

Superoxide Dismutase as an Intervention for Radiation Therapy-Associated Toxicities: Review and Profile of Avasopasem Manganese as a Treatment Option for Radiation-Induced Mucositis

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy

Stephen T Sonis ^{1,2}

¹Primary Endpoint Solutions, Waltham, MA, 02451, USA; ²Brigham and Women's Hospital and the Dana-Farber Cancer Institute, Boston, MA, 02215, USA

Abstract: Toxicities associated with radiation therapy are common, symptomatically devastating, and costly. The best chance to effectively mitigate radiation-associated normal tissue side effects are interventions aimed at disrupting the biological cascade, which is the basis for toxicity development, while simultaneously not reducing the beneficial impact of radiation on tumor. Oxidative stress is a key initiator of radiation-associated normal tissue injury as physiologic antioxidant mechanisms are overwhelmed by the accumulation of effects produced by fractionated treatment regimens. And fundamental to this is the generation of superoxide, which is normally removed by superoxide dismutases (SODs). Attempts to supplement the activity of endogenous SOD to prevent radiation-induced normal tissue injury have included the administration of bovine-derived SOD and increasing SOD production using gene transfer, neither of which has resulted in a clinically acceptable therapy. A third approach has been to develop synthetic small molecule dismutase mimetics. This approach has led to the creation and development of avasopasem manganese, a unique and specific dismutase mimetic that, in clinical trials, has shown promising potential to reduce the incidence, severity and duration of severe oral mucositis amongst patients being treated with concomitant chemoradiation for cancers of the head and neck. Further, avasopasem and related analogues have demonstrated mechanism-related antitumor synergy in combination with high dose per fraction radiotherapy, an observation that is also being tested in clinical trials. An ongoing Phase 3 trial seeks to confirm avasopasem manganese as an effective intervention for severe oral mucositis associated with chemoradiation in head and neck cancer patients.

Keywords: superoxide dismutase, mucositis, radiation, avasopasem manganese

Introduction

The May 1954 issue of the journal *Science* included a paper which would have a major impact on the understanding of the pathoetiology of ionizing radiation's toxic impact. For it was there that Rebecca Gerschman and her University of Rochester colleagues described a series of experiments demonstrating that radiation toxicity is associated with the generation of oxygen free radicals.¹ The results described the impact of radiation-induced hydrolysis on the generation of reactive oxygen species, a process occurring in cells with every fraction of radiation therapy.

Correspondence: Stephen T Sonis
Primary Endpoint Solutions, 360 Second
Avenue, Waltham, MA, 02451, USA
Email ssonis@pesclinical.com

Cells are 70% water, and thus water is an abundant target for ionizing radiation. Radiolysis during irradiation initiates the formation of both hydroxyl radical ($\bullet\text{OH}$), which plays a key role in radiation therapy tumor cell killing, and superoxide ($\text{O}_2^{\bullet-}$), a major cause of oxidative stress in normal cell damage, and uncontrolled, a conduit to impair normal cell survival.² In the hours following radiation, additional $\text{O}_2^{\bullet-}$ is generated as enzymes, such as the NADPH oxidases, are activated in irradiated cells.³ Then, for days and weeks after, inflammatory cells recruited in response to the immediate injury can continue to produce $\text{O}_2^{\bullet-}$ in substantial amounts.⁴

Given that cells are constantly under oxidative stress, including constitutive generation of $\text{O}_2^{\bullet-}$ from mitochondrial respiration, it is no surprise that internal mechanisms have evolved to maintain physiologic homeostasis. Among these mechanisms, $\text{O}_2^{\bullet-}$ converting enzymes were thought to play a major role and in 1969, McCord and Fridovich reported the discovery of the first superoxide dismutase (SOD).⁵ Superoxide dismutases are metalloproteins that are now known to exist in human cells in three isoforms: SOD1 (Cu/ZnSOD) in cytoplasm, SOD2 (MnSOD) in mitochondria, and SOD3 (EcSOD) in the extracellular space. While EcSOD is reported to be significantly decreased in several tumor types, MnSOD is probably the most significant in maintaining redox homeostasis and playing a protective role in response to RT as evidenced by the observation that its absence is incompatible with life.^{6,7}

Enzymatic degradation of superoxide occurs in two steps: first the dismutation of $\text{O}_2^{\bullet-}$ to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) which is mediated by SODs, and second the conversion of H_2O_2 by other enzymes. Radiation-induced $\text{O}_2^{\bullet-}$, however, represents an unusual and large burden that can overwhelm these mechanisms. So, based on these earlier findings and recognizing the potential of a SOD to potentially mitigate radiation-induced injury, in 1973 Lavelle et al assessed a protective role for SODs in preventing radiation-associated oxidative degradation of macromolecules and micro-organisms.⁸ Using *Photobacterium leiognathi* exposed to ultraviolet radiation they confirmed the importance of $\text{O}_2^{\bullet-}$ as a toxicity mediator and, importantly, observed that the addition of an exogenous source of SOD effectively mitigated the $\text{O}_2^{\bullet-}$ toxic effects.

Potential of SODs as a Radioprotectant

Shortly thereafter, Petkau and his collaborators took the important step of demonstrating that SOD had the potential to be applied therapeutically as a radioprotectant. They found that SOD (source not identified, but presumably bovine) injected intravenously into mice 1 hour before total body irradiation, effectively increased the 30-day LD_{50} by 12% from 627 rads [1 rad = 0.01 Gy] to 700 rads.⁹ Pre-radiation SOD also effectively resulted in a reduction in lethality following exposure to 650 rads from 72.5% in a saline control arm to 20.8% with exogenous SOD. Inactivated SOD was ineffective, confirming the role of dismutase enzymatic activity.

In a subsequent study, the same group reported that bovine SOD radioprotection was markedly impacted by the schedule of SOD administration.¹⁰ While SOD infusions immediately before (radioprotective factor 2.7) or after radiation (radioprotective factor 1.9) were protective, a schedule in which both times were used produced even more dramatic results (radioprotective factor 9.5).

Also, in 1975, Marberger et al¹¹ described a new drug with anti-inflammatory properties. Orgotein was a bovine-derived Cu/Zn SOD and within a year, the radioprotective potential of the drug was suggested by studies in patients receiving pelvic radiation who were at risk for treatment-associated cystitis.¹² By the end of 1977, a series of patents had been filed by Huber et al with the US Patent Office for Orgotein and its use to protect against radiation therapy toxicities. Additional clinical studies performed largely by the same group of investigators described the efficacy of Orgotein administered either intravenously or subcutaneously in mitigating radiation-associated therapy-induced cystitis and/or intestinal toxicity (diarrhea) in patients treated for cancers of the bladder, prostate, or rectum.¹³ A 2002 report also describes the efficacy (“some benefit”) of an aerosolized formulation of the drug in managing radiation-associated toxicities in patients being treated for cancers of the head and neck.¹⁴ Orgotein’s efficacy in mitigating late radiation toxicities was further described in a randomized trial of 100 patients who were treated with pelvic irradiation in which the drug was administered throughout the course of therapy. At two years following cancer therapy, patients who received Orgotein were 37% less likely to develop late toxicities vs controls.¹⁵

In 1994, Orgotein was granted orphan drug status by the US FDA for patients with mutations in Cu/Zn SOD and was approved for use in humans for a short period in some European countries. However, in contrast to the positive results noted above, a trial in which cystitis and proctitis were evaluated in patients being treated for bladder cancer had to be discontinued early (planned accrual 60 vs actual 30) due to unacceptable injection site side effects associated with subcutaneously administered Orgotein and no benefit was reported relative to the toxicity endpoints.¹⁶ As concerns grew about adverse reactions to the drug, perhaps due to its bovine origin, Orgotein was withdrawn as a human pharmaceutical.

Nonetheless, in aggregate, the results of the Orgotein clinical trials demonstrated two important findings which suggested the further development of SODs as an intervention for radiation-induced toxicities – one related to safety, the other to efficacy. With respect to safety, interventions for cancer regimen-related toxicities share a potential vulnerability, they could mitigate normal tissue toxicity at the cost of interfering with the anti-cancer effectiveness of the treatment. For example, palifermin (huKGF1) was successful in reducing the duration and incidence of severe oral mucositis in patients receiving concomitant chemoradiation for the treatment of head and neck cancers.¹⁷ But, given the presence of KGF1 receptors on tumor cells, it appeared to do so at the expense of CRT effectiveness. In contrast, Orgotein did not impact tumor response. In fact, as would be demonstrated later, SODs impact normal and tumor cells in different ways and may enhance the tumoricidal effects of CRT. With respect to efficacy, while not absolute, Orgotein appeared to confer normal tissue radioprotection consistently with different doses, formulations, and routes of administration. In agreement with pre-clinical findings, proximity of Orgotein administration to radiation was also associated with the protective signal.

Further confirmation of SOD utility for radiation-associated toxicities was suggested in 1994, when Delanian et al¹⁸ reported the results of an open-label clinical trial in 34 patients who received liposomal bovine Cu/Zn, months to years after radiation therapy for tumors at a range of sites. The liposomal preparation was given as twice weekly intramuscular injections for 3 weeks. The authors reported significant clinical benefit in the reduction of the long-standing post-irradiation fibrosis.

Despite these successes, the pharmacological value of native bovine SOD as a radioprotectant was undermined

by four factors: large molecular size which impaired permeability, limited half-life, antigenicity, and cost. Recognizing the therapeutic potential of SODs and seeking to bypass the drawbacks associated with simply infusing the enzyme, two approaches emerged: using gene therapy to boost host expression of SOD;¹⁹ and developing small molecule mimetics which not only avoided the limitations of the native enzymes but also could be engineered for improved behavior and efficacy.²⁰

SOD Gene Therapy

In 1991, Inoue et al described the delivery of a fusion gene coding for Cu/Zn SOD to harness the protective effects of SOD against tissue injury in an animal model of postischemic reflow arrhythmias.²¹

Subsequent applications of gene therapy to enhance SOD function have used both non-viral and viral vectors. As noted by Maier et al,¹⁹ each approach has both merits and drawbacks. While viral vectors tend to more efficiently affect gene transfer (and are therefore more popular in animal models), they come with baggage as they may invoke inflammatory and immune responses and are not easily produced. In contrast, non-viral vectors such as plasmid/liposomes are more biologically benign and cheaply produced, but at the expense of being less efficient.

Using topically applied MnSOD-plasmid liposome gene therapy, Greenberger's group successfully demonstrated radioprotective activity in the absence of adverse tumor outcomes for a range of radiation-induced tissue injuries in animal models, including oral mucositis. Application of the HuSOD2 transgene by orally administering a plasmid/liposome vector to mice an hour before a single 30 Gy radiation dose reduced the development of mucosal ulceration 5 days later compared to controls. Importantly, there was no radioprotection of orthotopic xenografts in mice overexpressing HuSOD2 after transgene administration.²² The same group demonstrated radioprotective effects on normal lung tissue using the MnSOD transgene. They also observed prolonged survival of mice radiated for the treatment of orthotopic tumors with intravenously transgene-treated animals, showing a reduction in the trajectory of tumor growth. This tumor growth inhibition was attributed to a radiosensitizing action on tumor cells due to H₂O₂ produced from O₂^{•-}, in contrast to the radioprotective effect on normal tissue from removing O₂^{•-}.²³

Greenberger subsequently tested the clinical safety and feasibility of a HuSOD2 plasmid liposome approach in a small (n=10) Phase 1 dose-escalation study in patients receiving concomitant chemoradiation for the treatment of locally advanced non-small cell lung cancer.²⁴ The objective of the trial was to identify a potential transgene dose which could be used to mitigate radiation-induced esophagitis as earlier pre-clinical results supported this indication.^{25,26} The plasmid liposome approach was safe in these patients but unfortunately failed to demonstrate expression of the plasmid.

Viral vectors have also been used to transfer SOD2 genes for radioprotection. Mice injected intraluminally with human MnSOD gene using a herpes virus vector were protected from injurious changes of the small intestine as evidenced by villi morphology after irradiation.²⁷ Radioprotection of hematopoiesis was also reported with retroviral transfer of the SOD2 genes in an erythroleukemic cell line.²⁸ And Yan et al confirmed the radioprotective potential of MnSOD using a murine model in which recombinant adeno-associated virus 2 was the gene transfer vector in a radiation skin injury model.²⁹

Development and Characterization of M40403 – A Small Molecule Superoxide Dismutase Mimetic

While the potential therapeutic value offered by the anti-inflammatory actions of the bovine SOD enzyme, Orgotein, was significant, this drug had several properties that limited its clinical utility. Most troubling was the immunogenicity of the whole protein which was sourced from cows and resulted in reported hypersensitivity reactions, the cessation of clinical trials, and ultimately removal from the market.³⁰ Its large size (MW of 32K), limited cell permeability, short circulating half-life, and bell-shaped dose-response were also problematic.³¹ But the mechanistic potential of increasing SOD activity as an anti-inflammatory and radiation cytoprotective agent remained obvious.

With the challenges facing gene therapy approaches to increasing SOD activity, especially when treating an acute syndrome such as radiation exposure rather than chronic disease, attention was focused on small molecule SOD mimetics. Following the acquisition of G.D. Searle in 1985, these observations spurred researchers at Monsanto led by Riley to seek to develop a synthetic SOD enzyme, or “dismutase mimetic,” building on the

chemical company’s expertise in catalysts. Their objective was to overcome the limitations of the naturally occurring SODs as human drugs, while simultaneously maintaining or increasing enzymatic activity and selectivity. In addition, they also noted that if the compound was to have therapeutic value, it needed to have chemical and kinetic stability.³² And in 1994, the group reported on their successful synthesis of a prototypical dismutase mimetic manganese pentaazamacrocyclic (MnPAM) complex.³³ Further development of this class increased SOD activity and stability, ultimately resulting in the first MnPAM dismutase mimetic to enter the clinic, M40403. This molecule’s dismutation rate of $O_2^{\bullet-}$ was comparable to natural SODs, its molecular weight was only 483, it was stable for up to 10 hours in whole blood at body temperature, and it demonstrated anti-inflammatory activity in animal models.

Further characterization of M40403 revealed its biological activities and its specificity.³⁴ Using a rat model for carrageenan-induced pleurisy, Salvemini³⁵ and her colleagues reported a reduction in pleurisy-associated histopathologic changes in M40403-treated rats which was accompanied by a reduction of pro-inflammatory cytokines, ICAM-1, P-selectin, nitrotyrosine and PARS. Importantly, and in contrast to other dismutase mimetics, M40403 demonstrated a selective effect on the removal of $O_2^{\bullet-}$ and did not react with other oxygen species, such as $\bullet OH$ and H_2O_2 . Functionally, such specificity avoided reactions with dismutation by-products and thus avoided the potential to produce more injurious radicals.³⁶

Recognizing the importance of oxidative stress as a common initiator, facilitator and catalyst of many diseases and the value of superoxide dismutase as a mitigator, M40403 was tested in animal models for a broad range of indications. Using a standard chemically induced IBD rat model (TNBS), rats receiving daily intraperitoneal administration of M40403 were observed to have a reduction in weight loss and bloody diarrhea, which coincided with amelioration of intestinal pathohistologic changes, foreshadowing the MnPAM class’ potential to protect oral mucosa.³⁷ Similar evidence of clinical potential was reported when the drug was used as monotherapy or synergistically with an immunosuppressive agent. In a ligature-induced model periodontal disease, a course of i.p. administered M40403 resulted in a reduction in inducible nitric oxide synthase activity, lipid peroxidation, nitrotyrosine formation, and neutrophil infiltration.³⁸ Concomitant administration of M40403 with methotrexate

also protected rats from the induction of erosive changes in articular cartilage and bone resorption following collagen challenge in an established rat model.³⁹ Similar findings were reported in the same model when M40403 was paired with dexamethasone.⁴⁰

M40403 and Avasopasem Manganese (GC4419) in the Mitigation of Radiation Therapy-Induced Mucosal Injury

Radiation therapy is a staple of current cancer therapy and will be used in half of the over 1.8 million patients with newly diagnosed cancers in 2021. While radiotherapy is critical to cancer management, its inability to differentiate between normal and cancer cells results in a significant toxicity profile. For patients treated with radiation therapy for cancers of the head and neck, or of the central lung, damage to the mucosa of the upper aerodigestive tract is common. Virtually all such patients develop ulcerative forms of mucositis, while two-thirds have such severe forms that their ability to eat solid foods is compromised and as many as one-third cannot drink liquids. This condition results in uncontrollable pain, loss of function, increased local and systemic infection risk, and increases in the cost of care. Additionally, patients' inability to tolerate these toxicities leads to breaks in the treatment continuity or the decision to stop treatment altogether.

Much has been learned about mucositis pathogenesis in the past two decades.⁴¹ It is now clear that the major impact of radiation on renewing epithelial stem cells is derived from the activation and potentiation of a biological cascade in which oxygen-free radicals such as $O_2^{\bullet-}$ play a critical initiating and potentiating role. Subsequently, activation of key transcription factors (NF- κ B) results in enhanced gene expression and pathway enablement which provide mediators leading to epithelial apoptosis and necrosis. Based on the impact of radiation on $O_2^{\bullet-}$ formation, the links between ROS, NF- κ B and tissue injury and M40403's mechanism of action and clinical impact, the molecule seemed to be a rational intervention for mucositis.

In 2006, Murphy et al reported using a highly translatable animal model⁴² to demonstrate the ability of M40403 to interfere with mucositis incidence, severity, and trajectory.⁴³ The cheek pouch mucosae of golden Syrian hamsters were irradiated with a single dose of 40 Gy in this model and the course of radiation-induced ulcerative

oral mucositis was consistent with peak ulceration occurring between 14 and 18 days after challenge. The clinical presentation of mucositis in the hamsters was also virtually identical to that seen in cancer patients, supporting the translatable value of the model. Mucosae were evaluated daily, and photographs were taken on alternate days beginning 6 days after radiation and continuing until day 28. At the conclusion of the in-life phase of the investigation, photographs were graded for mucositis severity by observers blinded to study group assignment. In addition to a vehicle control, three dose levels of M40403 were studied using different dosing schedules, and the results were compelling. M40403 significantly attenuated both the severity and duration of ulcerative mucositis across the range of schedules and doses tested compared to controls.

Confirmation of M40403's effectiveness as a radioprotectant of normal tissues was provided by Thompson et al in a subsequent pre-clinical assessment in which the molecule was shown to favorably impact the survival of mice receiving otherwise lethal doses of total body irradiation.⁴⁴ At the top M40403 dose in this study, 100% of treated mice survived, while all the saline control mice were dead within 18 days. Saline control mice also exhibited massive damage to intestinal mucosa, while the intestinal mucosa appeared normal in the M40403-treated mice. Subsequent experiments with other MnPAM dismutase mimetics, such as GC4401, have demonstrated that this radioprotection of normal tissues is a class activity.⁴⁵

Based on this extensive mechanistic and preclinical support, in 2013, the enantiomer of M40403, avasopasem manganese, was taken forward into clinical trials for reduction of radiation therapy normal tissue toxicity. Like M40403, avasopasem is a highly stable, low molecular weight (483) MnPAM dismutase mimetic with specificity for removing superoxide anions. Also, like M40403, Sishc has demonstrated that avasopasem is effective at preventing and mitigating radiation normal tissue toxicity in mouse models of radiation-induced pulmonary fibrosis and oral mucositis.^{46,47} Intriguingly, these reports also suggest potential for avasopasem in the particularly refractory setting of reirradiation. In addition, the question of whether older cancer patients are more susceptible to treatment toxicities has been ongoing for many years, with potential relevance in HNC radiation therapy given the age of some patients. So, it might be significant that Mapuskar noted a superoxide-driven difference in radiosensitivity in human fibroblasts the age of cell donors, and that avasopasem reverses that sensitivity.⁴⁸ Importantly,

a paper by El-Mahdy et al reported avasopasem's concurrent ability to protect normal epithelial cells, while sensitizing tumor cells to radiation-induced destruction.⁴⁹

As reported by Anderson et al in 2018, to assess the safety and preliminarily evaluate efficacy in patients at risk for severe oral mucositis (SOM), avasopasem was first tested in a trial of eleven different dose and schedule cohorts in patients being treated with chemoradiation for selected cancers of the head and neck.⁵⁰ The study population consisted of 46 patients who received concomitant chemoradiation (cisplatin weekly or tri-weekly with a standard daily 2 Gy fractionation schedule). Avasopasem was administered by a one-hour IV infusion before the delivery of each radiation fraction. To assure equivalence of mucositis risk, all patients had to have had two oral mucosal at-risk sites within the 50 Gy isodose line of the initial radiation therapy plan. Although the number of patients within each cohort was small because of the pilot nature of the study, both the incidence and duration of SOM (defined as WHO grade 3 or 4) were less than historical controls in the three cohorts totaling 14 patients who were treated throughout their total radiation course. While dosing for shorter periods during the earlier weeks of the radiotherapy course favorably impacted duration, the effect was not as consistent or dramatic compared to in these three cohorts dosing throughout the entire course.

These encouraging pilot Phase 1b/2a results supported a subsequent a Phase 2b double-blind, placebo-controlled trial in which two doses of avasopasem (30 mg and 90 mg) were compared to placebo in 223 patients recruited at 44 different North American study sites. As in the earlier trial, patients in the Phase 2b were those who received the highly stomatotoxic regimen of concomitant chemoradiation for the treatment of locally advanced oral cavity or oropharyngeal squamous cell cancers. Radiation was administered using standard intensity modulating technique (IMRT) in 2 Gy fractions for a minimum cumulative dose of 60 Gy (maximum 72 Gy) again with at least two oral sites at risk included in the 50 Gy isodose line. Radiation dosing was also confirmed by an independent radiation oncologist. Avasopasem or placebo was administered by a one-hour IV infusion immediately before each radiation dose. Oral mucositis was assessed twice weekly using WHO scoring criteria by a cadre of uniformly trained assessors at each study site. Scoring accuracy was evaluated for each visit by an independent subject expert.⁵¹

Avasopasem impacted SOM in a dose-responsive manner with the 90 mg dose significantly attenuating both the duration (the study's primary clinical endpoint), incidence and severity of SOM. Improved (reduced) SOM duration was statistically significant for the 90 mg group compared with the placebo group by a non-parametric test ($p=0.024$) but not significant for the 30 mg group vs the placebo group. The median SOM duration was 19 days for patients treated with placebo, but only 1.9 days in patients who received the 90 mg avasopasem dose and 8 days for patients receiving the 30 mg dose. Because study subjects who never developed SOM were considered to have an SOM duration of 0 days in this analysis, the difference in duration was partly attributable to the observation that 90 mg of avasopasem effectively prevented the development of SOM. Whereas 65% of placebo patients developed SOM at any time during IMRT, the incidence was reduced to 43% in individuals who received 90 mg of avasopasem (nominal $p = 0.009$). Incidence results for the 30 mg group were intermediate between those for 90 mg and placebo. While both WHO grades of 3 and 4 were included in the definition of SOM, a closer assessment demonstrated that the incidence of grade 4 mucositis (an assessment which has been described as the "severity" of SOM) was significantly blunted (16% for 90 mg avasopasem vs 30% for placebo; nominal $p=0.045$). This finding is of particular importance relative to its potential impact on mitigating the incremental cost of treatment (over \$32,000US)⁵² associated with the development of SOM in the study population. There was no apparent relationship between SOM results and patients' HPV status, cisplatin regimen, smoking status, or whether IMRT was given as definitive or adjunctive (ie, post-surgical resection) treatment. Safety was comparable between study groups. No significant avasopasem-specific toxicities were identified, nor were cisplatin-radiation toxicities exacerbated by the study drug. The incidence of the three most common adverse events (lymphopenia, nausea and fatigue) was equivalent across cohorts and consistent with those anticipated with concomitant chemoradiation.

Additionally, the results of the Phase 2b trial suggest that, in addition to its radioprotective impact on oral mucositis, avasopasem also reduced the incidence and severity of cisplatin-related nephrotoxicity.⁵³ This is consistent with the reported role of $O_2^{\bullet-}$ in cisplatin nephrotoxicity and avasopasem's ability in mouse models to prevent this.⁵⁴ Given the significant role of high-dose

cisplatin chemoradiation in this population, this could be an attractive additional benefit.

In addition to confirming the safety of GC4419, the Phase 2b trial answered two other pragmatic questions, one of formulation and route of administration, and the other of impact on tumor response. With respect to the first, daily IV infusion of GC-4419 was not a significant barrier for either site or patient study participation. In addition, clinical trial enrollment, subject retention, and adherence to the study treatment were excellent.

Mitigation of any supportive care indication which is based on the protection of normal cells from a cytotoxic challenge comes with the hypothetical risk that tumor tissue may also be protected. Earlier animal model studies have suggested that avasopasem not only does not decrease the anti-cancer efficacy of radiation, but with higher dose per fraction can increase it substantially⁴⁶ and, while simultaneously protecting the mucosa can enhance tumor kill following radiation and immunoradiotherapy.⁴⁷ In 2020, Anderson reported that long-term follow-up of Phase 2b participants confirmed that GC-4419 did not negatively impact tumor response (overall survival, progression-free survival, locoregional control, distant metastases) one and two years^{55,56} following treatment.

A phase 3 trial of avasopasem to reduce SOM in this same population (NCT03689712) is ongoing.

Conclusion

Oxidative stress driven by superoxide formation plays a critical role in the development and perpetuation of a broad range of maladies. Maintenance of homeostasis in the face of the controlled normal trickle of free radicals is largely provided by a series of enzymes of which the SODs are critical. While three human isoforms of SOD exist, it is the manganese metalloprotein which may be the most essential – its absence is incompatible with life. Given its value as in the neutralization of toxic free radicals, harnessing natural and synthesized forms for therapeutic use has been of interest for more than three decades. While naturally occurring proteins or gene transfer techniques have validated the potential therapeutic value of SODs, logistical, biological, functional and toxicity issues have limited their clinical applicability.

The development of a novel class of dismutase mimetics which provide superior pharmacokinetics, safety, duration, and enzymatic activity seems likely to serve as the therapeutic basis for an effective approach to intervene

in a range of pathologies. Among these is the tissue injury complex associated with the use of cytotoxic cancer therapies, and particularly toxicities associated with radiotherapy. In these settings, the superoxide levels may be very high, and that SOD mimetics have potential in this indication is recognized by interest in the area.^{57,58}

Of the compounds in the broad area of dismutase mimetics, the MnPAM class, and specifically avasopasem manganese, have the advantage of being both highly active and specific for the superoxide moiety. The utility of avasopasem to mitigate highly toxic radiation normal tissue injury has been suggested by the results of clinical trials in patients receiving chemoradiotherapy for cancers of the head and neck, without evidence that antitumor efficacy is at risk of being compromised. Accordingly, avasopasem has the potential to meet the unmet clinical need for an effective mucositis intervention. Separately, the observation of mechanism-related increased antitumor effect with high dose per fraction radiotherapy suggests promise for drugs of this class when used in combination with ultrahypofractionated radiotherapy, that is, radiotherapy regimens in which prescribed doses of 7–8 Gy or greater are administered for 1–5 fractions.

Disclosure

Dr Stephen T Sonis reports personal fees from Biomodels, LLC, personal fees from Primary Endpoint Solutions, during the conduct of the study. In addition, Dr. Sonis has the following issued patents: 6,458,777; 6,663,850; 6,713,463; 6,841,578B2; 7,297,123; and 10,475,53922; he is an employee of Biomodels LLC and Primary Endpoint Solutions LLC. Both companies assist government, academic and industry clients with the planning, implementation, execution and analysis of pre-clinical and clinical studies to assist and enable the development of new drugs, biologicals and devices for a wide range of indications, including side effects of cancer treatment. He does not receive direct payment for any client and he does not have equity in any of the companies which work with us. The author reports no other conflicts of interest in this work.

References

1. Gershman R, Gilbert DL, Nye SW, et al. Oxygen poisoning and X-irradiation: a mechanism in common. *Science*. 1954;119:623–626. doi:10.1126/science.119.3097.623
2. Azzam EI, Jay-Gerin JP, Pain D, et al. Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. *Cancer Lett*. 2012;327:48–60.

3. Collins-Underwood JR, Zhao W, Sharpe JG, et al. NADPH oxidase mediates radiation-induced oxidative stress in rat brain microvascular endothelial cells. *Free Radic Biol Med.* 2008;45:929–938. doi:10.1016/j.freeradbiomed.2008.06.024
4. Lei Y, Wang K, Deng L, et al. Redox regulation of inflammation: old elements, a new story. *Med Res Rev.* 2015;35:306–340.
5. McCord JM, Fridovich I. Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). *J Biol Chem.* 1969;244:6049–6055. doi:10.1016/S0021-9258(18)63504-5
6. Ighodaro OM, Akinloye OA. First line defence antioxidants – superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. *Alexandria J Med.* 2018;54:287–293. doi:10.1016/j.ajme.2017.09.001
7. Griess B, Tom E, Domann F, et al. Extracellular superoxide dismutase and its role in cancer. *Free Rad Biol Med.* 2017;112:464–479.
8. Lavelle F, Michelson AM, Dimitrijevic L, et al. Biological protection by superoxide dismutase. *Biochem Biophys Res Comm.* 1973;55:350–357. doi:10.1016/0006-291X(73)91094-2
9. Petkau A, Chelack WS, Pleskach SD, et al. Radioprotection of mice by superoxide dismutase. *Biochem Biophys Res Comm.* 1975;65:886–893. doi:10.1016/S0006-291X(75)80468-2
10. Petkau A, Chelack WS, Pleskach SD, et al. Protection of post-irradiated mice by superoxide dismutase. *Int J Radiat Biol.* 1976;29:297–299.
11. Marberger H, et al. Orgotein: a new anti-inflammatory metalloprotein drug: preliminary evaluation of clinical efficacy and safety in degenerative joint disease. *Curr Ther Res Clin Exp.* 1974;16:706–717.
12. Marberger H, Bartsch G, Huber W, et al. Orgotein: a new drug for the treatment of radiation cystitis. *Curr Ther Res Clin Exp.* 1975;18:466–475.
13. Edsmyr F, Menander-Huber KB. Orgotein efficacy in ameliorating side effects due to radiation therapy. *Eur J Rheumatol Inflamm.* 1981;4:228–236.
14. Escribano A, Garcia-Grande A, Montanes P, et al. Aerosol orgotein (Ontosein) for the prevention of radiotherapy-induced adverse effects in head and neck cancer patients: a feasibility study. *Neoplasma.* 2002;49:201–208.
15. Esco R, Valencia J, Coronel P, et al. Efficacy of Orgotein in prevention of late side effects of pelvic irradiation: a randomized study. *Int J Radiat Biol Phys.* 2004;60:10–1211. doi:10.1016/j.ijrobp.2004.04.038
16. Nielsen OS, Overgaard J, Overgaard M, et al. Orgotein in radiation treatment of bladder cancer. *Acta Oncologica.* 1987;26:101–105. doi:10.3109/02841868709091748
17. Le QT, Kim HE, Schneider CJ, et al. Palifermin reduces severe mucositis in definitive chemoradiotherapy of locally advanced head and neck cancer: a randomized, placebo-controlled study. *J Clin Oncol.* 2011;29(20):2808–2814. doi:10.1200/JCO.2010.32.4095
18. Delanian S, Baillet F, Huart J, et al. Successful treatment of radiation-induced fibrosis using liposomal Cu/Zn superoxide dismutase: clinical trial. *Radiother Oncol.* 1994;32:12–20. doi:10.1016/0167-8140(94)90444-8
19. Maier P, Veldwijk MR, Wenz F, et al. Radioprotective gene therapy. *Exp Opin Biol Ther.* 2011;11(9):1135–1151. doi:10.1517/14712598.2011.580271
20. Bonetta R. Potential therapeutic application of MnSODs and SOD-mimetics. *Chem.* 2018;24:5032–5041. doi:10.1002/chem.201704561
21. Inoue M, Watanabe N, Utsumi T, et al. Targeting SOD by gene and protein engineering and inhibition of free radical injury. *Free Radic Res Comm.* 1991;P1:391–399. doi:10.3109/10715769109145809
22. Guo H, Seixas-Silva JA, Epperly MW, et al. Prevention of radiation-induced oral cavity mucositis by plasmid/liposome delivery of the human manganese superoxide dismutase (SOD2) transgene. *Radiat Res.* 2003;159:361–370. doi:10.1667/0033-7587(2003)159[0361:PORIOC]2.0.CO;2
23. Epperly MW, Wegner R, Kanai AJ, et al. Effects of MnSOD-plasmid liposome gene therapy on antioxidant levels in irradiated murine oral cavity orthotopic tumors. *Radiat Res.* 2007;167:289–297. doi:10.1667/RR0761.1
24. Tarhini AA, Belani CP, Luketich JD, et al. A phase I study of concurrent chemotherapy (paclitaxel and carboplatin) and thoracic radiotherapy with swallowed manganese superoxide dismutase plasmid liposome protection in patients with locally advanced stage III non-small-cell lung cancer. *Human Gene Ther.* 2011;22:336–342. doi:10.1089/hum.2010.078
25. Epperly MW, Sikora C, Defilippi S, et al. Plasmid/liposome transfer of the human manganese superoxide dismutase transgene prevents ionizing irradiation-induced apoptosis in human esophagus organ explant culture. *Int J Cancer.* 2000;90:128–137. doi:10.1002/1097-0215(20000620)90:3<128::AID-IJC2>3.0.CO;2-U
26. Stickle RL, Epperly MW, Klein E, et al. Prevention of irradiation-induced esophagitis by plasmid/liposome delivery of the human manganese superoxide dismutase transgene. *Radiat Oncol Investig.* 1999;7(4):204–217. doi:10.1002/(SICI)1520-6823(1999)7:4<204::AID-ROI2>3.0.CO;2-S
27. Guo HL, Wolfe D, Epperly MW, et al. Gene transfer of human manganese superoxide dismutase protects small intestinal villi from radiation injury. *J Gastrointest Surg.* 2003;7:229–236. doi:10.1016/S1091-255X(02)00186-5
28. Southgate TD, Sheard V, Milsom MD, et al. Radioprotective gene therapy through retroviral expression on manganese superoxide dismutase. *J Gene Med.* 2006;8:557–565. doi:10.1002/jgm.890
29. Yan S, Brown SL, Kolozsvary A, et al. Mitigation of radiation-induced skin injury by AAV2-mediated MnSOD gene therapy. *J Gene Med.* 2008;10:1012–1018. doi:10.1002/jgm.1226
30. Petkau A. Scientific basis for the clinical use of superoxide dismutase. *Cancer Treat Rec.* 1986.
31. Salvemini D, Riley DP, Cuzzocrea S, et al. SOD mimetics are coming of age. *Nat Rev Drug Dis.* 2002;1:367–379. doi:10.1038/nrd796
32. Riley DP, Schall OF. Structure-activity studies and design of synthetic superoxide dismutase (SOD) mimetics as therapeutics. *Adv Inorganic Chem.* 2007;59:233–263.
33. Riley DR, Weiss RH. Manganese macrocyclic ligand complexes as mimics of superoxide dismutase. *J Am Chem Soc.* 1994;116:387–388. doi:10.1021/ja00080a051
34. Salvemini D, Wang ZQ, Zweier JL, et al. A nonpeptidyl mimic of superoxide dismutase with therapeutic activity in rats. *Science.* 1999;286:304–306. doi:10.1126/science.286.5438.304
35. Salvemini D, Mazzon E, Dugo L, et al. Pharmacological manipulation of the inflammatory cascade by the superoxide dismutase mimetic, M40403. *Brit J Pharmacol.* 2001;132:815–827. doi:10.1038/sj.bjp.0703841
36. Muscoli C, Cuzzocrea S, Riley DP, et al. On the selectivity of superoxide dismutase mimetics and its importance in pharmacological studies. *Brit J Pharmacol.* 2003;140:445–460. doi:10.1038/sj.bjp.0705430
37. Cuzzocrea S, Mazzon E, Dugo L, et al. Protective effects of M40403, a superoxide dismutase mimetic, in a rodent model of colitis. *Eur J Pharmacol.* 2001;432:79–89. doi:10.1016/S0014-2999(01)01427-3
38. DiPaola R, Mazzon E, Rotondo F, et al. Reduced development of experimental periodontitis by treatment with M40403, a superoxide dismutase mimetic. *Eur J Pharmacol.* 2005;516:151–157. doi:10.1016/j.ejphar.2005.04.039
39. Cuzzocrea S, Mazzon E, Di Paola R, et al. Synergistic interaction between methotrexate and a superoxide dismutase mimetic: pharmacologic and potential clinical significance. *Arthritis Rheum.* 2005;52:3755–3760. doi:10.1002/art.21480
40. Cuzzocrea S, Mazzon E, Paola RD, et al. Effects of combination of M40403 and dexamethasone therapy on joint disease in a rat model of collagen-induced arthritis. *Arthritis Rheum.* 2005;52:1929–1940. doi:10.1002/art.21044

41. Bowen J, Al-Dasoqi N, Bossi P, et al. The pathogenesis of mucositis: updated perspectives and emerging targets. *Support Care Cancer*. 2019;27:4023–4033. doi:10.1007/s00520-019-04893-z
42. Sonis ST. Animal models for mucosal toxicity. In: Teicher B, editor. *Animal Models in Cancer Research*. Totowa, NJ: Humana Press; 2002.
43. Murphy CK, Fey EG, Watkins BA, et al. Efficacy of superoxide dismutase mimetic M40403 in attenuating radiation-induced oral mucositis in hamsters. *Clin Cancer Res*. 2008;14:4292–4297. doi:10.1158/1078-0432.CCR-07-4669
44. Thompson JS, Chu Y, Glass J, et al. The manganese superoxide dismutase mimetic, M40403, protects adult mice from lethal total body irradiation. *Free Rad Res*. 2010;44:529–540. doi:10.3109/10715761003649578
45. Coleman MC, Olivier AK, Jacobus JA, et al. Superoxide mediates acute liver injury in irradiated mice lacking sirtuin 3. *Antioxid Redox Signalling*. 2014;20:1423–1435. doi:10.1089/ars.2012.5091
46. Sishc BJ, Polsdofer E, Bloom DA, et al. The radioprotector GC4419 ameliorates radiation induced lung fibrosis while enhancing the response of non-small cell lung cancer tumors to high dose per fraction radiation exposures. *Int J Rad Oncol Biol Phys*. 2019;105(1):E680. doi:10.1016/j.ijrobp.2019.06.1002
47. Sishc BJ, Saha D, Story MD, et al. Avasopasem manganese protects against radiation induced oral mucositis and enhances the response of squamous cell carcinoma of the head and neck to ionizing radiation and radioimmune therapy. *Int J Radiat Oncol Biol Phys*. 2020;108: S159. doi:10.1016/j.ijrobp.2020.07.921
48. Mapuskar KA, Flippo KH, Schoenfeld JD, et al. Mitochondrial superoxide dismutase increases age-associated susceptibility of human dermal fibroblasts to radiation and chemotherapy. *Cancer Res*. 2017;77:5054–5067. doi:10.1158/0008-5472.CAN-17-0106
49. El-Mahdy MA, Alzarie YA, Hemann C, et al. The novel SOD mimetic GC4419 increases cancer cell killing with sensitization to ionizing radiation while protecting normal cells. *Free Rad Biol Med*. 2020;160:630–642. doi:10.1016/j.freeradbiomed.2020.07.032
50. Anderson CM, Sonis ST, Lee CM, et al. Phase 1b/2a trial of superoxide dismutase mimetic GC4419 to reduce chemoradiotherapy-induced oral mucositis in patients with oral cavity or oropharyngeal carcinoma. *Int J Radiat Oncol Biol Phys*. 2018;100:427–435. doi:10.1016/j.ijrobp.2017.10.019
51. Anderson CM, Lee CM, Saunders DP, et al. Phase 2b, randomized, double-blind trial of GC4419 vs placebo to reduce severe oral mucositis in head and neck cancer patients receiving concurrent radiotherapy and cisplatin. *J Clin Oncol*. 2019;37:3256–3265. doi:10.1200/JCO.19.01507
52. Hoffbauer M, Fineberg J, Stattenfield R, Holmlund J. Cost of radiation-induced oral mucositis in head and neck cancer patients: an administrative claims analysis. *JMCP*. 2020;26(10–a Suppl):S31 (abs C48).
53. Steinbach EJ, Monga V, Furqan M, et al. Effects of GC4419 (avasopasem manganese) on chronic kidney disease in head and neck cancer patients treated with radiation and cisplatin. *J Clin Oncol*. 2020;38(15 suppl):12071. doi:10.1200/JCO.2020.38.15_suppl.12071
54. Mapuskar KA, Wen H, Holanda DG, et al. Persistent increase in mitochondrial superoxide mediates cisplatin chronic kidney disease. *Redox Biol*. 2019;20:98–106. doi:10.1016/j.redox.2018.09.020
55. Anderson CM, Lee CM, Saunders D, et al. Tumor outcomes of Phase IIb, randomized, double-blind trial of GC-4419 versus placebo to reduce severe oral mucositis due to concurrent radiotherapy and cisplatin for head and neck cancer. *Int J Radiat Oncol Biol Phys*. 2020;106:1221–1222. doi:10.1016/j.ijrobp.2020.02.004
56. Anderson CM, Lee CM, Saunders D, et al. 2-year outcomes of Phase IIb, randomized, double-blind trial of GC-4419 versus placebo to reduce severe oral mucositis due to concurrent radiotherapy and cisplatin for head and neck cancer. *Int J Radiat Oncol Biol Phys*. 2020;108:3836. doi:10.1016/j.ijrobp.2020.07.260
57. Ashcraft KA, Boss MK, Tovmasyan A, et al. A novel manganese-porphyrin superoxide dismutase-mimetic widens the therapeutic margin in a pre-clinical head and neck cancer model. *Int J Radiat Biol Phys*. 2015;93:892–900. doi:10.1016/j.ijrobp.2015.07.2283
58. Mapuskar KA, Anderson CM, Spitz DR, et al. Utilizing superoxide dismutase mimetics to enhance radiation therapy response while protecting normal tissues. *Semin Radiat Oncol*. 2019;29:72–80. doi:10.1016/j.semradonc.2018.10.005

Drug Design, Development and Therapy

Dovepress

Publish your work in this journal

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which has also

been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/drug-design-development-and-therapy-journal>