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Research Article

A New Proposal for Host-Directed Therapy in Ebola Virus Disease: Ozone Autohemotherapy as an Adjunctive Approach Within a Systems Biology Framework

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Abstract

Ebola Virus Disease (EVD) remains one of the most lethal viral hemorrhagic fevers, with mortality driven not only by viral replication but also by a dysregulated host response characterized by hyperinflammation, endothelial dysfunction, coagulopathy, metabolic derangement and multi-organ failure. Although monoclonal antibodies and vaccination have substantially improved outcomes, currently available interventions primarily target the virus and do not directly address the complex host-response networks that contribute to advanced disease and mortality.

Here, we propose a systems biology framework for evaluating Ozone Autohemotherapy (O_3-AHT), specifically Major Autohemotherapy (MAH), as a host-directed adjunctive strategy in EVD. The framework is based on the capacity of O_3-AHT to generate controlled redox signals through Reactive Oxygen Species (ROS) and Lipid Ozonation Products (LOPs), which activate adaptive stress-response pathways including the Nrf2/Keap1/Antioxidant Response Element (ARE) system, the AMP-Activated Protein Kinase (AMPK)-Forkhead Box O (FOXO)-mechanistic Target of Rapamycin (mTOR)-sirtuin 1 (Sirt1) axis and Nrf2-mediated modulation of Nuclear Factor Kappa B (NF-κB) signaling. These interconnected pathways regulate antioxidant defenses, mitochondrial adaptation, autophagy, inflammatory responses, endothelial homeostasis and immune regulation-biological processes that intersect with major pathogenic mechanisms of EVD. Particular attention is given to Heme Oxygenase-1 (HO-1), a downstream effector of Nrf2 activation for which experimental evidence demonstrates suppression of Ebola virus replication *in-vitro*, providing a mechanistic bridge between host-directed redox modulation and antiviral activity. By integrating current knowledge from virology, immunology, redox biology and systems medicine, this framework identifies multiple points of convergence between ozone-induced adaptive signaling and Ebola pathophysiology.

This work does not establish clinical efficacy but presents a hypothesis-generating model intended to guide future experimental investigation. As a conceptual framework, it proposes O_3-AHT as a candidate host-directed adjunctive approach that warrants systematic evaluation in preclinical models of Ebola virus disease.

Keywords: Ebola Virus Disease; Ozone Autohemotherapy; Major Autohemotherapy; Host-Directed Therapy; Systems Biology; Nrf2; Heme Oxygenase-1; Redox Signaling; Viral Hemorrhagic Fever; Immunomodulation

Abbreviations

EVD: Ebola Virus Disease; EBOV: Ebola Virus; MAH: Major Autohemotherapy; O_3-AHT: Ozone Autohemotherapy; HDT: Host-

Directed Therapy; DIC: Disseminated Intravascular Coagulation; TF: Tissue Factor; ROS: Reactive Oxygen Species; LOPs: Lipid Ozonation Products; H_2O_2: Hydrogen Peroxide; HO-1: Heme Oxygenase-1; ARE: Antioxidant Response Element; Nrf2: Nuclear Factor Erythroid 2-Related Factor 2; Keap1: Kelch-like ECH-associated Protein 1; AMPK: AMP-Activated Protein Kinase; FOXO: Forkhead Box O Transcription Factors; mTOR: Mechanistic Target of Rapamycin; Sirt1: Sirtuin 1; PGC-1α: Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha; HIF-1α: Hypoxia-Inducible Factor 1-alpha; eNOS: Endothelial Nitric Oxide Synthase; iNOS: Inducible Nitric Oxide Synthase; NO: Nitric Oxide; NF-κB: Nuclear Factor Kappa B; Treg: Regulatory T Cells; Th17: T Helper 17 Cells; FoxP3: Forkhead Box P3; NK cells: Natural Killer Cells; IFN: Interferon; IL: Interleukin; TNF-α: Tumor Necrosis Factor Alpha; GP: Glycoprotein (Ebola Surface Protein); SOD: Superoxide Dismutase; CAT: Catalase; GPx: Glutathione Peroxidase; GSTs: Glutathione S-Transferases; GCL: Glutamate-Cysteine Ligase; NPC1: Niemann-Pick C1 Receptor; RIG-I: Retinoic Acid-Inducible Gene I; MDA5: Melanoma Differentiation-Associated Protein 5; IRF: Interferon Regulatory Factor; STAT1: Signal Transducer and Activator of Transcription 1; VP35: Viral Protein 35; VP24: Viral Protein 24; VP40: Viral Protein 40; VP30: Viral Protein 30; L: RNA-Dependent RNA Polymerase (Large Protein); NP: Nucleoprotein.

Introduction

Ebola Virus Disease (EVD) is among the most lethal infectious diseases known, with case-fatality rates historically ranging from 25% to 90% depending on outbreak context and available medical infrastructure [1]. The 2014-2016 West African epidemic, responsible for more than 11,000 deaths and subsequent outbreaks in the Democratic Republic of the Congo have underscored the persistent inadequacy of therapeutic options [2]. Although two monoclonal antibody regimens-Inmazeb (atoltivimab/maftivimab/odesivimab) and Ebanga (ansuvimab)-received FDA approval in 2020 and the rVSV-ZEBOV vaccine demonstrated ~97.5% efficacy in ring vaccination, mortality in patients presenting with advanced disease or high viral load remains substantial [3]. These agents neutralise viral entry but do not address the host-side immunopathological cascade-cytokine storm, coagulopathy and multi-organ failure-that drives late-stage mortality [3,4].

Host-Directed Therapies (HDTs) represent a complementary paradigm: rather than targeting the virus directly, they modulate host cellular networks to enhance survival, limit immunopathology and support tissue repair [4,5]. Ozone autohemotherapy (MAH) is an established clinical procedure in which autologous blood is withdrawn, ozonated *ex-vivo* at controlled concentrations (10-80 μg/mL) and reinfused, generating ROS and LOPs that act as systemic redox messengers [6,7]. Its documented activation of Nrf2, AMPK, HO-1 and anti-inflammatory pathways positions it as a multi-target host-modulating intervention [6,8,9]. To our knowledge, this manuscript represents the first scientific proposal to apply ozone autohemotherapy as a host-directed adjunctive strategy specifically for EVD, constructing a systems biology framework that links ozone-induced host responses to the molecular pathogenesis of Ebola virus disease and defining the evidence gaps that must be addressed before clinical translation.

Ebola Virus Biology

Taxonomy, Morphology and Genome

Ebola viruses are enveloped, filamentous, negative-sense single-stranded RNA viruses of the family Filoviridae, genus Orthoebolavirus, comprising six species: Zaire, Sudan, Bundibugyo, Taï Forest, Reston and Bombali [1,2]. The viral particle is 80 nm in diameter and up to 14,000 nm in length, with a helical nucleocapsid enclosed in a host-derived lipid envelope studded with trimeric GP spikes [1]. The ~19 kb genome encodes seven structural proteins in the order 3′-NP-VP35-VP40-GP-VP30-VP24-L-5′, with non-coding intergenic regions regulating transcriptional gradients [2].

Viral Proteins and Functions

The Nucleoprotein (NP) encapsidates the viral genome and nucleates replication complexes [1]. VP35 functions as both a polymerase co-factor and a potent interferon antagonist by blocking RIG-I/MDA5 signalling and inhibiting IRF3/7 phosphorylation [1,10]. VP24 further suppresses innate immunity by competing with karyopherin-α for STAT1 binding, preventing nuclear import of phospho-STAT1 and blocking IFN-stimulated gene expression [1,10]. VP40, the major matrix protein, drives virion budding and has been shown to directly trigger inflammatory responses linked to ebolavirus virulence [11]. VP30 acts as a transcriptional activator and the RNA-dependent RNA polymerase L, together with VP35, NP and VP30, constitutes the replication-transcription complex [12]. The surface glycoprotein GP mediates receptor binding and membrane fusion via the NPC1 receptor in endolysosomes and directly induces T-lymphocyte death through TLR4-dependent mechanisms independent of productive infection [13].

Replication Cycle and Host Dependencies

EBOV enters cells via macropinocytosis and endocytosis, traffics to late endosomes where cathepsins B and L cleave GP to expose the NPC1-binding domain and fuses with the endosomal membrane to release the nucleocapsid [1]. Genome-wide siRNA screening identified de novo pyrimidine synthesis and multiple host kinase pathways as essential for viral replication, highlighting druggable host dependencies [12]. Proximity proteomics of the EBOV polymerase complex further resolved host interactors involved in RNA processing, translation and stress responses, expanding the landscape of host-directed targets [14].

Ebola Pathophysiology

Primary Cellular Targets and Immune Evasion

EBOV primarily infects monocytes, macrophages and dendritic cells-cells that normally initiate and coordinate antiviral immunity [1,15]. Infected macrophages undergo activation and contribute to systemic inflammatory signalling and tissue pathology throughout the course of infection [15]. VP35 and VP24 coordinate early antagonism of type I IFN responses, allowing high early viral replication and dysregulated immune activation; VP35 impairs RIG-I/MAVS sensing and IFN induction, while VP24 contributes to downstream blockade of interferon signalling [10,16]. Ebolaviruses additionally cause incomplete activation of dendritic cells and impair antigen presentation, contributing to defective adaptive immunity and unchecked viral spread [10].

Cytokine Storm and Lymphocyte Apoptosis

Infected macrophages and dendritic cells produce massive quantities of pro-inflammatory cytokines constituting a cytokine storm that drives systemic inflammation and vascular permeability [4,17]. Despite this hyper-inflammatory state, profound lymphopenia occurs through bystander apoptosis of uninfected T and B lymphocytes, mediated in part by direct GP-TLR4 interactions and by Fas/FasL and TRAIL pathways [13,17]. The resulting immunosuppression impairs viral clearance and antibody production, creating a paradox of simultaneous hyperinflammation and immune failure [4,17].

Endothelial Dysfunction and Coagulopathy

Ebola virus-like particles reprogram endothelial cell metabolism, inducing functional changes consistent with endothelial involvement in vascular leakage and disease progression [18]. Endothelial injury in EVD is linked to dysregulated haemostasis, microthrombus formation and a DIC-like consumptive coagulopathy; proposed mechanisms include endothelial activation with release of ultra-large von Willebrand factor and microthrombotic processes contributing to organ ischaemia [19]. EBOV-infected monocytes and macrophages overexpress Tissue Factor (TF), initiating the extrinsic coagulation cascade and driving Disseminated Intravascular Coagulation (DIC), characterised by simultaneous thrombosis and haemorrhage [20]. The convergence of coagulopathy, endothelial damage, cytokine-mediated organ injury and metabolic failure results in multi-organ failure the proximate cause of death in fatal EVD [2,16].

Current Standard Therapies

Inmazeb and Ebanga, both FDA-approved in 2020, target non-overlapping GP epitopes and demonstrated 28-day mortality reductions to 24% and 35%, respectively, versus 51% for ZMapp in the PALM randomised controlled trial [3]. The rVSV-ZEBOV-GP (Ervebo) vaccine demonstrated ~97.5% efficacy in ring vaccination during the 2018-2020 DRC outbreak [3]. Despite these advances, mortality in patients with high viral loads or late presentation remains high and no approved agent addresses the host-side pathological cascade cytokine storm, coagulopathy and endothelial failure-that dominates late-stage disease [3,4]. Drug discovery programmes continue to explore small molecules, RNAi platforms and host-targeting agents to address these gaps, motivating the investigation of host-directed adjunctive strategies that can modulate the immunopathological cascade [4,5].

Ozone Autohemotherapy: Procedure and Biochemistry

Major Autohemotherapy Procedure

In MAH, 100-250 mL of venous blood is withdrawn into a sterile glass bottle containing anticoagulant, exposed to a precisely metered oxygen-ozone gas mixture at concentrations of 10-80 μg/mL for 5-10 minutes with gentle agitation and reinfused intravenously [6,7]. The procedure is performed in a closed system to prevent ozone escape and concentrations are titrated to the clinical indication and patient tolerance [9]. Clinical protocols typically involve serial treatments administered over days to weeks, with the cumulative effect reflecting sustained activation of adaptive cellular programmes [7].

Generation of ROS, LOPs and Plasma Biochemistry

Ozone (O_3) reacts instantaneously with plasma components, particularly unsaturated fatty acids and proteins, generating Hydrogen Peroxide (H_2O_2) and a spectrum of Lipid Ozonation Products (LOPs) including hydroperoxides, aldehydes (4-hydroxynonenal, malondialdehyde) and isoprostanes [6,8]. Parenteral ozone exposure produces controlled ROS and LOPs that act as second messengers to trigger adaptive cellular responses (hormesis) rather than overt oxidative damage at therapeutic doses [9, 7]. H_2O_2 acts as a short-lived, membrane-permeant redox messenger activating downstream signalling cascades, while LOPs serve as longer-lived systemic transducers that persist in circulation after reinfusion [6]. Albumin and plasma antioxidants buffer the initial oxidative stimulus, permitting controlled cellular signalling without systemic oxidative damage [9]. The antioxidant capacity of plasma follows a characteristic biphasic pattern: an initial transient decrease is followed by a sustained rebound above baseline, reflecting activation of endogenous antioxidant systems [9].

Obeid (2020) provided a mechanistic synthesis of O_3-AHT's antiviral and antioxidant properties that is directly relevant to the framework proposed here. At the antiviral level, O_3 has been shown *in-vitro* to oxidise the lipoproteins, glycoproteins and surface proteins of lipid-enveloped viruses, thereby disrupting virus-to-cell contact and interfering with viral reproductive cycles. This mechanism is of particular relevance to EBOV, a lipid-enveloped filovirus whose surface glycoprotein GP mediates receptor binding and membrane fusion-both processes susceptible to oxidative disruption. At the antioxidant level, Obeid, described how LOPs, especially 4-Hydroxynonenal (4-HNE), form adducts with Keap1 cysteine residues (C272 and C288), releasing Nrf2 from proteasomal degradation and enabling its nuclear translocation and ARE-driven transcription of SOD, GPx, CAT, HO-1 and NQO-1. Additionally, O_3-induced cytokine release-including IFN-γ, IL-2 and TNF-α from ozonated leukocytes was proposed to enhance NK cell activity and T-cell proliferation, partially restoring the antiviral immune capacity that EBOV systematically dismantles [21]. Obeid, concluded that O_3 therapy warrants investigation across the spectrum of lipid-enveloped viral infections; to our knowledge, the present manuscript constitutes the first systematic application of this rationale to Ebola virus disease, integrating these mechanisms within a comprehensive systems biology framework.

Mitohormesis and Redox Signalling

Hormesis describes the phenomenon whereby low-dose stressors elicit adaptive responses that enhance cellular resilience [6,7]. Ozone-derived ROS and LOPs produce a mild, transient oxidative perturbation that activates mitochondrial stress sensors, triggering mitohormetic programmes including mitochondrial biogenesis, enhanced oxidative phosphorylation efficiency and upregulation of antioxidant and cytoprotective gene networks [9]. Controlled ozone exposure can alter mitochondrial function and bioenergetics, producing hormetic mitochondrial signalling that underpins therapeutic claims in experimental studies [7,9]. This controlled oxidative preconditioning is mechanistically distinct from pathological oxidative stress: the dose, duration and cellular context of the oxidative signal determine whether the outcome is adaptive or injurious [6, 8]. The temporal dynamics of mitohormetic responses involve both immediate kinase-level signalling and sustained transcriptional adaptations that can persist for days to weeks, providing the mechanistic basis for cumulative therapeutic effects with serial MAH treatments [9].

Nrf2/Keap1/ARE System

The Nuclear Factor erythroid 2-related factor 2 (Nrf2) pathway is the master regulator of cellular antioxidant defence and a primary molecular target of ozone-induced redox signalling [22,23]. Under basal conditions, Nrf2 is constitutively ubiquitinated by the Keap1-Cullin3 E3 ligase complex and targeted for proteasomal degradation [23]. Ozone-generated ROS and LOPs oxidise critical cysteine sensor residues in Keap1 (C151, C273, C288), inducing conformational changes that disrupt Keap1-Nrf2 binding and permit Nrf2 stabilisation, nuclear translocation and heterodimerisation with small Maf proteins [22,23]. MAH treatment cycles have been associated with increased activities of superoxide dismutase and catalase in patients, consistent with Nrf2 pathway engagement and antioxidant enzyme upregulation [24]. Nuclear Nrf2 binds Antioxidant Response Elements (ARE) in target gene promoters, activating transcription of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glutathione S-Transferases (GSTs), Glutamate-Cysteine Ligase (GCL) for glutathione synthesis and Heme Oxygenase-1 (HO-1) [22,23]. The coordinated induction of these enzymes and their NADPH-regenerating cofactor systems creates a comprehensive antioxidant network capable of neutralising oxidative stress and maintaining redox homeostasis under pathological conditions [23,24].

AMPK-FOXO-mTOR-Sirt1 Pathway

AMP-Activated Protein Kinase (AMPK), activated by the elevated AMP:ATP ratio associated with ozone-induced mitochondrial perturbation, coordinates cellular energy homeostasis and stress adaptation [8, 9]. AMPK phosphorylates and activates FOXO transcription factors, promoting expression of stress-resistance genes including manganese SOD and catalase and inhibits mTORC1, thereby activating autophagy and removing damaged organelles and proteins [8]. Sirt1, a NAD^+-dependent deacetylase upregulated under conditions of metabolic stress, deacetylates and activates both FOXO and PGC-1α, the master regulator of mitochondrial biogenesis, further enhancing mitochondrial number and function [8]. This AMPK-FOXO-mTOR-Sirt1 axis orchestrates a comprehensive metabolic adaptation programme conserving ATP, enhancing mitochondrial quality, activating autophagy and increasing stress resistance that could support cellular survival under the metabolic demands imposed by severe viral infection [8,9].

Nrf2-NF-κB Crosstalk

Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) is the principal transcriptional driver of the pro-inflammatory cytokine cascade in EVD [4,17]. Ozone-induced modulation of inflammatory cytokines (reductions in TNF-α and IL-1β) and the cross-talk between Nrf2 activation and NF-κB suppression constitute a key part of the mechanistic framework for ozone's anti-inflammatory action [8,24]. Nrf2 activation antagonises NF-κB signalling through multiple convergent mechanisms: direct protein-protein interaction between Nrf2 and the p65 NF-κB subunit sequesters p65 in the cytoplasm; Nrf2-induced HO-1 generates carbon monoxide and bilirubin, which inhibit IκB kinase and block NF-κB nuclear translocation; and competition between Nrf2 and NF-κB for the shared transcriptional co-activator CBP/p300 limits inflammatory gene expression [22,25]. Ozone autohemotherapy-driven Nrf2 activation therefore has the potential to shift the transcriptional environment from pro-inflammatory toward anti-inflammatory and cytoprotective states, directly opposing the cytokine storm that characterises fatal EVD [17,22].

Secondary Adaptive Pathways

HIF-1α

Hypoxia-Inducible Factor 1-alpha (HIF-1α) is stabilised under conditions of cellular hypoxia and oxidative stress, activating transcription of genes for angiogenesis (VEGF), glycolytic enzymes and erythropoietin [8]. In the context of EVD-associated tissue hypoxia and endothelial dysfunction, ozone-induced HIF-1α activation could theoretically support tissue oxygenation and vascular repair, though direct evidence in EBOV models is lacking [8, 9].

Heme Oxygenase-1

HO-1 is the most clinically relevant secondary adaptive target of ozone autohemotherapy in the context of EVD. Induced by Nrf2 activation, HO-1 catalyses the degradation of pro-oxidant heme to Carbon Monoxide (CO), biliverdin (subsequently reduced to bilirubin) and free iron, with CO and bilirubin exerting potent anti-inflammatory, anti-apoptotic and cytoprotective effects [25]. Critically, pharmacological induction of HO-1 by hemin or Cobalt Protoporphyrin (CoPP) directly suppresses EBOV replication in cell-based models by approximately 3-4 $\log_{10}$ and therapeutic administration of hemin reduced viral titres in EBOV-infected cell systems [26,27]. This makes HO-1 the most experimentally validated mechanistic intersection between ozone-induced host responses and direct antiviral activity against EBOV.

Nitric Oxide Signalling

Ozone-induced redox signalling modulates endothelial Nitric Oxide Synthase (eNOS) activity, influencing bioavailable Nitric Oxide (NO) levels [8]. Physiological NO produced by eNOS promotes vasodilation, inhibits platelet aggregation and maintains endothelial barrier integrity functions directly compromised in EVD-associated endothelial dysfunction [20, 19]. Inducible NOS (iNOS), upregulated during inflammatory states, generates supraphysiological NO that can contribute to oxidative stress via peroxynitrite formation; ozone-induced Nrf2 activation may help regulate this balance [8,22].

Effects on Blood Cell Populations

Red Blood Cells

Ozone treatment of erythrocytes increases intracellular ATP production and elevates 2,3-diphosphoglycerate (2,3-DPG) concentrations, which allosterically reduces haemoglobin-oxygen affinity (rightward Bohr shift), facilitating oxygen unloading

in peripheral tissues [6,7]. These effects optimise oxygen delivery to hypoxic tissues-a potentially significant benefit given the tissue hypoxia associated with EVD coagulopathy and endothelial dysfunction [6,20].

White Blood Cells and NK Cells

Ozone-derived LOPs activate macrophages, enhancing phagocytic capacity and modulating cytokine secretion profiles toward more regulated, less hyperinflammatory states [8]. Natural Killer (NK) cell activity is supported by ozone-induced cytokine modulation, potentially enhancing cytotoxic surveillance of virally infected cells [7,8].

T-Lymphocytes

Ozone-driven redox signalling promotes regulatory T-cell (Treg) expansion through FoxP3 induction and PPAR-γ activation, suppresses pathological Th17 responses and optimises $CD8^+$ cytotoxic T-cell function through modulation of IL-2 and IFN-γ signalling [8,24]. These effects are mechanistically relevant to EVD, where lymphocyte apoptosis and immune dysregulation impair viral clearance [13,17].

B-Lymphocytes and Platelets

Ozone-induced immunoregulation preserves B-lymphocyte viability and supports humoral immune homeostasis through indirect T-cell guidance, potentially maintaining antibody production capacity during severe infection [8]. Platelet activation by ozone-modulated blood components promotes alpha-granule release, PDGF and TGF-β secretion and tissue repair signalling functions relevant to the haemostatic dysregulation of EVD [7,8].

Effects on Endothelial Cells and Bone Marrow

Endothelial cells are primary targets of ozone-induced adaptive responses. Nrf2 activation in endothelial cells increases HO-1 expression, enhances eNOS-derived NO bioavailability and strengthens tight-junction protein expression, collectively stabilising the endothelial barrier [22,25]. These effects directly oppose the endothelial dysfunction increased permeability, barrier disruption, microvascular thrombosis-that is a central pathological feature of EVD [19,20]. Metabolic reprogramming of endothelial cells by EBOV components further underscores the importance of targeting endothelial metabolism as a host-directed strategy [18]. Ozone-induced AMPK activation additionally supports endothelial metabolic resilience and mitochondrial function under inflammatory stress conditions [9]. Systemic anti-inflammatory and redox-balancing effects of MAH are proposed to support erythropoiesis and haematopoietic stem cell function through improved bone marrow microenvironment signalling though direct experimental evidence in viral haemorrhagic fever models is not yet available [9,24].

Integrated Ebola-Ozone Systems Biology Model

The systems biology framework proposed here maps ozone-induced molecular events onto the pathological cascade of EVD at multiple levels:

1. Redox messenger generation: MAH produces H_2O_2 and LOPs that enter systemic circulation and engage redox-sensitive sensors in multiple cell types simultaneously [6,8]
2. Nrf2 node activation: Keap1 cysteine oxidation releases Nrf2, which drives ARE-dependent transcription of SOD, CAT, GPx, GCL and HO-1 across immune cells, endothelial cells and hepatocytes [22,23]
3. AMPK-Sirt1 metabolic reprogramming: Concurrent AMPK activation promotes autophagy, mitochondrial biogenesis and energy conservation, supporting cellular survival under viral-infection metabolic stress [8,9]
4. NF-κB suppression: Nrf2-mediated NF-κB antagonism and HO-1-derived CO/bilirubin reduce transcription of pro-inflammatory cytokines, attenuating cytokine storm [22,25]
5. HO-1 antiviral activity: Nrf2-induced HO-1 directly suppresses EBOV replication-the only experimentally validated direct antiviral mechanism in this framework [26,27]
6. Treg expansion and immune recalibration: PPAR-γ and FoxP3-driven Treg expansion counteracts lymphocyte apoptosis-induced immunosuppression and restores immune regulation [8,24]
7. Endothelial stabilisation: NO bioavailability restoration and tight-junction reinforcement oppose vascular leakage and microvascular thrombosis [19,20,22]
8. Oxygen delivery optimisation: RBC 2,3-DPG elevation improves tissue oxygenation in hypoxic organ beds [6,7]

This multi-node, multi-level intervention profile distinguishes ozone autohemotherapy from single-target antivirals and positions it as a candidate systems-level HDT adjunct.

Theoretical Impact on Ebola Symptoms (Table 1)

EVD Feature	Ozone-Induced Counter-Mechanism	Key Pathways	Evidence Level
Cytokine storm	NF-κB suppression via Nrf2-HO-1 axis	Nrf2/NF-κB [22,25]	Mechanistic
Lymphocyte apoptosis	Treg expansion; FoxP3/PPAR-γ induction	Immunomodulatory [8,24]	Indirect
Endothelial dysfunction	eNOS/NO restoration; HO-1; tight junctions	Nrf2/HO-1/NO [22,25]	Mechanistic
Vascular leakage	Barrier stabilisation; NO bioavailability	Endothelial [19,20]	Mechanistic
Tissue hypoxia	2,3-DPG elevation; HIF-1α activation	RBC/HIF-1α [6,8]	Mechanistic
Coagulopathy/DIC	Platelet modulation; anti-inflammatory	Haemostatic [8,20]	Theoretical
Multi-organ failure	AMPK/autophagy; metabolic resilience	AMPK-Sirt1 [8,9]	Theoretical
Viral replication	HO-1 direct antiviral suppression	HO-1 [26,27]	In vitro

Table 1: Impact on Ebola symptoms.

Discussion

The systems biology framework presented here demonstrates that ozone autohemotherapy engages a network of host pathways-Nrf2/Keap1/ARE, AMPK-FOXO-mTOR-Sirt1, Nrf2-NF-κB crosstalk, HIF-1α, HO-1 and NO signalling-that collectively oppose the principal molecular drivers of EVD pathogenesis [6,8,9,22]. The most compelling mechanistic intersection is HO-1: ozone therapy induces HO-1 as a cytoprotective and antiviral effector, while EBOV pathogenesis creates conditions that HO-1 is biologically designed to counteract [22,25-27]. The discovery that VP40 directly triggers inflammatory responses linked to ebolavirus virulence further expands the landscape of host-directed targets that ozone-induced anti-inflammatory pathways could address [11].

From a network biology perspective, the advantage of a host-directed approach over single-target antiviral therapy lies in its ability to modulate multiple disease-relevant nodes simultaneously [4,5]. Monoclonal antibodies such as Inmazeb and Ebanga are highly effective at neutralising viral entry but do not address the downstream immunopathological cascade [3]. The metabolic reprogramming of endothelial cells by EBOV components [18] and the central role of macrophage activation in tissue pathology represent additional host-side targets that ozone-induced redox signalling could theoretically modulate [15]. A combination strategy-approved antivirals to reduce viral burden plus MAH to modulate host immunopathology-could therefore address both arms of EVD lethality [3,4].

Several critical limitations must be acknowledged. First, no direct preclinical or clinical evidence demonstrates that ozone autohemotherapy reduces viral load, cytokine levels or mortality in EBOV infection. Second, the hormetic dose-response relationship of ozone is highly context-dependent; the immunosuppressive environment of advanced EVD may alter cellular responses to ozone-derived signals in unpredictable ways [7]. Third, the coagulopathy of EVD is a complex, multi-factorial process involving TF overexpression, endothelial activation and microthrombosis that may not be substantially modifiable by redox-based interventions alone [19,20]. Fourth, logistical constraints-the requirement for specialised equipment and trained personnel-limit MAH applicability in resource-limited outbreak settings. These gaps define a clear research agenda: controlled in vitro studies in EBOV-infected primary human cells, followed by non-human primate models, are prerequisites before any clinical investigation.

Conclusion

Ebola Virus Disease (EVD) remains a devastating viral hemorrhagic fever in which mortality is driven not only by viral replication but also by a profound dysregulation of host biological systems, including hyperinflammation, endothelial injury, coagulopathy, metabolic collapse and immune dysfunction. While currently approved monoclonal antibodies have significantly improved outcomes by targeting viral entry, they do not directly address the complex host-response network responsible for late-stage organ failure and death.

The systems biology framework presented in this manuscript identifies a compelling mechanistic convergence between the pathophysiology of EVD and the biological effects induced by Ozone Autohemotherapy (O_3-AHT). Through the controlled generation of Reactive Oxygen Species (ROS) and Lipid Ozonation Products (LOPs), O_3-AHT activates adaptive redox-signalling pathways, particularly the Nrf2/Keap1/ARE system, the AMPK-FOXO-mTOR-Sirt1 metabolic axis and Nrf2-mediated suppression of NF-κB-driven inflammation. Collectively, these pathways promote antioxidant defense, mitochondrial resilience, autophagy, immune recalibration, endothelial stabilization and restoration of redox homeostasis-biological processes that directly counteract major drivers of EVD pathology. Among the mechanisms discussed, induction of Heme Oxygenase-1 (HO-1) represents the strongest experimental link between ozone-mediated host responses and Ebola virus biology. Existing *in-vitro* evidence demonstrating that HO-1 induction can suppress EBOV replication provides a biologically plausible bridge between host-directed redox modulation and antiviral activity. Furthermore, the potential effects of O_3-AHT on regulatory T-cell expansion, endothelial barrier integrity, nitric oxide signaling, oxygen delivery and metabolic adaptation suggest a unique capacity to simultaneously influence multiple disease-relevant pathways that are not targeted by current antiviral therapies.

Importantly, the present work does not establish clinical efficacy for ozone autohemotherapy in Ebola virus disease. Rather, it provides a hypothesis-generating and mechanistically grounded model that integrates contemporary knowledge from virology, immunology, redox biology and systems medicine. The proposed benefits remain theoretical and must be validated through rigorous experimental investigation. Critical next steps include studies in EBOV-infected primary human cells, mechanistic analyses of cytokine and endothelial responses, evaluation in validated animal models and ultimately carefully designed clinical trials if preclinical evidence proves favorable. This manuscript represents the first comprehensive systems-biology framework that integrates the molecular mechanisms of ozone autohemotherapy with the host-pathophysiological pathways of Ebola Virus Disease and proposes O_3-AHT as a host-directed adjunctive therapeutic strategy. The convergence of ozone-induced adaptive signaling pathways with key pathogenic mechanisms of EVD supports further investigation of this approach as a potential complement-not a replacement-to established antiviral and supportive therapies. If validated experimentally, O_3-AHT could represent a novel systems-level therapeutic modality capable of enhancing host resilience, attenuating immunopathology and improving outcomes in severe viral hemorrhagic fever.

Conflict of Interest
The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Data Availability Statement
The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement
The project did not meet the definition of human subject research under the purview of the IRB according to federal regulations and therefore was exempt.

Informed Consent Statement
Informed consent was obtained from all participants included in the study.

Authors' Contributions
All authors contributed equally to this paper.

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