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Review article

Ozone Therapy Supports the Glutathione System as an Integrated Metabolic Network: From Physiological Regulation to Oncological Implications.

La ozonoterapia como apoyo al sistema del glutatión como red metabólica integrada: desde la regulación fisiológica hasta las implicaciones oncológicas

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Keywords

Ozone therapy
Glutathione
Oxidative stress
Reductive stress
Nrf2
Antioxidants
Hormesis
Ferroptosis
Supportive oncology

Abstract

Ozone therapy has long been discussed within an “antioxidant” framework, yet this framing obscures how it actually engages the glutathione system. This review reframes ozone therapy not as a mere antioxidant intervention but as a qualitatively distinct redox intervention that supports the glutathione system as an integrated metabolic network, and examines its implications in settings — such as oncology — where exogenous glutathione (GSH) and ozone must be sharply distinguished. Low-grade, transient oxidative signals generated during ozone therapy — including lipid electrophiles such as 4-hydroxynonenal — modify Kelch-like ECH-associated protein 1 (Keap1) and promote nuclear factor erythroid 2-related factor 2 (Nrf2) nuclear translocation, which in turn supports the GSH system at four coordinated levels: cystine import via the cystine/glutamate antiporter (xCT), de novo synthesis via glutamate–cysteine ligase, regeneration of reduced glutathione by glutathione reductase, and supply of nicotinamide adenine dinucleotide phosphate (NADPH) from the pentose phosphate pathway. This network-level perspective reorganizes the clinical meaning of glutathione across eight interlocking axes, spanning reactive oxygen and reactive nitrogen species handling, thiol switches (S-nitrosoglutathione [GSNO] and its reductase [GSNOR], and S-glutathionylation), immune and inflammatory modulation, infection defense, anti-glycation, xenobiotic detoxification, and mitochondrial integrity. In oncology, the same GSH system that protects the host can also support tumor survival through the xCT–GSH–glutathione peroxidase 4 (GPX4) axis and ferroptosis resistance. This dual nature requires that exogenous intravenous glutathione and ozone therapy — often grouped together as antioxidants — be sharply distinguished: intravenous glutathione produces supraphysiological plasma concentrations that raise conceptual concerns about reductive stress, whereas ozone therapy has been shown to elicit physiological, adaptive glutathione responses. Building on evidence that cluster of differentiation 8 ($CD8^+$) T-cell-derived interferon-gamma (IFN-γ) suppresses xCT, this paper proposes that ozone therapy may act upstream of the IFN-γ–xCT axis through immune priming rather than direct tumoricidal activity. Within this framework, a clinical perspective on relatively high-concentration, low-total-dose ozone regimens in oncology, together with biomarker-guided titration, is outlined. Prospective clinical validation of this framework remains to be established.

Palabras clave

Ozonoterapia
Glutatión
Estrés oxidativo
Estrés reductor
Nrf2
Antioxidantes
Hormesis
Ferroptosis
Oncología de soporte

Resumen

La ozonoterapia se ha interpretado tradicionalmente dentro de un marco conceptual de “antioxidante”. Sin embargo, esta visión simplifica y oculta la forma en que realmente interactúa con el sistema del glutatión. Esta revisión propone una nueva perspectiva, considerando la ozonoterapia no como una mera intervención antioxidante, sino como una intervención redox cualitativamente distinta que favorece el sistema del glutatión como una red metabólica integrada. Asimismo, analiza sus implicaciones en contextos como la oncología, donde es fundamental diferenciar claramente entre el glutatión exógeno (GSH) y la ozonoterapia. Las señales oxidativas leves y transitorias generadas durante la ozonoterapia —incluidos electrófilos lipídicos como el 4-hidroxinonenal (4-HNE)— modifican la proteína Keap1 y favorecen la translocación nuclear del factor Nrf2. Este proceso potencia el sistema del glutatión a cuatro niveles coordinados: Importación de cistina mediante el intercambiador cistina/glutamato (xCT); Síntesis de novo a través de la glutamato-cisteína ligasa; Regeneración del glutatión reducido mediante la glutatión reductasa; Aporte de NADPH procedente de la vía de las pentosas fosfato; Esta visión basada en redes metabólicas reorganiza el significado clínico del glutatión a través de ocho funciones interrelacionadas, que incluyen: Manejo de especies reactivas de oxígeno y nitrógeno; Regulación de los interruptores tiol (GSNO, GSNOR y S-glutationilación); Modulación inmunitaria e inflamatoria; Defensa frente a infecciones; Acción antiglicación; Desintoxicación de xenobióticos; Mantenimiento de la integridad mitocondrial.

En oncología, el mismo sistema del glutatión que protege al organismo también puede favorecer la supervivencia tumoral mediante el eje xCT–GSH–GPX4 y la resistencia a la ferroptosis. Esta doble naturaleza obliga a diferenciar claramente entre el glutatión intravenoso exógeno y la ozonoterapia, aunque ambos se agrupen con frecuencia bajo el término de “antioxidantes”.

Mientras que la administración intravenosa de glutatión genera concentraciones plasmáticas suprafisiológicas que pueden plantear preocupaciones relacionadas con el estrés reductor, la ozonoterapia parece inducir respuestas fisiológicas y adaptativas del sistema del glutatión.

Basándose en evidencias que muestran que el interferón gamma (IFN-γ) derivado de los linfocitos T CD8+ suprime la actividad de xCT, los autores proponen que la ozonoterapia podría actuar aguas arriba del eje IFN-γ–xCT mediante un mecanismo de activación inmunitaria, más que a través de una acción tumoral directa.

Dentro de este marco conceptual, se plantea una perspectiva clínica sobre el uso de regímenes de ozono de concentración relativamente alta y dosis total baja en oncología, junto con estrategias de ajuste terapéutico guiadas por biomarcadores. No obstante, los autores señalan que esta hipótesis requiere todavía validación clínica prospectiva.

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1. Introduction

Medical ozone has long been used in clinical practice, primarily as a modulator of reactive oxygen species (ROS) via major auto-haemotherapy (MAH) and adjacent administration routes (Sagai & Bocci, 2011). Its clinical rationale was framed historically as a controlled oxidative stimulus that elicits an adaptive cellular response, and this hormesis-based logic remains the cornerstone of the dominant mechanistic account (Bocci, Borrelli, Travagli, & Zanardi, 2009). Within this tradition, ozone therapy has often been positioned, at least in patient-facing communication, as an antioxidant intervention — a framing that is intuitively accessible but increasingly difficult to reconcile with current redox biology.

That ozone therapy supports the glutathione system is well documented and not in dispute. The conceptual question is how it does so. The simplified label "ozone = antioxidant therapy" carries a specific clinical hazard: it invites pharmacological conflation with substrate supplementation strategies such as intravenous glutathione (IV-GSH; reduced glutathione, GSH), as if the two acted along a common axis differing only in dose. Recent conceptual work has argued that this conflation is mechanistically misleading and that the two interventions are better understood as qualitatively different forms of redox modulation (Chirumbolo, Franzini, Ricevuti, & Valdenassi, 2025). This paper shares this reservation, and develops its implications in detail in §5.5.

Modern oxidative-stress biology no longer treats antioxidant defence as a single layer; it distinguishes between an enzymatic, preventive layer that constrains hydroxyl radical generation upstream and a radical-scavenging layer in which glutathione operates (Sies, Berndt, & Jones, 2017).[3] This two-layer view, which is established at §4.1 as a dual-layer antioxidant defence framework, allows ozone therapy to be re-positioned not as an intervention that contributes to the scavenging pool but as a transcriptional inducer that engages both layers — a positioning developed progressively across §2 to §5.

To address this conceptual gap, this paper proposes a reframing that takes the integrated nature of the glutathione system seriously and revisits the implications of this reframing for clinical settings where exogenous GSH and ozone are often conflated.

> *"This review reframes ozone therapy not as a mere antioxidant intervention but as a qualitatively distinct redox intervention that supports the glutathione system as an integrated metabolic network, and examines its implications in settings — such as oncology — where exogenous GSH and ozone must be sharply distinguished."*

In what follows, the argument is developed by tracing the mechanistic basis of ozone–GSH interaction, the integrated structure of the GSH system, its non-oncology physiological axes, and finally the oncology setting where the proposed distinction carries the greatest clinical weight. §2 establishes the mechanistic basis on which ozone therapy acts as a redox intervention rather than a mere antioxidant approach, §3 then articulates the GSH system as an integrated metabolic network rather than a single molecule, §4 surveys the non-oncology physiological axes through which this network operates — including a dual-layer antioxidant defence framework that is established at §4.1 — and §5 returns to the oncology setting, where the host-versus-tumour asymmetry of GSH biology requires the sharpest distinction between ozone therapy and exogenous GSH supplementation.

2. Ozone Therapy as a Redox Intervention, Not a Mere Antioxidant Approach

2.1 The 4-HNE / Keap1 / Nrf2 axis

Ozone therapy is often described in popular accounts as an "antioxidant" treatment, yet its primary chemistry is unambiguously oxidative. When medical-grade ozone (O_3) contacts plasma during major autohemotherapy or other delivery routes, it reacts rapidly with polyunsaturated fatty acids (PUFA) and thiol groups to generate

a defined family of secondary messengers: hydrogen peroxide (H_2O_2), short-lived ozonides, and α,β-unsaturated lipid electrophiles, of which 4-hydroxy-2-nonenal (4-HNE) is the prototypical example (Sagai & Bocci, 2011). These products, rather than ozone itself, constitute the actual biological signal delivered to the cell.

Among them, 4-HNE is mechanistically pivotal. As a soft electrophile, it covalently modifies reactive cysteine residues on Kelch-like ECH-associated protein 1 (Keap1), disrupting Keap1-mediated ubiquitination of nuclear factor erythroid 2-related factor 2 (Nrf2) and allowing Nrf2 to translocate to the nucleus and engage antioxidant response elements (Saito et al., 2016; Dayalan Naidu & Dinkova-Kostova, 2020). This electrophile-driven activation has been independently demonstrated in vivo following ozonated autohemotherapy (Re et al., 2014).

Crucially, the oxidative challenge produced by therapeutic ozone is calibrated rather than overwhelming. The "ozone paradox" — that a controlled oxidant elicits a net cytoprotective response — operates within a narrow therapeutic window characteristic of hormetic stimuli (Bocci et al., 2009), and clinical work in peripheral arterial disease has been shown to reproduce the U-shaped dose–response predicted by hormesis theory (Bocci, Zanardi, & Travagli, 2011). In short, ozone therapy does not supply antioxidants; it delivers low-grade, transient oxidative signals that instruct the cell to upregulate its own redox machinery — a distinction that frames every subsequent argument in this review.

2.2 Nrf2 Supports the Glutathione System at Four Levels

Beyond initiating Nrf2 nuclear translocation (§2.1), the more substantive question is what Nrf2 actually does for the glutathione (GSH) system once activated. Nrf2 functions as a master transcriptional coordinator that, via antioxidant/electrophile response elements (ARE/EpRE) in target promoters, simultaneously upregulates an array of GSH-related genes (Tebay et al., 2015). This action is best framed as a coordinated four-level expansion of the GSH system rather than a simple increase in steady-state GSH concentration.

First, cystine import via xCT — Nrf2 directly induces the cystine/glutamate antiporter (xCT, encoded by SLC7A11) through an EpRE-1 element in its promoter, securing the substrate that limits subsequent biosynthesis (Sasaki et al., 2002; Koppula, Zhuang, & Gan, 2021). Second, de novo synthesis via GCL — Nrf2 transactivates both subunits of γ-glutamylcysteine ligase (GCL), the rate-limiting enzyme of GSH biosynthesis (Lu, 2013).[11] Third, regeneration by GR — Nrf2 has been shown to upregulate glutathione reductase (GR), which recycles glutathione disulfide (GSSG) back to GSH (Harvey et al., 2009). Fourth, NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) supply from PPP — Nrf2 drives the transcription of glucose-6-phosphate dehydrogenase (G6PD) and other pentose phosphate pathway (PPP) enzymes, providing the reducing equivalents that GR and other thiol-recycling reactions consume (Mitsuishi et al., 2012). Comparable Nrf2-mediated upregulation of these axes has been observed in ozone-exposed systems (Pecorelli et al., 2013).[14]

The cumulative effect is an expansion of system capacity — not a stockpile of antioxidant molecules but a strengthened machinery for substrate import, biosynthesis, regeneration, and cofactor supply. Yet this same Nrf2-driven expansion exhibits a U-shaped dose–response: insufficient activation predisposes to disease, while constitutive or excessive activation can be co-opted in oncological settings — a dual nature revisited in §5 (Tebay et al., 2015)

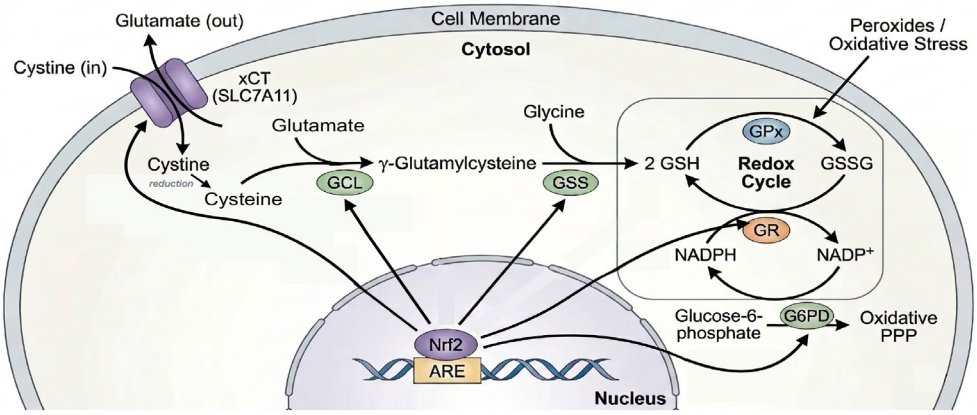

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Figure 1. Nrf2-regulated glutathione system. Schematic representation of the Nrf2-mediated transcriptional control through which ozone therapy supports glutathione system maintenance via a four-level cascade: (1) cystine import via xCT (SLC7A11); (2) de novo synthesis through γ-glutamylcysteine ligase (GCL) and glutathione synthetase (GSS); (3) regeneration of reduced GSH from GSSG by glutathione reductase (GR); and (4) NADPH supply from the oxidative pentose phosphate pathway via glucose-6-phosphate dehydrogenase (G6PD). The redox cycle (GPx → GSSG → GR → GSH) is sustained by NADPH consumption. Abbreviations are defined in the main text and listed in the Abbreviations section. Source: Original figure created by the author.

3. The Glutathione System as an Integrated Metabolic Network

3.1 Synthesis, Structure, and the Cysteine-Limited Step

Glutathione is a tripeptide composed of glutamate, cysteine, and glycine, with two structural features that together define its biochemical role: an atypical γ-carboxyl peptide bond between glutamate and cysteine, which renders the molecule resistant to most intracellular peptidases and thereby allows GSH to accumulate within cells at the millimolar concentrations characteristic of the cytosolic pool, and a free thiol on the cysteine residue, which provides the reactive electron donor central to thiol-redox chemistry (Lushchak, 2012).

GSH biosynthesis proceeds through two ATP-dependent steps. Glutamate-cysteine ligase (GCL) couples glutamate to cysteine to form γ-glutamylcysteine, and glutathione synthetase (GS) appends glycine to yield GSH. GCL catalyzes the rate-limiting reaction and is subject both to feedback inhibition by GSH and to transcriptional control via Nrf2 (Lu, 2009; Lushchak, 2012). Once synthesized, GSH is consumed by glutathione peroxidase (GPx) during peroxide reduction, generating GSSG that is restored to GSH by GR at the cost of NADPH. Beyond this intracellular cycle, γ-glutamyl transpeptidase (γ-GT) cleaves extracellular GSH at the cell surface, allowing constituent amino acids — particularly cysteine — to be reabsorbed and reincorporated into newly synthesized GSH, providing an extracellular salvage route that conserves cysteine across tissues (Bjørklund et al., 2021).

Cellular cysteine availability is sustained by dietary intake, by transsulfuration from methionine, and by xCT-mediated cystine import — the last being the dynamically regulated component that responds to redox-stress signaling (Koppula et al., 2021). The availability of cysteine via the cystine/glutamate antiporter xCT (SLC7A11), which will emerge as a pivotal shared regulatory hub in §2.2- ① and §5.6, is a critical rate-limiting factor that couples GSH biosynthesis to the broader redox network.

3.2 Functional Framework — Eight Interlocking Axes

The biochemistry outlined above is a necessary but insufficient account of what GSH does in a living cell. A more complete picture emerges when GSH is viewed not as an antioxidant molecule operating in isolation but as the shared currency of a metabolic network that intersects multiple physiological do-

mains. Aquilano, Baldelli, and Ciriolo (2014) have argued for precisely this reframing, in which GSH is repositioned from a passive radical scavenger to an active node in cellular metabolism; this paradigm shift establishes the conceptual basis for the present review.

Within this framework, eight functional axes can be distinguished, as illustrated in Figure 2: (i) ROS/RNS scavenging and antioxidant defense; (ii) thiol switches, including S-nitrosylation and S-glutathionylation; (iii) immune and inflammatory modulation; (iv) infection defense against bacterial and viral pathogens; (v) anti-glycation and carbonyl stress containment; (vi) detoxification through the mercapturic acid pathway; (vii) mitochondrial integrity; and (viii) cancer biology, where GSH exhibits a dual nature. Each axis depends on a distinct set of effector enzymes — peroxidases, transferases, glutaredoxins, methylglyoxal-handling glyoxalases, and others — yet draws on the same cytosolic GSH pool and the same GSH/GSSG redox currency (Tebay et al., 2015). This shared substrate-and-currency architecture is framed as the operational meaning of an integrated metabolic network: perturbation in one axis propagates, through pool depletion or redox-state shift, to all others.

Axes (i) through (vii) are taken up in §4, while axis (viii) forms the focus of §5.

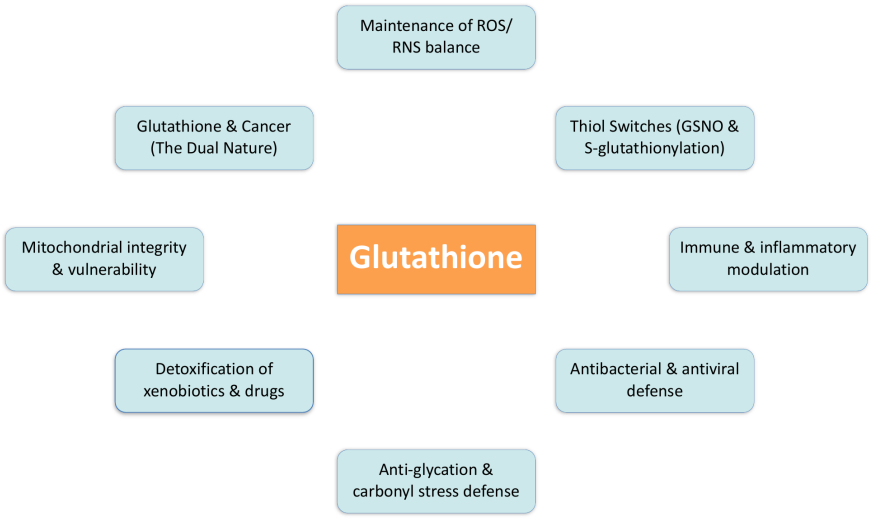

Figure 2. The glutathione system as an integrated metabolic network. Glutathione functions as a shared cellular pool and shared metabolic currency across eight functional axes: (i) ROS/RNS scavenging and antioxidant defense; (ii) thiol switches (GSNO/GSNOR and S-glutathionylation); (iii) immune and inflammatory modulation; (iv) antibacterial and antiviral defense; (v) anti-glycation and carbonyl stress containment; (vi) detoxification of xenobiotics and drugs through the mercapturic acid pathway; (vii) mitochondrial integrity and vulnerability; and (viii) cancer biology, where GSH exhibits a dual nature. Abbreviations are defined in the main text and listed in the Abbreviations section. Source: Original figure created by the author.

4. Non-Oncology Axes

4.1 ROS/RNS Balance and the Antioxidant Network

Antioxidant defense organizes around hydroxyl radical (HO•) reactivity in two coordinated layers. Layer-1 — superoxide dismutase (SOD), catalase, GPx, peroxiredoxin (Prx), thioredoxin reductase

(TrxR) — prevents HO• upstream. Layer-2 (the GSH antioxidant network) — GSH, ascorbate (vitamin C), α-tocopherol (vitamin E), α-lipoic acid (ALA), coenzyme Q10 (CoQ10) — scavenges HO• once formed. GSH bridges both layers as scavenger, GPx co-substrate, and GR product, the GSH/GSSG couple anchoring redox buffering (Sies et al., 2017; Schafer & Buettner, 2001; Halliwell & Gutteridge, 2015).

Low-grade, transient oxidative signals from ozone induce both layers via Nrf2: layer-1 enzymes are transcriptionally upregulated, while the layer-2 GSH system is supported at four sequential levels — cystine import via xCT, de novo synthesis via GCL, regeneration by GR, and NADPH supply from PPP (§2.2). Ozone therapy thus engages both layers, distinguishing it from substrate-only supplementation (IV-GSH, vitamin C, ALA), which engages only the scavenging layer without inducing the upstream enzymatic machinery (Bocci et al., 2009).

4.2 Thiol Switches I — GSNO/GSNOR Axis

Beyond ROS handling, the GSH system also governs nitric oxide (NO) signaling through a dedicated thiol switch. NO and GSH combine via transnitrosylation to form S-nitrosoglutathione (GSNO), the principal low-molecular-weight NO reservoir, which transmits NO bioactivity by S-nitrosylation of protein cysteines — a reversible post-translational switch. S-nitrosoglutathione reductase (GSNOR; alcohol dehydrogenase 5, ADH5), an NADH-dependent enzyme, decomposes GSNO to GSSG and ammonia, thereby setting the steady-state GSNO pool and the global protein-SNO landscape (Barnett & Buxton, 2017; Stomberski, Hess, & Stamler, 2019). In asthma, airway GSNOR activity is elevated and bronchoalveolar S-nitrosothiols are depleted, a pattern correlating with airway hyperresponsiveness — establishing GSNO/GSNOR as a clinically tractable thiol switch (Que et al., 2009).

Whether ozone's transient oxidative pulse modulates GSNO turnover is not directly established. This paper proposes that ozone-driven Nrf2 activation, by reshaping thiol homeostasis, may shift the GSNO/GSNOR balance and tune downstream S-nitrosylation — a hypothesis that remains to be tested experimentally.

4.3 Thiol Switches II — S-Glutathionylation

A second class of thiol switch operates through reversible S-glutathionylation, by which the glutathione redox system caps reactive protein cysteines as protein-S-glutathionylated species (PSSG), shielding them from over-oxidation and modulating activity. Glutaredoxin (Grx) catalyzes reverse deglutathionylation under reducing conditions, regenerating protein-SH, so PSSG occupancy is set by the prevailing GSH/GSSG ratio (Mieyal, Gallogly, Qanungo, Sabens, & Shelton, 2008; Dalle-Donne, Rossi, Colombo, Giustarini, & Milzani, 2009). For example, endothelial nitric oxide synthase (eNOS) S-glutathionylation causes NOS uncoupling and impaired endothelium-dependent relaxation in hypertensive rat aorta, reversible by dithiothreitol (Chen et al., 2010); other targets (GAPDH, PTP1B) have been shown to undergo analogous redox-tunable inhibition.

Free and protein-bound GSH thus form a redistributing pool within the glutathione redox system, not separate compartments. This paper proposes that ozone's transient oxidative pulse may engage this signaling domain by tilting PSSG occupancy, in contrast to IV-GSH, which expands the free pool without comparable switch engagement.

4.4 Immune and Inflammatory Modulation

Intracellular GSH levels in immune cells regulate inflammatory and adaptive responses. In antigen-presenting cells (APC), GSH availability has been shown to govern interleukin-12 (IL-12) production and T helper 1 / T helper 2 (Th1/Th2) cytokine balance (Peterson, Herzenberg, Vasquez, & Waltenbaugh, 1998). The transcription factor nuclear factor-κB (NF-κB) is itself thiol-redox sensitive, with S-glutathionylation of the p65 subunit suppressing its DNA-binding activity. Consistent with this, GSH-C4 (a cell-permeable GSH prodrug) has been shown to attenuate NF-κB phosphorylation and nuclear translocation in lipopolysaccharide (LPS)-stimulated macrophages, indicating that intracellular GSH levels causally tune NF-κB signaling and downstream cytokine output (Limongi et al., 2019).
Ozone's transient oxidative pulse may engage this signaling axis hormetically, plausibly redirecting immune polarization without the bulk substrate inflation entailed by IV-GSH (Bocci et al., 2009). This paper proposes that ozone-induced cellular thiol adjustment, rather than antioxidant supply per se, is one of the mechanisms accounting for its immunomodulatory effects.

4.5 Other GSH Roles: Infection Defense and Anti-Glycation

GSH supports host defense in viral and bacterial infections. Low intracellular GSH has been shown to enhance viral replication and impair antiviral immunity, while in bacterial infection the GSH/GSSG redox cycle sustains NADPH-dependent processes — including the phagocyte oxidative burst — through glutathione reductase activity (Yan et al., 2012). A randomized controlled trial (RCT) of N-acetylcysteine (NAC), a GSH precursor, has been shown to reduce influenza-like illness in older high-risk adults (De Flora, Grassi, & Carati, 1997). For COVID-19, a recent NAC meta-analysis rated clinical benefit as GRADE VERY LOW (Liu et al., 2024). By contrast, a meta-analysis of ozone adjuvant therapy in COVID-19, pooling four randomised controlled trials and four case-control studies (371 patients), reported a significant reduction in mortality and a 41% higher rate of polymerase chain reaction (PCR) negativization in the ozone arm relative to standard treatment alone, alongside improvements in inflammatory markers (Jafari-Oori et al., 2022). This contrast supports the broader argument of this review: substrate antioxidant supply addresses only one face of host defense, whereas ozone therapy engages the integrated redox-signaling network.

GSH also defends against carbonyl stress driven by methylglyoxal (MGO), a key glycation precursor. The glyoxalase system uses GSH as a co-factor: Glyoxalase 1 (Glo1) and Glyoxalase 2 (Glo2) sequentially convert MGO via S-D-lactoylglutathione to D-lactate, regenerating GSH without depleting the pool (Thornalley, 2003). Nrf2 induces Glo1 (Xue et al., 2012). In a 6-month RCT, oral GSH supplementation has been shown to lower hemoglobin A1c (HbA1c) in elderly type 2 diabetes (T2D) patients (Kalamkar et al., 2022), with effect confined to subjects ≥55 years. Clinical evidence consistent with this mechanism comes from a randomised controlled trial in patients with diabetic foot syndrome, in which ozone therapy improved glycaemic control alongside reduced oxidative stress and elevated antioxidant enzyme activity (Martínez-Sánchez et al., 2005). This paper proposes that ozone-driven Nrf2 activation may contribute via Glo1 induction. Across both axes GSH operates as a defense currency, consumed as substrate in infection-driven oxidative loss but recycled as a co-factor in glyoxalase activity.

4.6 Detoxification — The Shared GSH Currency

Detoxification of electrophilic xenobiotics is the canonical GSH function in which the molecule is consumed stoichiometrically as substrate rather than recycled as a co-factor. Glutathione S-transferase (GST) catalyzes conjugation of GSH with reactive electrophiles — alkylating agents, chemotherapy

drug metabolites, and environmental toxicants — to yield GS-conjugates that are extruded by ATP-dependent multidrug efflux pumps and processed sequentially via the mercapturic acid pathway to urinary mercapturic acids (Hayes, Flanagan, & Jowsey, 2005). Each conjugation event spends one GSH molecule one-for-one, so sustained substrate flux progressively depletes the cytosolic pool, with replenishment depending on xCT-mediated cystine import and GCL-driven de novo synthesis (§2.2). xCT thus serves as a shared upstream node feeding both detoxification and broader GSH-dependent cellular functions (Koppula et al., 2021). This same GSH-substrate consumption pathway acquires clinical significance in oncology contexts, where GST-mediated drug detoxification and xCT-driven cystine supply jointly modulate chemotherapy response (§5.4) (Townsend & Tew, 2003).

4.7 Mitochondrial Integrity and Vulnerability.

Mitochondria cannot synthesize GSH and depend on cytosolic import, so the mitochondrial GSH (mGSH) pool constitutes an independent compartment whose status need not parallel total cellular GSH (Marí et al., 2020). Recent work identifies SLC25A39 (solute carrier family 25 member 39) and its paralog SLC25A40 (solute carrier family 25 member 40) as the principal inner membrane GSH carrier (Wang et al., 2021). Within the matrix, glutaredoxin 5 (Grx5) uses GSH as an obligate co-factor for Fe-S cluster assembly (Ye et al., 2010); mGSH depletion destabilizes Fe-S biogenesis, weakens the respiratory chain, and primes lipid peroxidation-driven cell death. Intravenous GSH does not cross the inner membrane and has been shown to leave mGSH largely unaltered, in contrast to ozone therapy, which upregulates Nrf2-driven cysteine supply and whole-cell GSH synthesis (Bocci et al., 2009). A practical working hypothesis is that this systemic increase in GSH availability may support mGSH through mass-action loading of the SLC25A39 carrier — qualitatively distinct from substrate infusion. This mGSH vulnerability establishes a premise for §5.2, where the xCT–GSH–GPX4 axis is dissected in the tumour ferroptosis context.

5. GSH and Cancer: The Dual Nature

5.1 Classic View vs Paradigm Shift: Guardian → Survival Fuel

For decades, glutathione (GSH) has been framed as a generic guardian against carcinogenesis. Early epidemiological work associated higher antioxidant intake with lower cancer incidence (Ames, Shigenaga, & Hagen, 1993), and circulating GSH or its precursors have been associated with reduced cancer risk in dietary cohorts (Flagg et al., 1994). Within this classic view, “more GSH” was implicitly equated with “less cancer.”

This framing has since been substantially revised. In tumours driven by KRAS (Kirsten rat sarcoma viral oncogene), BRAF (B-Raf proto-oncogene), or MYC (MYC proto-oncogene), constitutive Nrf2 activation drives a coordinated expansion of the glutathione system — cystine import, de novo synthesis, GSSG regeneration, and NADPH supply through the pentose phosphate pathway — and supports proliferation under oxidative load (DeNicola et al., 2011; Mitsuishi et al., 2012). The Nrf2 module that protects normal tissue can therefore be hijacked as a survival circuit, a phenomenon now described as “Nrf2 addiction” (Harris & DeNicola, 2020). High intratumoural GSH establishes a reproducible high-risk axis: it is associated with poor prognosis and chemoresistance across multiple solid tumours, and Nrf2 hyperactivity segregates aggressive subsets (Tebay et al., 2015). The corollary is direct: in cells that have completed transformation, the same biosynthetic and recycling capacity that buffers oxidative load also licenses chemoresistance and persistence under treatment.

The implication is that GSH is not a one-directional cancer-protective molecule. It guards the host but can simultaneously fuel established tumours. This dual nature establishes the frame in which

the remainder of §5 should be read: every subsequent question — about ferroptosis vulnerability, exogenous GSH, ozone therapy, and clinical translation — turns on which side of this duality is being engaged.

5.2 The xCT–GSH–GPX4 Axis and Ferroptosis

The dual nature established in §5.1 finds its first concrete mechanistic expression in the xCT–GSH–GPX4 axis. Ferroptosis is an iron-dependent, regulated form of cell death driven by phospholipid hydroperoxide accumulation rather than by caspase activation or pore formation (Dixon et al., 2012; Stockwell et al., 2017). The defence against this lethality is anchored on glutathione peroxidase 4 (GPX4), which uses reduced GSH to convert membrane phospholipid hydroperoxides (PLOOH) to the corresponding alcohols (PLOH) and so prevents propagation of lipid radicals on the membrane surface. GPX4 is the only mammalian enzyme capable of repairing oxidised phospholipids in situ, and its activity is gated by the size and turnover of the cellular GSH pool.

Tumours sustain this defence by upregulating xCT (SLC7A11), the cystine/glutamate antiporter that imports the rate-limiting precursor for GSH biosynthesis (Koppula et al., 2021). Because GPX4 has no alternative thiol cofactor, the axis collapses to a single linear dependency: cystine import, GSH synthesis, hydroperoxide repair. This dependency makes the xCT–GSH–GPX4 axis a therapeutic pressure point — pharmacological xCT inhibition or buthionine sulfoximine-mediated GSH depletion preferentially suppresses growth in RAS-driven cells that have already configured themselves around this axis (Yang et al., 2014). The converse is also direct: tumours that constitutively elevate xCT and GSH are those that depend on this defence to remain viable.

Read in this light, what classic epidemiology framed as “antioxidant capacity” is, in transformed cells, more accurately understood as the membrane-survival arm of the integrated GSH network. The mitochondrial GSH vulnerability established earlier thus enters oncology as a defining axis of tumour redox physiology rather than a generic stress response.

5.3 Persister Cells and Ferroptosis Vulnerability

The clinical relevance of the xCT–GSH–GPX4 axis emerges most clearly when treatment is applied. After tyrosine kinase inhibitor or BRAF/MEK (mitogen-activated protein kinase kinase) exposure, a fraction of cells survive in a slow-cycling, drug-tolerant “persister” state from which relapses subsequently arise. Persisters exhibit a recurrent transcriptional signature characterised by loss of epithelial markers and acquisition of mesenchymal features (Hangauer et al., 2017). This phenotypic shift is not a passive byproduct of stress; it carries a distinct redox commitment.

Mesenchymal cells configure their lipid metabolism toward polyunsaturated phospholipids and become disproportionately dependent on GPX4 for membrane integrity. Pharmacological GPX4 inhibition or genetic GPX4 ablation triggers selective ferroptotic death in this state, while parental epithelial cells remain comparatively unaffected (Hangauer et al., 2017; Viswanathan et al., 2017). The same GSH-anchored axis that supports tumour proliferation thus narrows, after treatment, into a focal vulnerability: GPX4 dependence becomes a discriminating feature of the surviving population.

The therapeutic implication that follows is sequential rather than alternative. Conventional cytotoxic or targeted therapies remain effective at debulking the tumour mass, but they leave behind precisely the cells that have become ferroptosis-vulnerable. Lipid-peroxidation-promoting strategies — xCT inhibition, GPX4 inhibition, cystine restriction — are therefore most plausible as adjuncts that target

the residual persister population rather than as primary cytotoxic agents (Tebay et al., 2015). This sequential framing — bulk debulking followed by selective ferroptotic elimination — anticipates the role assigned to ozone therapy in this review as an upstream rather than direct anti-tumour modality.

5.4 Direct, Indirect, and Prodrug Strategies Targeting the GSH Axis

The dual nature framework articulated in §5.1 — guardian and survival fuel — suggests three distinct logics for therapeutic intervention against the tumour glutathione system. Each has been developed independently and operates through a different mechanism, but all three converge on the integrated network introduced earlier rather than on a single molecule.

Direct depletion targets the upstream supply or the glutathione pool itself. Sulfasalazine inhibits xCT and reduces cystine import, suppressing GSH synthesis in a manner most pronounced in cells with high baseline xCT (Lo, Wang, & Gout, 2008). Buthionine sulfoximine (BSO) inhibits γ-glutamylcysteine ligase (GCL) and produces direct GSH depletion; early-phase clinical experience established proof-of-principle for systemic GSH lowering (Bailey et al., 1994). More recently, recombinant cyst(e)inase enzymatically degrades extracellular cyst(e)ine, depriving tumours of the rate-limiting precursor and slowing growth in xenograft models (Cramer et al., 2017).

Indirect modulation operates on the tumour microenvironment rather than on tumour cells directly, shifting the redox and inflammatory context within which the GSH network is configured (Liu et al., 2023).

Prodrug exploitation inverts the logic of depletion: rather than lowering tumour GSH, it uses tumour-high GSH or upregulated glutathione-S-transferases as activators, releasing cytotoxic species preferentially within the tumour. Glutathione-S-transferase upregulation, set out as a chemoresistance mechanism in §4.6, becomes here the basis for selective bioactivation.

These three logics establish a baseline against which any new intervention — including ozone therapy — must be positioned: not as another antioxidant, but as a redox-active modality engaging the same network through a distinct mode of action.

5.5 Exogenous Intravenous Glutathione versus Ozone Therapy: A Qualitative Distinction

The qualitative distinction between exogenous GSH supplementation and ozone therapy is the operational pivot of this review. Recent work using ordinary differential equation modelling has framed the difference quantitatively: an intravenous bolus of exogenous GSH produces a supraphysiological plasma spike that is roughly two orders of magnitude above physiological levels, whereas ozone therapy generates a controlled, transient rise on the order of micromoles per litre that decays within minutes (Chirumbolo et al., 2025). The first acts as a pharmacological excess; the second mimics a hormetic physiological signal.

This distinction matters because reductive shifts of the cellular thiol pool are not benign. Reductive stress disrupts disulfide-dependent protein folding, suppresses the mitochondrial reactive species needed for redox signalling, and in transgenic mouse models triggers cardiomyopathy (Rajasekaran et al., 2007; Xiao & Loscalzo, 2020). From the modelling perspective, even modest IV-GSH doses are predicted to push the cellular redox poise in a reductive direction. Such observations raise conceptual concerns about reductive stress and motivate distinguishing supplementation from ozone exposure on principle.

A clinical reality, however, qualifies the strength of this argument: intravenous GSH at 200 mg has been used routinely under Japanese insurance-based practice without systematic adverse events, so the model-derived threshold for reductive stress cannot be equated with a clinical safety threshold. A categorical contraindication is therefore not warranted. The argument is not that exogenous GSH is unsafe, but that its logic — substrate flooding — differs fundamentally from the logic of ozone therapy, which relies on transient oxidative signals to engage the host's own redox machinery.

Table 1. Intravenous reduced glutathione (IV-GSH) and ozone therapy compared as redox interventions. Abbreviations are defined in the main text and listed in the Abbreviations section. Source: Original table compiled by the author.

Dimension	Intravenous reduced glutathione (IV-GSH)	Ozone therapy
Pharmacological category	Substrate supplementation	Hormetic redox signaling
Mode of action	Direct expansion of the free GSH pool	Transcriptional induction of the integrated GSH network via Keap1–Nrf2
Engagement with the integrated GSH network	Free GSH pool only; no induction of upstream cystine import, biosynthesis, regeneration, or NADPH supply	Coordinated four-level support: cystine import (xCT), de novo synthesis (GCL), regeneration (GR), and NADPH supply from the pentose phosphate pathway
Antioxidant defense layer engaged	Layer-2 (scavenging) only	Both Layer-1 (enzymatic defense) and Layer-2 (GSH-centred scavenging) via Nrf2-driven transcription
Plasma / cellular redox kinetics	Supraphysiological plasma spike, approximately two orders of magnitude above physiological levels per ODE modelling	Controlled, transient rise on the order of micromoles per litre, decaying within minutes
Implication in oncology	May support tumour antioxidant capacity or ferroptosis resistance in some contexts; the GSH × chemotherapy interaction is drug-specific	Direct anti-tumour activity remains to be established; positioned as upstream modulator of the IFN-γ–xCT axis
Clinical positioning	Substrate-flooding strategy; legitimate where reductive support is the therapeutic goal (e.g., cisplatin neurotoxicity attenuation), with caution required during active cancer therapy	Qualitatively distinct redox conditioning rather than antioxidant supplementation; the two are not interchangeable

Conceptual comparison. The two interventions are qualitatively distinct rather than interchangeable, and neither is universally superior. Substrate flooding (IV-GSH) and transient oxidative signaling (ozone therapy) engage the glutathione system at different layers and with different kinetics; clinical relevance depends on diagnosis, treatment phase, tumour biology, dose, route, timing, and patient condition.

5.6 Ozone Therapy in Cancer: Upstream of the IFN-γ–xCT Axis

The position of ozone therapy in this landscape follows from the redox architecture established in §2 and §3. Rather than supplying reduced thiols or scavenging reactive species, oxygen-ozone therapy delivers low-grade, transient oxidative signals that engage the cellular adaptive machinery — Nrf2

activation, transcriptional support of cystine import, de novo GSH synthesis, GSSG regeneration, and NADPH supply through the pentose phosphate pathway, together with induction of layer-1 enzymatic defences (Bocci et al., 2009; Tebay et al., 2015). In the tumour setting, this dual-layer inducer profile places ozone therapy upstream of the GSH-anchored ferroptosis axis rather than alongside the cytotoxic strategies catalogued in §5.4.

Several mechanisms have been proposed in the literature for ozone therapy in cancer: (i) microenvironmental normalization through improvement of regional hypoxia (Clavo et al., 2004); (ii) metabolic reorientation away from sustained glycolysis toward oxidative phosphorylation; (iii) redox signaling that conditions Nrf2-mediated host defence; and (iv) protection of normal tissue against treatment-induced damage (Clavo et al., 2019). Direct anti-tumour activity of ozone therapy itself remains to be established; the available rationale supports adjunctive, immune- and metabolism-supportive use.

Beyond these established mechanisms, the present review proposes that ozone therapy may operate upstream of the interferon-gamma (IFN-γ)–xCT axis. CD8+ (cluster of differentiation 8) T cell-derived IFN-γ activates STAT1 (signal transducer and activator of transcription 1) in tumour cells, suppresses xCT (SLC7A11), reduces cystine import and intratumoural GSH, and sensitises tumour cells to lipid peroxidation (Wang et al., 2019). This circuit ties immunological activation directly to ferroptotic vulnerability through the same shared regulatory hub introduced in §2.2 and §5.2. To the extent that ozone therapy supports CD8+ T cell function and IFN-γ output, it would act as an upstream modulator of this axis rather than as a direct tumour-killing agent. Direct experimental support for ozone-driven interferon-class signalling comes from in vitro and in vivo Bocci-group work, in which ozone exposure of human leucocytes induced IFN-γ ex vivo (Bocci & Paulesu, 1990) and ambulatory autohaemotherapy in normal volunteers activated the interferon-induced Mx protein (Bocci, Luzzi, Corradeschi, & Paulesu, 1993). However, plasma IFN-γ itself does not appear to be consistently elevated by ozonated autohemotherapy in healthy subjects (Boczkowska-Radziwon et al., 2022), suggesting that any upstream IFN-γ–related effect, if present, is likely to operate through localised or transient signalling rather than sustained systemic cytokine elevation. This proposal prepares the ground for the dosing perspective developed in §5.7.

5.7 [PERSPECTIVE] Relatively High-Concentration, Low-Total-Dose Ozone in Oncology

The following subsection presents a clinical perspective — a proposed framework rather than an established standard.

(a) Existing literature support

Three findings frame the proposal. First, ozone exerts its biological effects through hormesis, where stimulus magnitude determines whether the response is adaptive or injurious (Bocci et al., 2009). Second, concentration and cumulative dose can be dissociated, and the AEPROMO/ISCO3 Madrid Declaration treats them as independently titratable parameters (ISCO3, 2025). Third, in oncology contexts, Clavo and colleagues demonstrated that high-concentration, high-volume ozone exposure (60 µg/mL with 200 mL blood and 200 mL gas) achieved oxygenation of the most hypoxic tumour cells (Clavo et al., 2004) — a finding that establishes the upper feasibility of the operational range. Whether comparable per-session concentration and dose are required for every oncology patient, however, remains an open question, and motivates exploration of regimens that preserve per-session concentration while reducing cumulative exposure. This open question gains specific weight from the early Bocci-group finding that ozone-induced IFN-γ production from human leucocytes is concentration-

critical, with low concentrations failing to induce IFN-γ in whole blood and concentrations near 42 μg/mL required to elicit the response (Bocci & Paulesu, 1990).

(b) Reasoned extrapolation

Three frameworks must not be conflated: the AEPROMO/ISCO3 Madrid Declaration on ozone therapy (ISCO3, 2025), the low-dose ozone concept articulated by Viebahn-Hänsler, León Fernández, and Fahmy (2012), and the relatively high-concentration / low-total-dose framework proposed here. The first two share the goal of minimising overall oxidative burden and have legitimately shaped current oncology practice. The framework proposed here departs from them in one specific way: it accepts that a sufficient concentration may be required to engage IFN-γ. From the author's clinical perspective, this can be reconciled with the goal of minimising burden by holding total dose low while preserving per-session concentration. In operational terms, this corresponds to ozone concentrations in the 10–40 μg/mL range, blood volumes near 50 mL, and total per-session doses in the 500–2000 μg range — quantitatively distinct from the uniformly low-concentration approach.

(c) Untested clinical hypothesis

The framework remains a working hypothesis. No randomised controlled trial has compared concentration-dominant against dose-dominant titration in oncology, and the proposal is therefore offered to motivate prospective evaluation rather than to revise practice. Biomarker-guided titration, developed in §5.8, provides the operational feedback loop for safe exploration. In the author's own practice, this loop is operationalised by starting from low concentration and low total dose and titrating cautiously upwards under biomarker guidance. A two-by-two design crossing concentration (10–40 μg/mL) against total dose (500–2000 μg), with quality of life, biomarker trajectories, and progression-free survival as candidate endpoints, would represent a feasible first test. Prospective validation is warranted before any clinical adoption.

5.8 Biomarker-Guided Titration

Operationalising §5.7 requires a feedback loop sensitive to both excessive oxidative load and intended metabolic effects. The panel proposed here is dual-purpose: each marker is read against two axes — (1) cancer-related prognostic information and (2) systemic oxidative load monitoring. The two are not always cleanly separable in clinical practice, and that ambiguity is part of the framework.

Four operational categories populate the panel. Oxidative balance is captured by d-ROMs (derivatives of reactive oxygen metabolites) / BAP (biological antioxidant potential), a (2)-axis monitor of systemic oxidant/antioxidant balance (Pigazzani et al., 2022). Inflammatory load is tracked through neutrophil-to-lymphocyte ratio (NLR) (Templeton et al., 2014), C-reactive protein (Heikkilä, Ebrahim, & Lawlor, 2007), albumin/globulin ratio (AGR) (He et al., 2017), and serum copper / ceruloplasmin (Arenas de Larriva et al., 2020) — all dual-purpose biomarkers. Iron metabolism, inflammation, and oxidative stress converge on ferritin (Pfeifhofer-Obermair, Tymoszuk, Petzer, Weiss, & Nairz, 2018), which complements ceruloplasmin's ferroxidase function, links to the ferroptosis vulnerability of §5.6, and additionally serves as a marker of inflammatory and oxidative status in oncology. Tumour metabolism and erythrocyte damage are reflected in lactate dehydrogenase (LDH) — primarily a Warburg-to-oxidative phosphorylation (OXPHOS) shift monitor, secondarily a tumour marker (Claps et al., 2022) — and in reticulocyte index, which captures eryptosis and erythrocyte membrane peroxidation (Bissinger, Bhuyan, Qadri, & Lang, 2019; Orrico et al., 2023). 8-hydroxy-2′-deoxyguanosine (8-OHdG, also known as 8-oxodG) and F2-isoprostanes provide direct oxidative DNA and lipid damage validation (Lee, Cai, Shu, & Nechuta, 2017).

Decision-making does not rest on any single biomarker but integrates trajectories across sessions. Other candidate indices, including the systemic immune-inflammation index (SII) (Tian et al., 2022; Yang, Chang, Meng, Gao, & Wang, 2018), have been proposed but are not formally included here. Standard safety monitoring follows the AEPROMO/ISCO3 framework articulated in the Manual of Clinical Ozone Therapy (Schwartz, 2020) and the Madrid Declaration on Ozone Therapy (4th edition) (ISCO3, 2025). The framework reflects a pragmatic monitoring strategy informed by the author's clinical practice; prospective validation is warranted.

5.9 Doxorubicin–Glutathione as a Supporting Analogue

The qualitative distinction set out in §5.5 finds an instructive analogue in the doxorubicin–glutathione interaction. In a syngeneic murine breast tumour model, intragastric GSH at 5, 50, and 500 mg/kg dose-dependently attenuated doxorubicin-induced cardiac and hepatic toxicity, but at the same time produced a parallel dose-dependent reduction in antitumour efficacy: tumour inhibition fell from 91% with doxorubicin alone to 65%, 38%, and 31% with co-administration of GSH at the three escalating doses (Shen et al., 2019). Intratumoural doxorubicin concentrations were not altered by GSH co-administration, indicating that the loss of efficacy was pharmacodynamic — a redox-level interference with the cytotoxic mechanism — rather than pharmacokinetic. The investigators concluded that exogenous GSH attenuates doxorubicin toxicity but should not be used to counteract its activity in clinical applications.

This finding contrasts sharply with the cisplatin setting, in which intravenous GSH attenuated neurotoxicity without compromising antitumour response (Cascinu et al., 1995). Together, the two studies indicate that the GSH × chemotherapy interaction is drug-specific and remains context-dependent; no universal rule applies.

The analogy lies in the structural pattern, not in the intervention itself: a single redox-level intervention can produce opposite effects on host tissue and tumour. Direct experimental evidence applies this same asymmetry pattern to ozone therapy. In a Sprague-Dawley rat model, ozone-oxidative preconditioning prevented doxorubicin-induced dilated cardiomyopathy, preserving left ventricular morphology, reducing pro-brain natriuretic peptide elevation, and restoring antioxidant enzyme activity (Delgado-Roche et al., 2014). Independently, in vitro work on a Luminal-A breast cancer cell line showed that ozone applied after doxorubicin enhanced the viability of L929 fibroblasts while further reducing the viability of MCF-7 tumour cells relative to doxorubicin alone (Karagülle & Yurttaş, 2022). Read together, these findings — one in vivo, one in vitro — are consistent with a profile in which ozone therapy attenuates doxorubicin toxicity in host tissue without compromising, and possibly enhancing, antitumour activity. Both effects are mechanism-inferred for clinical practice at present and remain to be established in prospective clinical trials. The relevance of the doxorubicin–glutathione literature to this review lies in what it demonstrates about asymmetry: a single intervention against the GSH axis can have opposite consequences for host and tumour, and the clinical implications cannot be read from the antioxidant label alone.

6. Discussion and Clinical Implications

6.1 Integrative Restatement

The argument developed across §2 to §5 can now be restated in integrated form. At the mechanistic level, ozone therapy operates not by donating reducing equivalents but by generating low-grade, transient oxidative signals that engage the Keap1–Nrf2 axis through 4-HNE-mediated cysteine modification

(Saito et al., 2016) and, downstream, recruit a transcriptional programme that simultaneously induces the layer-1 enzymatic defence established at §4.1 and the layer-2 glutathione-centred scavenging system articulated at §2.2 (Sies et al., 2017). On this view, characterising ozone therapy as an antioxidant intervention captures only part of its action and risks locating the intervention at a different layer of redox biology from the one it actually engages. The glutathione system, in turn, is not a single antioxidant molecule but an integrated metabolic network — cystine import via xCT, *de novo* synthesis via GCL, regeneration by GR, and NADPH supply from the pentose phosphate pathway — whose capacity expands and contracts as a coordinated whole (Tebay et al., 2015; Mitsuishi et al., 2012). In oncology, the same network protects host tissue and shelters the tumour, and this asymmetry is what makes ozone therapy and exogenous IV-GSH supplementation qualitatively, not merely quantitatively, distinct (Chirumbolo et al., 2025; Wang et al., 2019). The central thesis of this review is therefore re-affirmed: ozone therapy is best understood as a qualitatively distinct redox intervention that supports the glutathione system as an integrated metabolic network, with implications that are particularly consequential where exogenous GSH and ozone must be sharply distinguished.

6.2 Clinical Implications

For practising clinicians using oxygen-ozone therapy, three implications follow. First, patient-facing language matters: framing ozone therapy as “an antioxidant approach” invites the inference that it is interchangeable with — or additive to — IV-GSH or other scavenger supplementation, a conflation that the present analysis specifically rejects. Standard safety profiles and accepted indications continue to be governed by the AEPROMO/ISCO3 Manual of Clinical Ozone Therapy (Schwartz, 2020) and the Madrid Declaration on Ozone Therapy (4th edition) (ISCO3, 2025), and the reframing proposed here operates within, not against, this institutional framework. Second, in oncology, the dual nature of the glutathione system means that the working logic of ozone administration cannot be borrowed wholesale from non-oncology indications; from the author’s clinical perspective, articulated as a perspective in §5.7, a relatively high concentration combined with a low total dose is one principled way to retain hormetic redox signalling while limiting cumulative oxidative burden, but this remains explicitly framed as a proposed framework rather than an established standard. Third, decisions about whether to combine ozone therapy with exogenous GSH or with antioxidant chemotherapy adjuncts cannot be reduced to a universal rule: as the Cascinu–Shen comparison shows, the GSH × chemotherapy interaction is drug-specific (Cascinu et al., 1995; Shen et al., 2019). Biomarker-guided titration along the dual-purpose framework introduced in §5.8 — addressing both cancer-related change and excess oxidative load — is therefore offered as a pragmatic monitoring scaffold informed by clinical practice rather than as a validated protocol.

6.3 Future Research Directions

Three directions warrant priority. The relatively high-concentration / low-total-dose framework proposed in §5.7 has not been tested in a prospective randomised trial; a factorial design dissociating concentration (10–40 μg/mL) from total dose (500–2000 μg) is the cleanest way forward. The dual-purpose biomarker panel of §5.8 needs prospective validation, with d-ROMs/BAP trajectories (Pigazzani et al., 2022) anchoring the oxidative-load axis and established prognostic markers anchoring the cancer axis. Finally, whether ozone therapy modulates the IFN-γ–xCT axis upstream of tumour ferroptosis sensitivity remains, on present evidence, mechanism-inferred rather than directly demonstrated (Wang et al., 2019), and direct measurement in patient-derived material is the natural next step.

7. Limitations

This review has several limitations that should be acknowledged. First, the central distinction between ozone therapy as a transcriptional inducer of the glutathione system and IV-GSH as substrate-only supplementation is mechanistically grounded but has not been resolved by a head-to-head prospective clinical trial; the strongest direct comparison currently available is conceptual rather than empirical (Chirumbolo et al., 2025). Second, the relatively high-concentration / low-total-dose framework outlined in §5.7 is explicitly framed as a clinical perspective informed by the author's practice rather than as a validated standard, and prospective randomised testing of the proposed concentration-versus-total-dose dissociation is required before the framework can be recommended outside the present perspective. Third, the proposition that ozone therapy may attenuate doxorubicin (DOX) cardiotoxicity is, on present evidence, a mechanism-inferred possibility extrapolated from the asymmetric pharmacology of GSH and DOX (Shen et al., 2019); direct clinical demonstration is not claimed in this review. Fourth, the dual-purpose biomarker panel of §5.8 has not been prospectively validated, and the proposed cross-chapter linkage between §2.1 ozone–PUFA chemistry and §5.8 d-ROMs measurement remains a methodological convergence rather than a tested clinical correlation.

8. Conclusion

This review concludes that ozone therapy is most usefully understood not as an intervention that contributes to the antioxidant pool, but as a transient redox signal that engages the glutathione system at the regulatory layer through Nrf2-mediated transcriptional induction (Saito et al., 2016; Sies et al., 2017). Three take-home messages follow. First, ozone therapy is qualitatively distinct from substrate-only antioxidant supplementation: it acts as a transcriptional inducer of both the layer-1 enzymatic defence established at §4.1 and the layer-2 glutathione-centred scavenging system articulated at §2.2, whereas IV-GSH and related strategies operate only on the latter. Second, the glutathione system is best treated as an integrated metabolic network — cystine import, *de novo* synthesis, regeneration, and NADPH supply functioning as a coordinated whole — rather than as a single antioxidant molecule (Tebay et al., 2015; Mitsuishi et al., 2012). Third, in oncology, where the same network protects host tissue and shelters the tumour, ozone therapy and exogenous GSH must be sharply distinguished; their effects are not interchangeable, and decisions about combining either with cytotoxic chemotherapy remain drug-specific rather than governed by any universal antioxidant rule (Wang et al., 2019; Cascinu et al., 1995; Shen et al., 2019). Future work should test the relatively high-concentration / low-total-dose framework in a factorial randomised design, prospectively validate the dual-purpose biomarker panel, and measure the IFN-γ–xCT axis directly in patient-derived material. Within these directions, this paper proposes that the reframing developed here can inform clinical practice in ways that the older framing of ozone therapy as an antioxidant intervention cannot.

Abbreviations

4-HNE: 4-Hydroxy-2-Nonenal
8-OHdG: 8-Hydroxy-2′-Deoxyguanosine (also known as 8-oxodG)
AGR: Albumin/Globulin Ratio
ALA: α-Lipoic Acid
ARE: Antioxidant Response Element
BAP: Biological Antioxidant Potential
BRAF: B-Raf Proto-Oncogene
BSO: Buthionine Sulfoximine
CD8: Cluster of Differentiation 8

CoQ10: Coenzyme Q10
COVID-19: Coronavirus Disease 2019
d-ROMs: Derivatives of Reactive Oxygen Metabolites
DOX: Doxorubicin
EpRE: Electrophile Response Element
G6PD: Glucose-6-Phosphate Dehydrogenase
GCL: γ-Glutamylcysteine Ligase
Glo1: Glyoxalase 1
Glo2: Glyoxalase 2
GPx: Glutathione Peroxidase
GPX4: Glutathione Peroxidase 4
GR: Glutathione Reductase
GRADE: Grading of Recommendations Assessment, Development and Evaluation
Grx5: Glutaredoxin 5
GS: Glutathione Synthetase
GSH: Glutathione (reduced form)
GSNO: S-Nitrosoglutathione
GSNOR: S-Nitrosoglutathione Reductase
GSSG: Glutathione Disulfide (oxidized form)
GST: Glutathione S-Transferase
γ-GT: γ-Glutamyl Transpeptidase
HbA1c: Hemoglobin A1c
IFN-γ: Interferon-gamma
IV-GSH: Intravenous Glutathione
Keap1: Kelch-Like ECH-Associated Protein 1
KRAS: Kirsten Rat Sarcoma Viral Oncogene
L929: L929 Murine Fibroblast Cell Line
LDH: Lactate Dehydrogenase
MAH: Major Auto-Haemotherapy
MCF-7: Michigan Cancer Foundation-7 Breast Cancer Cell Line
MEK: Mitogen-Activated Protein Kinase Kinase
mGSH: Mitochondrial Glutathione
MGO: Methylglyoxal
MYC: MYC Proto-Oncogene
NAC: N-Acetylcysteine
NADPH: Nicotinamide Adenine Dinucleotide Phosphate (reduced form)
NLR: Neutrophil-to-Lymphocyte Ratio
NO: Nitric Oxide
Nrf2: Nuclear Factor Erythroid 2-Related Factor 2
OXPHOS: Oxidative Phosphorylation
PCR: Polymerase Chain Reaction
PLOH: Phospholipid Alcohols
PLOOH: Phospholipid Hydroperoxides
PPP: Pentose Phosphate Pathway
Prx: Peroxiredoxin
PSSG: Protein S-Glutathionylation
PUFA: Polyunsaturated Fatty Acid
RCT: Randomized Controlled Trial
RNS: Reactive Nitrogen Species
ROS: Reactive Oxygen Species
SII: Systemic Immune-Inflammation Index

SLC7A11: Solute Carrier Family 7 Member 11
SLC25A39: Solute Carrier Family 25 Member 39
SLC25A40: Solute Carrier Family 25 Member 40
SOD: Superoxide Dismutase
STAT1: Signal Transducer and Activator of Transcription 1
T2D: Type 2 Diabetes
TrxR: Thioredoxin Reductase
xCT: Cystine/Glutamate Antiporter

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Conflict of Interests

The author declares no conflict of interest.

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