a randomized trial of motexafin gadolinium and whole-brain radiation therapy in brain metastases. *J Clin Oncol* 21,2529-2536 (2003)
107. Meyers CA, J.A. Smith, A. Bezjak M. P. Mehta, J. Liebmann,T. Illidge,I. Kunkler,J. M. Caudrelier,P. D. Eisenberg,J. Meerwaldt,R. Siemers,C. Carrie,L. E. Gaspar,W. Curran,S. C. Phan,R. A. Miller,M. F. Renschler: Neurocognitive function and progression in patients with brain metastases treated with whole-brain radiation and motexafin gadolinium: results of a randomized phase III trial. *J Clin Oncol* 22,157-165 (2004)
108. Lin TS, L. Naumovski, P. Lecane, M.S. Lucas, M.E. Moran, A.P. Mone, D.M. Lucas, S. Phan , R.A. Miller, J.C. Byrd : Effects of the Redox Mediator Motexafin Gadolinium in a Pilot Phase I Trial in Refractory Chronic Lymphocytic Leukemia. American Society of Hematology (ASH). San Diego, CA (2004)
109. Evens AM, L. Naumovski, R.A. Miller, L.I. Gordon: Motexafin gadolinium (MGd) induces apoptosis in lymphoma cell lines: rationale for use of a redox-active drug in the treatment of lymphoma. 6th International Symposium and Expert Workshops on Leukemia and Lymphoma, Amsterdam, Netherlands, (2005 Mar)
110. Bellosillo B, N. Villamor, A. Lopez-Guillermo, S. Marce, J. Esteve,E. Campo,D. Colomer,E. Montserrat: Complement-mediated cell death induced by rituximab in B-cell lymphoproliferative disorders is mediated *in vitro* by a caspase-independent mechanism involving the generation of reactive oxygen species. *Blood* 98,2771-7 (2001)
111. Pei XY, Y. Dai, S. Grant: Synergistic induction of oxidative injury and apoptosis in human multiple myeloma cells by the proteasome inhibitor bortezomib and histone deacetylase inhibitors. *Clin Cancer Res* 10,3839-52 (2004)
112. Fribley A, Q. Zeng , C.Y. Wang: Proteasome inhibitor PS-341 induces apoptosis through induction of endoplasmic reticulum stress-reactive oxygen species in head and neck squamous cell carcinoma cells. *Mol Cell Biol* 24, 9695-704 (2004)
113. Ungerstedt JS, Y. Sowa, W.S. Xu, Y. Shao, M. Dokmanovic,G. Perez,L. Ngo,A. Holmgren,X. Jiang,P. A. Marks: Role of thioredoxin in the response of normal and transformed cells to histone deacetylase inhibitors. Proc *Natl Acad Sci U S A* 102,673-8 (2005)
114. Shanafelt TD, Y.K Lee, N.D. Bone, A. K. Strege,V. L. Narayanan,E. A. Sausville,S. M. Geyer,S. H. Kaufmann,N. E. Kay: Adaphostin-induced apoptosis in CLL B cells is associated with induction of oxidative

stress and exhibits synergy with fludarabine. Blood 105,2099-2106 (2005)

**Abbreviations:** ROS, reactive oxygen species; BSO, buthionine sulfoximine; $As_2O_3$, arsenic trioxide; MGd, motexafin gadolinium; GSH, glutathione; ATP, adenosine triphosphate; DNA, deoxyribonucleic acid; $H_2O_2$, hydrogen peroxide; $O_2^-$, superoxide anion; NADPH, nicotinamide adenine dinucleotide phosphate; CDDP, cis-dichlorodiammineplatinum; AA, ascorbic acid; APL, acute promyelocytic leukemia; NAC, N-acetyl-L-cysteine; $O_2$, 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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