Hypothesis

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Glucosodiene as a Non-Enzymatically Generated Glucose-Derived Glycosidic System: Multi-Scale Reprogramming of the Glycolysis–pH–Glycocalyx Network from Molecular Design to Nanotechnology-Enabled Translational Oncology

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Glucosodiene as a Non-Enzymatically Generated Glucose-Derived Glycosidic System: Multi-Scale Reprogramming of the Glycolysis–pH–Glycocalyx Network from Molecular Design to Nanotechnology-Enabled Translational Oncology

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Abstract

Background and Purpose: Tumor progression is sustained by a tightly coupled biochemical network linking aerobic glycolysis, extracellular acidification, and glycocalyx-dependent membrane signaling. This study evaluates glucosodiene, a non-enzymatically generated glucose-derived glycosidic system, as a multi-axis perturbant capable of simultaneously modulating metabolic flux, pH homeostasis, and glycan architecture. **Methods:** A translational framework was constructed integrating chemical synthesis modeling, spectroscopic validation (^{1}H NMR, ^{13}C NMR, FTIR), nanostructured formulation analysis (glucosodiene-loaded nanoferrites, GLONF), in vitro cytotoxicity assessment, preclinical murine data, and early human case-based observations. **Results:** Structural analysis supports glucosodiene as a 1→2 glycosidic glucose derivative with preserved hydroxyl-rich architecture and altered stereochemical properties. In vitro, no detectable cytotoxicity was observed in BJ1 fibroblasts up to 100 μg/mL (LC_{50} not reached). In vivo, GLONF administration (50.4 mg/kg/day) in Ehrlich solid tumor-bearing mice resulted in a significant reduction in tumor weight and size compared to untreated controls ($p < 0.01$), with the most pronounced effect observed in the post-treatment group. This was accompanied by normalization of hepatic biomarkers, as ALT decreased from 47.0 to 34.8 U/L and AST from 306.4 to 199.0 U/L. Antioxidant systems were restored, with GSH increasing from 0.67 to 2.07 mmol/g and SOD from 58.7 to 97.7 U/g, alongside a marked reduction in lipid peroxidation, reflected by a decrease in MDA from 166.8 to 79.1 nmol/g. Molecular analysis demonstrated attenuation of proliferative signaling (PCNA) and modulation of p53-associated stress response pathways. Early clinical observations (n = 3) showed rapid symptomatic improvement by day 5 and metabolically favorable imaging responses within 15–20 days, including PET-documented regression and biomarker reduction, where ALP declined from 700 to 280 U/L and CA15-3 from 146.6 to 78.1 KU/L. **Mechanistic Interpretation:** Glucosodiene is proposed to reduce effective glycolytic throughput, attenuate lactate-driven extracellular acidosis, and disrupt glycocalyx integrity via altered glycosylation dynamics, thereby reconditioning tumor metabolic and signaling states. **Conclusion:** Glucosodiene represents a glucose-derived systems-level perturbant targeting the integrated metabolism–microenvironment–glycocalyx network, warranting further mechanistic and translational validation.

Keywords: glucosodiene; non-enzymatic glycosylation; 1→2 glycosidic linkage; tumor metabolism reprogramming; glycocalyx disruption; metabolic cancer therapy

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1. Introduction

Cancer is increasingly understood as a systems-level disease in which malignant progression is sustained not only by genetic and epigenetic alterations, but also by coordinated reprogramming of cellular metabolism, extracellular chemistry, stromal interactions, and immune regulation [1]. One of the most consistent features of this reprogramming is the shift toward high-flux aerobic glycolysis, even in the presence of oxygen, which permits malignant cells to maintain rapid ATP turnover, support anabolic biosynthesis, preserve redox balance, and survive under fluctuating nutrient and oxygen conditions [2,3]. This metabolic phenotype is not merely a bioenergetic adaptation; it is a structural and signaling determinant of the tumor microenvironment [4].

A major consequence of sustained glycolysis is the overproduction of pyruvate-derived lactate, together with coupled proton export through monocarboxylate transport systems and other membrane acid-handling mechanisms [5]. The resulting extracellular acidification is a defining biochemical feature of many tumors. In contrast to normal tissues, where extracellular pH is tightly regulated to preserve enzyme activity, immune function, and tissue organization, tumor tissue frequently evolves toward extracellular acidosis with relative intracellular alkalinization [6]. This reversed pH gradient has profound biological consequences. It supports local invasion through activation of pH-sensitive proteases, promotes extracellular matrix remodeling, weakens drug efficacy, and alters the signaling behavior of both malignant and non-malignant cells [7]. It also exerts potent immunoregulatory effects, including inhibition of $CD8^+$ T-cell proliferation and cytokine production, impairment of natural killer cell cytotoxicity, promotion of regulatory T-cell persistence, and reinforcement of suppressive myeloid phenotypes [8].

In this way, acidity is not a passive metabolic byproduct but a functional component of tumor persistence [9]. At the same time, glucose in cancer is not simply oxidized or fermented; a substantial fraction of glucose-derived carbon is redirected into biosynthetic pathways that construct the tumor cell surface [10]. Among these, the hexosamine biosynthetic pathway is especially important because it converts fructose-6-phosphate into UDP-N-acetylglucosamine and related activated sugar donors that are required for N-linked glycosylation, O-linked glycosylation, glycolipid synthesis, and proteoglycan assembly [11,12]. These pathways feed the construction of the glycocalyx, a dense carbohydrate-rich layer composed of glycoproteins, glycolipids, mucins, and extracellular glycoconjugates that surrounds the tumor cell membrane [13]. In malignant tissues, this glycocalyx is often enlarged, compositionally distorted, and functionally optimized to support receptor clustering, mechanotransduction, integrin signaling, migration, stromal engagement, and immune concealment [14]. Surface glycans also participate directly in suppressive immune interactions, including binding to lectin receptors such as galectins and siglecs, thereby reducing effective immune synapse formation and promoting immune tolerance [15,16].

These observations indicate that tumor metabolism, extracellular acid–base balance, and membrane glycosylation are not independent processes. Rather, they form a coupled biochemical continuum [17]. The same glucose flux that feeds glycolysis also sustains glycosylation; the same glycolytic state that generates lactate also creates an extracellular environment that reinforces immune dysfunction; and the same biosynthetic pathways that preserve membrane glycan structure help stabilize malignant signaling and protect tumor cells from host attack [18,19]. Accordingly, the tumor cell may be viewed as being maintained by an integrated metabolic–chemical–glyco-structural system, rather than by energy metabolism alone. Within this context, glucosodiene is considered here as a glucose-derived glycosidic system provisionally assigned as 1–2-O-β-D-glucopyranosyl-α-D-glucose. The significance of this compound lies not simply in its origin from glucose, but in the possibility that it retains enough structural familiarity to enter glucose-associated biological handling while possessing sufficient chemical divergence to alter downstream processing. In mechanistic terms, glucosodiene is therefore considered not as a conventional cytotoxic compound, but as a candidate metabolic and glyco-structural perturbant. Its proposed biological relevance lies in the possibility that it may influence three tightly linked domains: effective glycolytic throughput, extracellular pH homeostasis, and glucose-dependent construction of the tumor glycocalyx [20]. The

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purpose of the present manuscript is therefore to define glucosodiene within a scientifically precise translational framework. Specifically, this review aims to: (i) examine the chemical and spectroscopic basis for its assignment as a glucose-derived glycosidic structure; (ii) develop a biologically grounded model linking glucosodiene to glycolytic regulation, extracellular pH remodeling, and glycocalyx biology; (iii) integrate the currently available in vitro, in vivo, and human observations without overstating their level of proof; and (iv) position glucosodiene within a broader oncology framework centered on the coupled metabolism–microenvironment–glycosylation axis.

2. Mechanistic Hypothesis: Glucosodiene as a Modulator of Glycolytic Flux, pH Homeostasis, and Glycocalyx Integrity

The central working hypothesis of this review is that glucosodiene may act as a multi-layered biochemical perturbant within a tumor system whose survival depends on uninterrupted glucose handling. This model does not assume that glucosodiene functions as a direct poison. Rather, it proposes that the compound may alter the behavior of tumor cells by disturbing the biochemical continuity between glucose uptake, glycolytic carbon flow, extracellular acidification, and glycan-dependent cell-surface organization. The first component of this hypothesis concerns the regulation of glycolytic flux. In highly glycolytic tumor cells, glucose uptake is typically increased through facilitative transporters such as GLUT1 and, in some contexts, GLUT3 [21]. Once internalized, glucose is rapidly phosphorylated by hexokinase, retained intracellularly, and committed to downstream processing through phosphofructokinase-driven glycolysis [22]. In malignant cells, this pathway often operates under the influence of HIF-1α, MYC, PI3K/AKT/mTOR signaling, and growth factor receptor amplification, thereby linking glucose metabolism directly to proliferation, survival, and biosynthesis [23]. A glucose-derived glycosidic structure that preserves partial substrate resemblance while diverging in stereochemical accessibility may interfere with this high-flux system at one or more levels [24,25]. Such interference could occur at the level of uptake competition, altered enzymatic recognition, ineffective downstream phosphorylation compatibility, or perturbation of intracellular carbon routing [26].

Reduced glycolytic productivity would be expected to diminish pyruvate-to-lactate conversion and therefore decrease extracellular lactate accumulation [27]. This leads directly to the second component of the hypothesis, namely modulation of tumor pH homeostasis. The acidic tumor microenvironment is sustained not only by lactate production but by coordinated proton export, carbonic anhydrase activity, bicarbonate handling, and membrane transporter function [28]. This acidified extracellular compartment contributes to selective immune paralysis, impairs antigen-directed cytotoxicity, alters membrane receptor behavior, and changes the kinetics of many enzyme systems [29,30]. Acid stress can also reshape intracellular signaling networks, affecting AMPK, mTOR, NF-κB, MAPK stress responses, and p53-associated signaling [31]. Although p53 is often considered primarily in the context of genomic stress, its functional state is also influenced by broader metabolic and chemical context, including oxidative load, nutrient pressure, and stress microenvironment [32].

Under this framework, partial reduction of extracellular acid burden could help restore a biochemical environment in which growth restraint, cell-cycle regulation, and apoptosis-related pathways are more responsive [33]. This is mechanistically relevant to the observed association between glucosodiene-containing treatment and reduction of proliferative stress markers such as PCNA together with modulation of p53-associated signals in preclinical tissue [34].

The third component concerns the tumor glycocalyx, which represents one of the most underappreciated but mechanistically important consequences of glucose-dependent tumor biology. The glycocalyx is not merely a surface coating; it is a dynamic molecular interface that governs receptor spacing, ligand accessibility, membrane curvature, mechanochemical signaling, and immune-cell contact. In tumors, the glycocalyx is often enriched in highly branched N-glycans, aberrant O-glycans, sialylated structures, mucins, and proteoglycan-associated side chains [35]. These structures enhance receptor tyrosine kinase clustering, stabilize growth signaling through

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PI3K/AKT and MAPK cascades, and shield tumor cells from immune recognition by increasing steric hindrance and by engaging inhibitory glycan-sensing receptors on immune cells [36,37]. Because the construction and renewal of this structure depend on activated sugar donors derived from glucose metabolism, especially via the hexosamine biosynthetic pathway, glycocalyx integrity is inseparable from metabolic state [38].

Under this model, glucosodiene may affect glycocalyx biology in two nonexclusive ways. First, by lowering effective glucose flux into biosynthetic pathways, it may reduce the substrate supply needed to sustain high-rate glycan synthesis. Second, if glucose-derived but structurally altered intermediates are incorporated imperfectly into glycosylation-associated processing, glucosodiene may introduce qualitative disturbance into glycan maturation, branching, or glycoconjugate assembly [20].

Such disruption would be expected to alter receptor organization, weaken membrane-associated signaling platforms, and reduce immune concealment. In immunological terms, this could favor more effective immune synapse formation, reduce galectin- and siglec-mediated dampening, and partially restore cytotoxic engagement by $CD8^+$ T cells and NK cells [39,40]. Thus, the proposed effect is not merely depletion of the glycocalyx, but potential glycocalyx destabilization and remodeling [41,42].

These three layers glycolytic attenuation, pH remodeling, and glycocalyx disruption should not be viewed as parallel but independent pathways. They are metabolically and functionally coupled. Reduced glycolysis lowers lactate burden; reduced acid burden improves signaling conditions for cell stress and immune function; disturbed glycosylation weakens cell-surface shielding and oncogenic receptor organization; and the combination of these changes may shift the tumor from a chemically protected and structurally stabilized state toward one that is metabolically strained, less immunologically concealed, and less permissive to malignant persistence [43–45]. This integrated working model is summarized in Table 1.

Table 1. Integrated mechanistic model of glucosodiene activity in tumor systems.

Biological axis	Tumor baseline state	Effect of glucosodiene	Downstream consequence
Glycolytic metabolism	Increased glucose uptake, high glycolytic flux, elevated lactate	Disruption of glucose-compatible metabolic processing	Reduced glycolytic throughput, metabolic stress, decreased lactate production
pH homeostasis	Extracellular acidosis with relative intracellular alkalinization	Modulation of proton dynamics and partial attenuation of acid load	Restoration of apoptosis-permissive conditions and improved signaling responsiveness
p53 / proliferation signaling	Dysregulated stress signaling, elevated proliferation	Modulation of p53-associated pathways; reduced PCNA expression	Decreased proliferation, enhanced stress response
Glycosylation / glycocalyx	Hyperglycosylation, receptor clustering, immune shielding	Altered glycan precursor availability and glycan processing	Glycocalyx destabilization, altered receptor organization
Tumor–immune interface	Suppressed $CD8^+$ and NK activity, lectin-mediated inhibition	Reduced acidity and altered glycan presentation	Improved immune recognition and cytotoxic response

Taken together, this model positions glucosodiene not as a classical single-node inhibitor, but as a candidate systems-level modulator of the tumor metabolic–chemical–glyco-structural continuum.

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3. Chemical Structure, Non-Enzymatic Formation, Spectroscopic Validation, and Nanostructured Optimization of Glucosodiene

Glucosodiene is herein defined as a glucose-derived glycosidic entity formed through a non-enzymatic condensation process under thermally induced, mildly alkaline conditions. Based on integrated mechanistic reasoning and spectroscopic evidence, the compound is most consistently interpreted as a disaccharide-like structure characterized by a 1→2 interglycosidic linkage, provisionally assigned as 1–2-O-β-D-glucopyranosyl-α-D-glucose. This assignment reflects the preservation of the glucopyranose scaffold while introducing a chemically induced bond between two glucose units in the absence of enzymatic catalysis, thereby generating a molecule that retains carbohydrate identity yet exhibits altered stereochemical and biochemical behavior (Figure 1).

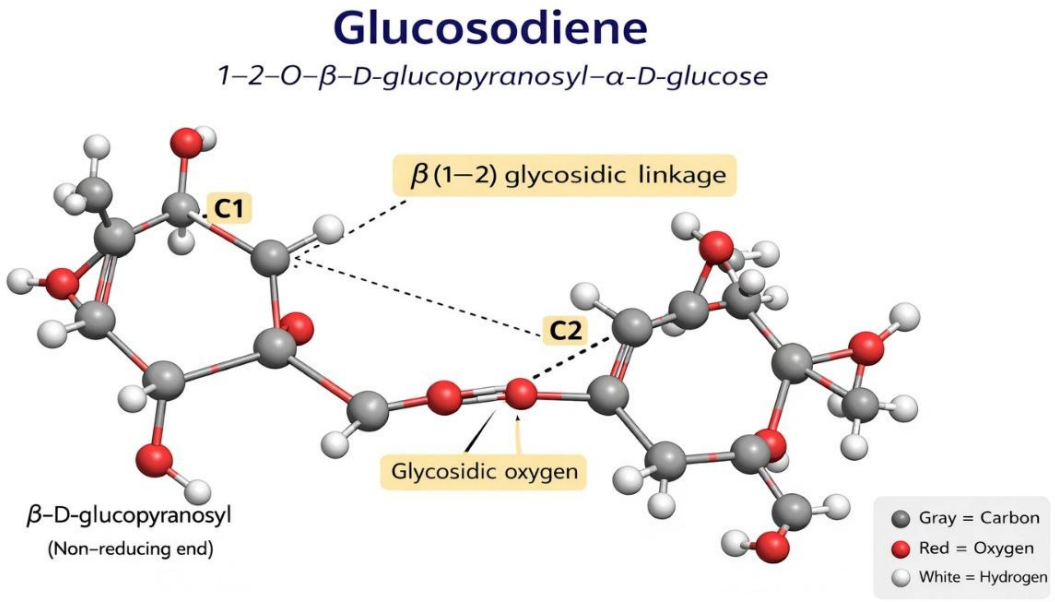

Figure 1. Three-dimensional molecular representation of the proposed glucosodiene structure, illustrating a β-(1→2) glycosidic linkage between a β-D-glucopyranosyl (non-reducing) unit and an α-D-glucose (reducing) unit, with explicit identification of the glycosidic oxygen and participating carbons (C1→C2).

The formation of glucosodiene proceeds via a controlled alkaline-assisted thermal reaction that can be reproducibly implemented at the microscale. In a representative protocol, approximately 35 mg of dextrose monohydrate and 25 mg of sodium bicarbonate are dissolved in 1 mL of ultrapure water under continuous agitation. Upon heating to 95–100 °C, effervescence is observed as a consequence of bicarbonate decomposition and carbon dioxide release, generating a transient alkaline microenvironment.

This localized increase in pH promotes partial deprotonation of glucose hydroxyl groups, particularly enhancing the nucleophilicity of the secondary hydroxyl at the C2 position. Concurrently, the anomeric carbon (C1) of another glucose molecule becomes transiently electrophilic through equilibrium with its open-chain aldehyde form. Under these conditions, a nucleophilic substitution-like condensation occurs, resulting in formation of a 1→2 glycosidic bond with elimination of water, yielding a structurally modified glucose dimer. The reaction is subsequently quenched by rapid cooling, and the product is isolated via lyophilization to obtain a stable amorphous compound suitable for downstream characterization [20]. The conventional microscale preparation is contrasted with the later nanostructured assembly strategy in Table 2.

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Table 2. Conventional synthesis versus GLONF nanostructured formulation.

Feature	Conventional synthesis	GLONF system
Process type	Alkaline thermal condensation	Multi-step nanoassembly
Conditions	95–100 °C, aqueous alkaline	Sol–gel + chitosan coating + loading
Core material	None	Spinel nanoferrite ($Sr_{0.5}Mn_{0.5}La_{0.02}Fe_{1.98}O_4$)
Polymer coating	None	Chitosan
Structural scale	Molecular	~25 nm nanoparticles
Stability	Moderate	Enhanced
Delivery efficiency	Limited	Improved cellular uptake and retention
Functional role	Metabolic perturbation	Targeted metabolic–structural modulation

The structural assignment of glucosodiene is strongly supported by nuclear magnetic resonance spectroscopy. The ^{13}C NMR spectrum demonstrates a prominent resonance near ~97 ppm, characteristic of an anomeric carbon involved in glycosidic linkage formation, accompanied by a dense cluster of signals within the 70–75 ppm range corresponding to oxygenated carbons typical of carbohydrate systems. A signal at approximately ~61 ppm is consistent with the presence of CH_2OH groups, confirming preservation of the glucose backbone. Importantly, the absence of peaks in the carbonyl region excludes oxidative degradation, supporting the integrity of the glycosidic structure. Complementary ^1H NMR analysis reveals a dominant distribution of proton signals between 3.0–4.5 ppm, indicative of protons bound to oxygen-bearing carbons, alongside an anomeric proton signal near ~4.8–5.0 ppm. Broad downfield signals in the ~9–10 ppm region are attributed to exchangeable hydroxyl protons rather than aldehydic functionalities, reinforcing the conclusion that the compound remains within a reduced carbohydrate framework **(**Figure 2) (Figure 3). Independent spectroscopic descriptions reported in the *Scientific Reports* study further corroborate this structural interpretation [20].

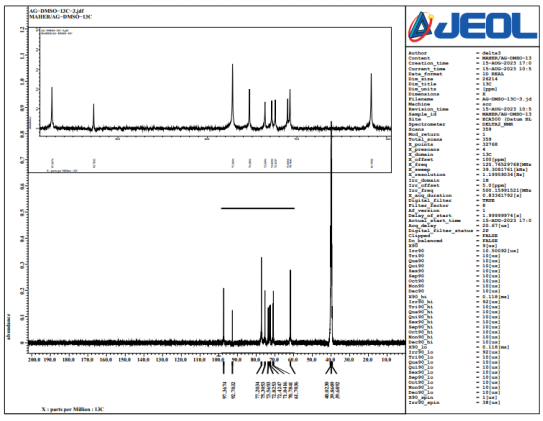

Figure 2.C

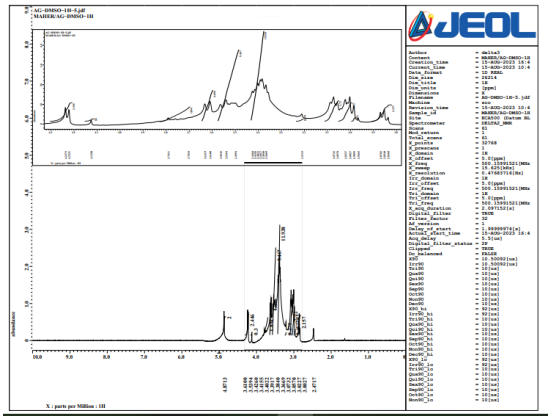

Figure 2.B

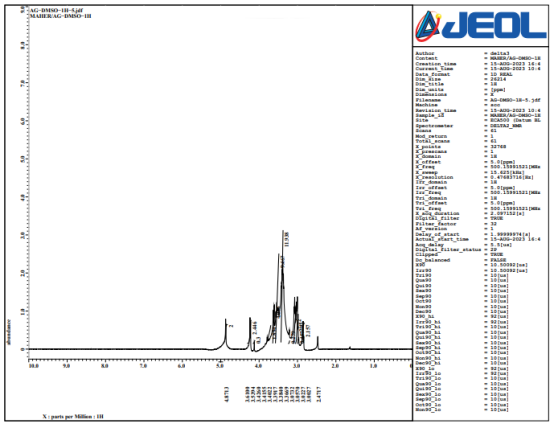

Figure 2.A

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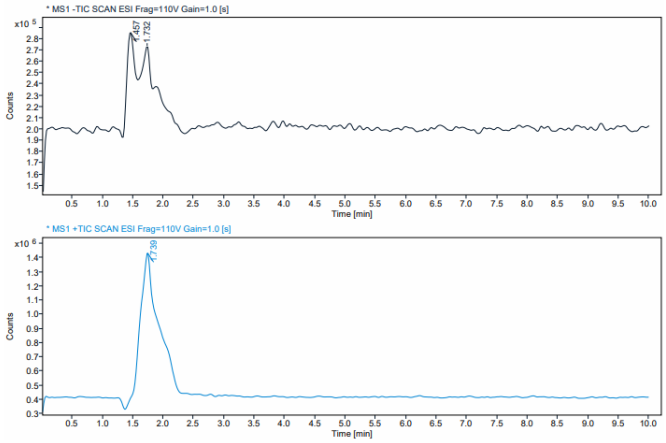
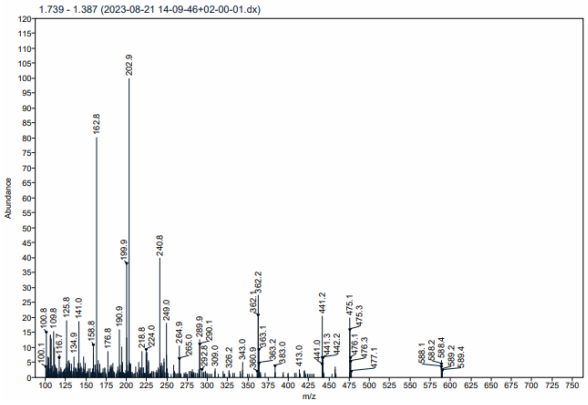
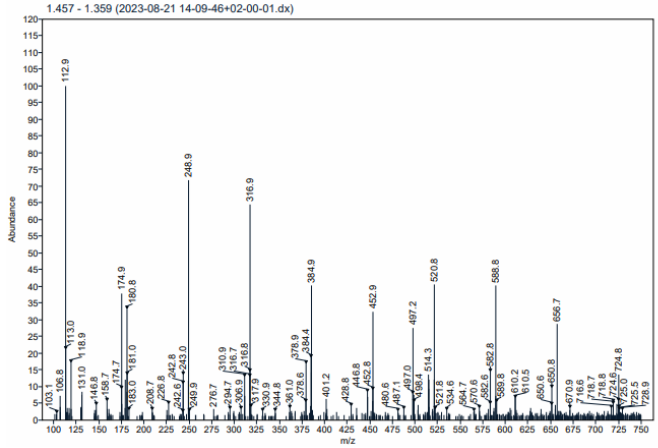
Figure 2. NMR spectroscopic characterization of glucosodiene (A) Full ^{1}H NMR spectrum (500 MHz, DMSO-d_6) showing the characteristic carbohydrate proton envelope dominated by signals in the δ 3.0–4.5 ppm region, with an anomeric proton resonance near δ ~4.8–5.0 ppm. (B) Expanded ^{1}H NMR region highlighting multiplet structure and proton coupling patterns associated with glucopyranose ring protons. (C) ^{13}C NMR spectrum (125 MHz, DMSO-d_6) demonstrating a diagnostic anomeric carbon signal at ~97 ppm, a cluster of oxygenated carbons between 70–75 ppm, and a CH_2OH signal near ~61 ppm, with no evidence of carbonyl functionalities. Collectively, the NMR data confirm the formation of a glycosidic, hydroxyl-rich, non-aromatic carbohydrate structure consistent with a glucose-derived dimeric system featuring a preserved pyranose framework and non-enzymatically generated glycosidic linkage.







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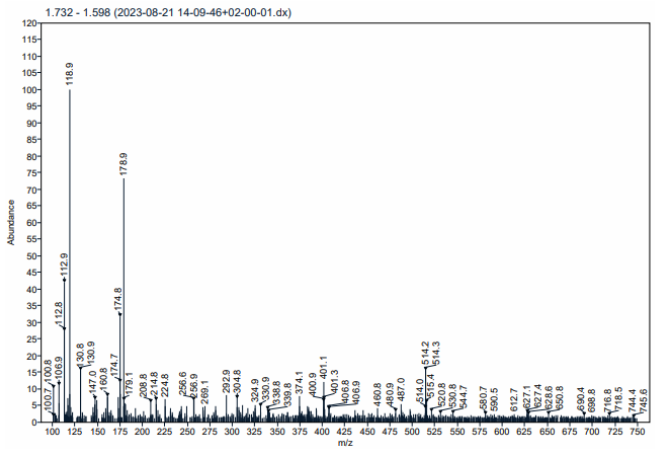

Figure 3. LC–MS analysis of glucosodiene (diglucose) showing a dominant early elution profile (~1.4–1.7 min) consistent with a highly polar carbohydrate-derived compound. The mass spectra exhibit characteristic fragmentation patterns, including low m/z carbohydrate ions (~100–250), intermediate fragments (~250–400) corresponding to glycosidic bond cleavage, and higher m/z signals (~360–380 and above) indicative of disaccharide-like structures and possible oligomeric species. These findings support the presence of a glucose-derived glycosidic system with preserved carbohydrate backbone and limited structural heterogeneity.

The published spectra demonstrated clear anomeric signals consistent with glycosidic bond formation, multiplet patterns reflecting preserved cyclic glucose conformations, and CH_2OH-associated resonances confirming intact primary alcohol groups. Notably, the presence of minor additional peaks was interpreted as evidence of selective chemical modifications and structural heterogeneity, which is entirely consistent with a non-enzymatic condensation mechanism. The reported spectral complexity, including coupling patterns and chemical shift distributions, aligns closely with those obtained for glucose-derived glycosides and supports the conclusion that glucosodiene is a polymeric or oligomeric glucose derivative with preserved ring integrity and partial chemical modification rather than a simple degradation product [20,34]. The principal structural and physicochemical features of glucosodiene are summarized in Table 3.

Table 3. Structural and physicochemical characteristics of glucosodiene.

Property	Description
Chemical designation	Proposed 1→2 linked glucose dimer
Structural class	Disaccharide-like glucose derivative
Formation	Non-enzymatic alkaline condensation
Linkage	1→2 glycosidic bond
NMR (^{1}H)	3.0–4.5 ppm; anomeric ~4.8–5.0 ppm
NMR (^{13}C)	~97 ppm (anomeric), 70–75 ppm (C–O), ~61 ppm (CH_2OH)
Polarity	Highly polar, water-soluble
Structural nature	Partially heterogeneous glycoside
Functional class	Metabolically incompatible glucose analogue

From a physicochemical perspective, glucosodiene retains high polarity and aqueous solubility due to its multiple hydroxyl functionalities, yet the non-enzymatically generated 1→2 linkage introduces stereochemical irregularities likely to impair enzymatic recognition. As a result, glucosodiene can be conceptualized as a metabolically incompatible glucose analogue, capable of interacting with glucose-dependent transport and biosynthetic systems while disrupting their normal processing. This duality structural similarity combined with functional deviation forms the

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basis of its proposed biological activity. A concise comparison between glucosodiene and the classical glycolytic analogue 2-deoxy-D-glucose is provided in Table 4 [46,47].

Table 4. Comparative profile: Glucosodiene vs 2-deoxy-D-glucose.

Feature	Glucosodiene	2-Deoxy-D-Glucose
Structural type	Glycosidic glucose dimer	Modified monosaccharide [46,47]
Formation	Non-enzymatic	Synthetic [46,47]
Cellular entry	Likely GLUT-mediated	GLUT-mediated [46,47]
Glycolysis impact	Indirect disruption	Direct inhibition (hexokinase trapping) [46,47]
Lactate production	Potentially reduced	Reduced [46,47]
Extracellular pH	Potential modulation	Limited [46,47]
Glycosylation	Potential interference	Partial inhibition [46,47]
Glycocalyx impact	Structural remodeling	Minimal [46,47]

Building upon this molecular framework, the most advanced formulation of glucosodiene reported to date involves its incorporation into a multifunctional nanostructured delivery system described in *Scientific Reports*, commonly referred to as glucosodiene-loaded nanoferrites (GLONF). In this system, glucosodiene is integrated into a tri-component nanocomposite consisting of a spinel nanoferrite core ($Sr_{0.5}Mn_{0.5}La_{0.02}Fe_{1.98}O_4$), a chitosan biopolymer coating, and the active glycosidic molecule. The nanoferrite core is synthesized via a citrate-based sol–gel auto-combustion method, yielding nanocrystalline particles with controlled composition and magnetic properties. These particles are subsequently coated with chitosan under mildly acidic conditions, producing a biocompatible polymeric shell that enhances dispersion stability and provides reactive functional groups for molecular incorporation [34]. Glucosodiene is then introduced into this system through controlled dissolution and gradual addition to the nanoferrite–chitosan suspension, often followed by crosslinking stabilization using agents such as glutaraldehyde. The mixture is subjected to prolonged stirring and sonication to ensure homogeneous distribution and interaction between the glycosidic molecule and the polymeric matrix. The final nanocomposite is typically isolated via freeze-drying, yielding a stable nano-ferrite/chitosan/glucosodiene hybrid platform [34].

This architecture does not alter the intrinsic chemical structure of glucosodiene but significantly enhances its physicochemical behavior, including stability, dispersion, and interaction with biological systems. Functionally, the nanostructured formulation is expected to enhance the bioavailability and cellular interaction of glucosodiene by facilitating its retention near cell membranes and promoting uptake through endocytic mechanisms. The chitosan coating contributes to membrane adhesion and permeability, while the nanoscale dimensions enable more efficient localization within biological environments. Within this context, glucosodiene is hypothesized to exert activity across the same three interconnected biochemical axes outlined above: interference with glycolytic flux, indirect modulation of extracellular proton dynamics and pH, and alteration of glycosylation processes and glycocalyx organization. These effects are expected to be amplified in the nanostructured formulation due to enhanced delivery efficiency and sustained molecular availability.

4. Experimental and Translational Evidence

4.1. In-Vitro Safety Assessment of Glucosodiene on Normal Human Fibroblasts

As an initial step toward evaluating the biological acceptability of glucosodiene, its cytotoxic potential was assessed in vitro using the human normal fibroblast cell line BJ1. Cell viability was determined by the standard MTT reduction assay, which measures mitochondrial metabolic activity through the conversion of yellow tetrazolium salt into purple formazan.

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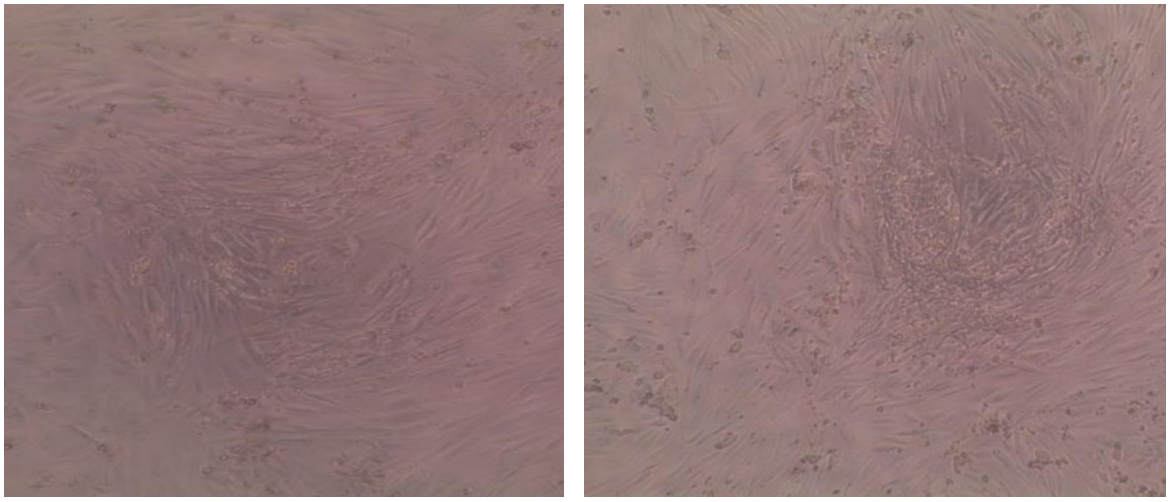
This assay was selected as a primary screening tool to determine whether glucosodiene exerts overt toxicity on non-malignant cells under controlled culture conditions. BJ1 fibroblasts were cultured in DMEM-F12 medium supplemented with antibiotics and L-glutamine, then seeded into 96-well plates and exposed for 48 hours to a serial concentration range of glucosodiene spanning 0.78 to 100 µg/mL. DMSO was used as the vehicle control at a final concentration below 0.3%, while doxorubicin served as a positive lethal control under the same experimental conditions. After incubation, cell viability was quantified spectrophotometrically following MTT reduction and solubilization of the formed formazan crystals. Across the tested concentration range, glucosodiene did not show measurable cytotoxicity toward BJ1 fibroblasts. Neither LC_{50} nor LC_{90} could be derived, indicating that the compound did not induce overt lethality under the assay conditions (Figure 4). The vehicle control also remained biologically acceptable at the working concentration, supporting the interpretation that the observed profile was attributable to the compound rather than solvent effects [20]. A concise summary of the in-vitro cytotoxicity findings is provided in Table 5.



A Control B Glucosodiene

Figure 4. In vitro cytotoxicity assessment of glucosodiene on normal human fibroblast (BJ1) cells using the MTT assay. Cells were exposed to increasing concentrations of the compound (0.78–100 µg/mL) for 48 hours, and viability was determined based on mitochondrial reduction of MTT to formazan. The results demonstrate maintained cell viability across the tested concentration range, with no significant reduction compared to the negative control. The vehicle (DMSO, <0.3%) showed no detectable effect on cell viability, while doxorubicin (100 µg/mL) was used as a positive control to confirm assay responsiveness. These findings indicate the absence of detectable cytotoxicity of glucosodiene toward normal fibroblasts under the experimental conditions.Top of Form.

Table 5. In vitro cytotoxicity of glucosodiene (BJ1 fibroblasts).

Sample	LC50 (µg/mL)	LC90 (µg/mL)	Interpretation
Glucosodiene	Not reached	Not reached	No detectable cytotoxicity
DMSO	Not reached	Not reached	No toxicity at working concentration
Control	—	—	Baseline viability maintained

These findings support a favorable preliminary safety profile for glucosodiene in normal fibroblasts and provide a necessary foundation for subsequent preclinical and translational interpretation.

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4.2. Preclinical In-Vivo Evidence from the GLONF Murine Model

The strongest preclinical evidence currently available for glucosodiene derives from the recently reported GLONF platform evaluated in the Ehrlich solid tumor mouse model. In that study, glucosodiene was assessed as the active component of a nanostructured formulation rather than as a free compound, enabling simultaneous evaluation of biological activity and delivery-enhanced performance.

The animal study included five experimental conditions: untreated controls, GLONF-only exposure, tumor-bearing mice, a co-treatment arm in which GLONF was given at the time of tumor induction, and a post-treatment arm in which treatment followed tumor establishment. This design is notable because the post-treatment window approximates the short response interval later described in the clinical observations. Before biological outcomes were assessed, the nanoplatform itself was characterized physicochemically. The reported data indicated preservation of a single-phase spinel ferrite structure after coating and loading, with progressive peak broadening consistent with chitosan deposition and glucosodiene incorporation.

The stepwise increase in crystallite size, nanoscale particle morphology, and altered magnetic behavior after loading together supported successful assembly of the final nanocomposite system. FTIR findings were also consistent with retention of a hydroxyl-rich carbohydrate framework within the loaded platform. Biologically, the tumor-bearing condition was associated with marked hepatic dysfunction and oxidative disturbance, whereas glucosodiene-containing nanotherapy attenuated these abnormalities. The most pronounced improvement was consistently observed in the post-treatment arm, suggesting that the nanostructured system remained biologically active even after tumor establishment. In biochemical terms, the diseased state was accompanied by elevations in ALT, AST, and ALP together with reductions in albumin and total protein, while glucosodiene-containing treatment shifted these values toward the control range [34]. These liver function data are summarized in Table 6.

Table 6. Liver function parameters (GLONF model).

Group	ALT	AST	Albumin	ALP	Total protein
Control	33.1 ± 1.26	153.5 ± 3.75	4.28 ± 0.03	143.1 ± 5.97	5.81 ± 0.03
GLONF	28.9 ± 1.42	142.3 ± 8.07	4.19 ± 0.07	133.3 ± 3.99	5.97 ± 0.05
EST	47.0 ± 1.47	306.4 ± 6.80	2.79 ± 0.11	234.9 ± 5.44	5.21 ± 0.05
GLONF + EST	41.5 ± 1.37	222.3 ± 9.44	3.65 ± 0.12	192.6 ± 4.18	5.70 ± 0.03
EST + GLONF	34.8 ± 0.97	199.0 ± 6.33	4.27 ± 0.04	144.5 ± 3.94	6.09 ± 0.07

A similar pattern was observed in redox-related biomarkers. Tumor-bearing animals showed increased malondialdehyde together with depletion of reduced glutathione, superoxide dismutase, and catalase, indicating substantial oxidative stress. Treatment with GLONF shifted these markers toward biochemical recovery, again with the post-treatment condition showing the strongest effect. The oxidative stress and antioxidant profile is summarized in Table 7.

Table 7. Oxidative stress and antioxidant markers.

Group	MDA	GSH	SOD	CAT
Control	71.77 ± 1.93	2.44 ± 0.05	109.9 ± 0.86	93.8 ± 0.64
GLONF	74.2 ± 2.23	2.61 ± 0.06	115.0 ± 2.98	96.3 ± 1.04
EST	166.8 ± 1.92	0.67 ± 0.01	58.7 ± 1.87	60.97 ± 1.33
GLONF + EST	122.5 ± 2.75	1.04 ± 0.03	73.6 ± 2.75	70.67 ± 2.73
EST + GLONF	79.07 ± 1.44	2.07 ± 0.04	97.7 ± 2.45	89.4 ± 3.23

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The same study also reported that tumor-bearing tissue displayed substantial structural injury and increased immunohistochemical expression of PCNA and P53, whereas glucosodiene-containing nanotherapy was associated with attenuation of these abnormalities. Because this is a review manuscript rather than a reproduction of the original experimental report, the key point is not the exact histological phrasing of the original work, but the broader result: the glucosodiene nanoplatform was associated with coordinated improvement in physicochemical integrity, liver biochemistry, oxidative balance, tissue morphology, and proliferation/stress-associated markers.

Collectively, these observations provide the strongest current in-vivo support for glucosodiene as a biologically active glucose-derived system, particularly when incorporated into a nanostructured delivery platform [34].

4.3. Clinical Case-Based Observations and Early Human Signals

Although the current human evidence remains preliminary and case-based, the available reports are notable for the temporal consistency and imaging-supported nature of the observed responses. Across the reported cases, clinically meaningful changes tended to emerge within approximately 5 days, whereas radiologic or metabolic reassessment after 15–20 days often indicated measurable improvement. This timing is broadly concordant with the biological window observed in the preclinical post-treatment model.

4.3.1. Case 1. Metastatic Triple-Negative Breast Cancer with Bone Involvement

The first reported case involved a 43-year-old woman with right-sided triple-negative invasive ductal carcinoma that later progressed to osseous metastases following surgery and chemotherapy. At the time glucosodiene was initiated, the patient had severe leg pain and marked functional limitation. Glucosodiene was given orally at 100 mL daily for 15 days in conjunction with a low-carbohydrate dietary regimen. Clinically, the most notable early change was improvement in mobility and reduction in severe pain beginning around day 5. Follow-up bone scintigraphy suggested stabilization of tracer distribution without clear evidence of newly aggressive destructive activity [48]. A structured summary of this case is presented in Table 8.

Table 8. Summary of Case 1: metastatic triple-negative breast cancer with bone metastases.

Parameter	Observation
Age	43
Diagnosis	Triple-negative breast cancer
Status	Bone metastases
Treatment	Glucosodiene 100 mL/day × 15 days
Response	Reduced pain, improved mobility (~day 5)
Imaging	Stabilized disease

4.3.2. Case 2. Stage II Triple-Positive Breast Cancer with Nodal Involvement

The second case involved a 35-year-old woman with biologically active triple-positive breast carcinoma and axillary nodal disease. Baseline PET/CT demonstrated FDG-avid breast lesions and nodal involvement. Glucosodiene was introduced at 100 mL orally once daily for 20 days alongside a strict carbohydrate-restricted nutritional plan and limited concomitant chemotherapy exposure.

Visible external improvement reportedly began near day 5 and progressed during the treatment period. Follow-up PET/CT after 20 days was interpreted as showing a marked metabolic response, with disappearance of previously metabolically active nodal and distant lesions (Figure 5) [49]. A structured summary is provided in Table 9.

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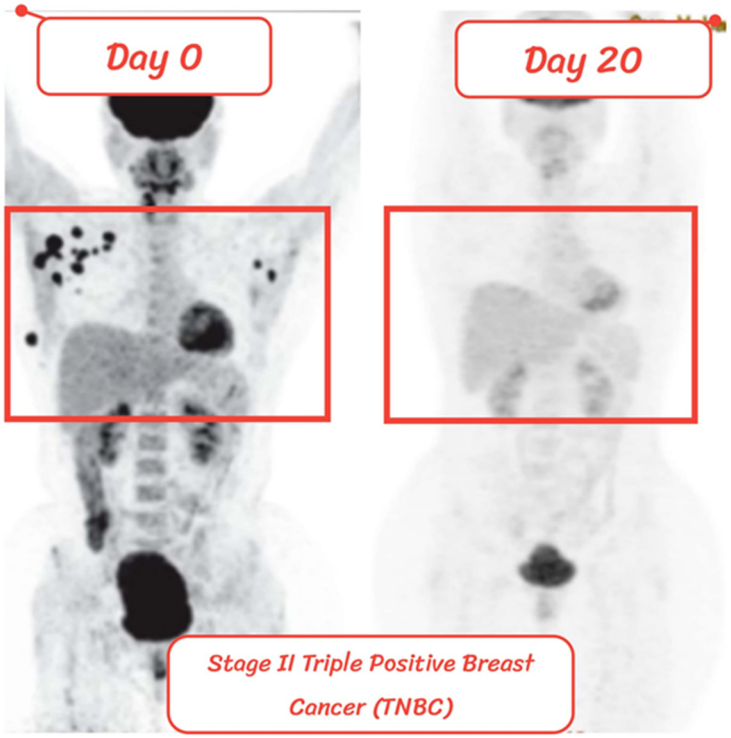

Figure 5. Baseline and post-treatment PET/CT imaging of a patient with stage II triple-positive breast cancer with axillary nodal involvement. The initial scan (Day 0) demonstrates multiple FDG-avid lesions within the breast and regional lymph nodes, indicating metabolically active disease. Following 20 days of oral glucosodiene administration, the follow-up scan (Day 20) shows a marked reduction in metabolic activity, with resolution of previously hypermetabolic nodal and distant lesions and near absence of abnormal FDG uptake. These findings are consistent with a significant metabolic response over a short treatment interval.

Table 9. Summary of Case 2: stage II triple-positive breast cancer.

Parameter	Baseline	Post-treatment
Tumor status	Multifocal + nodal	No active lesions
Imaging	FDG-avid	PET-negative
Clinical	Distortion	Morphology restored
Duration	—	20 days

4.3.3. Case 3. Metastatic Triple-Positive Breast Cancer with Disseminated Bone Disease

The third case involved a 36-year-old woman with metastatic triple-positive breast carcinoma and extensive skeletal disease despite multiple prior lines of therapy. After carbohydrate-restricted preparation, glucosodiene was administered at 100 mL orally every 24 hours for 15 doses.

Bone pain and mobility reportedly improved by approximately the fifth day. PET imaging performed after treatment indicated a partial metabolic response, including regression of breast and nodal activity and marked reduction in hypermetabolic osseous lesions (Figure 6). Biomarker changes were also documented, including decline of ALP from 700 U/L to 280 U/L and CA 15-3 from 146.6 KU/L to 78.1 KU/L [49]. A concise summary is presented in Table 10.

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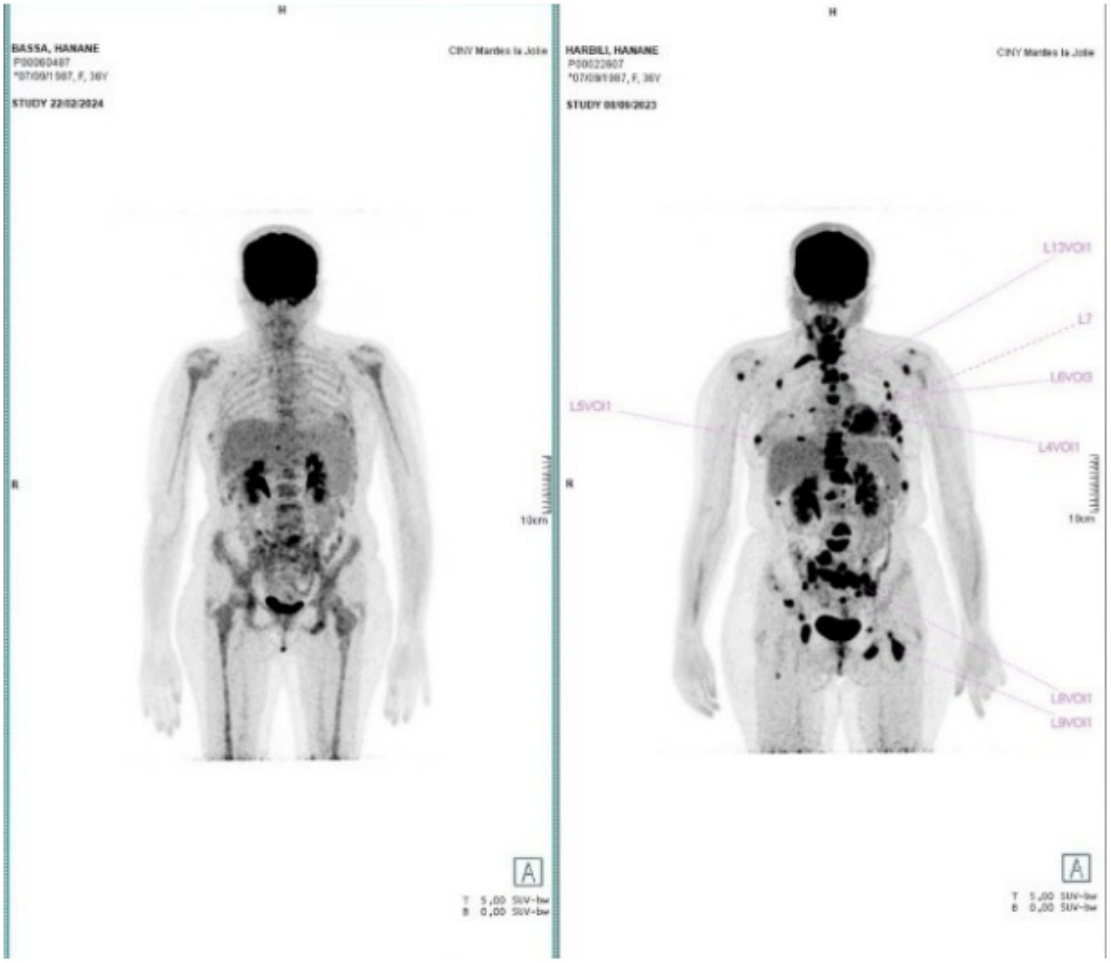

Figure 6. Baseline and post-treatment whole-body PET/CT imaging of a patient with metastatic triple-positive breast cancer and extensive skeletal involvement. The pre-treatment scan demonstrates widespread FDG-avid lesions involving the axial and appendicular skeleton, as well as nodal and breast-associated metabolic activity, consistent with disseminated metastatic disease. Following 15 days of oral glucosodiene administration, the post-treatment scan shows a clear reduction in the number, intensity, and distribution of hypermetabolic foci, particularly within osseous sites, indicating a partial metabolic response. These imaging findings are concordant with the observed clinical improvement in pain and mobility and are supported by concurrent biochemical changes, including a marked decline in alkaline phosphatase (ALP) and CA 15-3 levels.

Table 10. Summary of Case 3: metastatic triple-positive disease with diffuse bone involvement.

Parameter	Pre	Post
Disease burden	Bone + nodal metastases	Partial regression
ALP	700	280
CA 15-3	146.6	78.1
Clinical	Severe pain	Improved mobility
Imaging	Progressive	Partial response

5. Discussion

The present synthesis of chemical, preclinical, and early clinical evidence supports the view that glucosodiene should not be interpreted as a conventional glucose analogue acting through a single inhibitory node, but rather as a candidate multi-domain metabolic and glyco-structural perturbant

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whose potential biological significance lies in its capacity to influence the coupled axes of glycolytic flux, extracellular acid–base balance, redox homeostasis, and glycocalyx organization.

What emerges from the currently available dataset is not simply a molecule associated with isolated anticancer observations, but a coherent systems-level hypothesis in which a glucose-derived glycosidic structure may exploit the very metabolic dependencies that tumors use to maintain survival, membrane signaling, and immune protection.

At the chemical level, the importance of glucosodiene begins with its intermediate biological identity. Spectroscopic evidence supports the interpretation of the compound as a glucose-derived glycosidic structure with preserved carbohydrate architecture but altered linkage geometry [20]. This point is mechanistically important because tumor cells are not merely high consumers of glucose; they are cells whose survival programs are tightly coupled to continuous glucose recognition, import, retention, and redistribution into both energetic and biosynthetic pathways [50]. A compound that remains sufficiently "glucose-like" to enter or compete within this system, yet sufficiently altered to resist normal enzymatic handling, has the potential to produce broader biological consequences than a classic poison or simple transport blocker. In this context, glucosodiene may be best understood as a structurally familiar but functionally discordant substrate [20].

The first major mechanistic implication of this interpretation is disturbance of effective glycolytic productivity [51]. In glycolysis-dominant tumors, high glucose uptake through GLUT transporters is integrated with enhanced phosphorylation by hexokinase, increased flux through phosphofructokinase and pyruvate kinase, and preferential conversion of pyruvate to lactate via LDHA [52]. This metabolic configuration is often stabilized by HIF-1α, MYC, PI3K/AKT/mTOR signaling, and receptor tyrosine kinase activity, thereby linking glucose metabolism directly to growth, proliferation, and biosynthesis [53]. If glucosodiene engages glucose-handling pathways imperfectly whether by transporter competition, altered phosphorylation compatibility, reduced processing efficiency, or diversion of carbon flow it could reduce the net yield of glycolytic flux without necessarily acting through a single canonical inhibitory step. This broader kind of interference is conceptually distinct from that of 2-deoxy-D-glucose, which primarily acts through intracellular phosphorylation and dead-end trapping. Whereas 2-DG is largely a glycolytic inhibitor, glucosodiene may instead behave as a glucose-processing perturbant, affecting not only ATP-generating metabolism but also the downstream biochemical landscape that depends on glucose-derived intermediates [46,47].

This point becomes especially relevant when considering extracellular acidification. Lactate is not merely a terminal metabolic byproduct; it is a major determinant of tumor microenvironment chemistry and a biologically active mediator of stromal and immune behavior. Excessive lactate export through MCT systems is coupled to proton movement and contributes directly to extracellular acidosis [54].

In turn, this acidic environment supports invasion, protease activity, extracellular matrix degradation, and immune paralysis. Low extracellular pH suppresses $CD8^+$ T-cell proliferation, decreases IFN-γ production, weakens NK-cell cytotoxicity, and helps sustain immunosuppressive cell populations. Acidic stress also affects tumor-intrinsic signaling, including AMPK, MAPK stress responses, mTOR regulation, and p53-associated pathways [55]. Under the mechanistic model proposed here, any reduction in effective lactate generation by glucosodiene would therefore be expected to alter not only metabolism, but also the chemical permissiveness of the tumor environment itself. In this framework, glucosodiene may exert biologically meaningful effects by partially relieving acid stress and thereby restoring a state in which cell-cycle restraint, death signaling, and immune recognition become more feasible [20].

The preclinical findings from the GLONF study are broadly compatible with this interpretation. Tumor-bearing animals exhibited severe oxidative and biochemical injury, whereas treatment with glucosodiene-loaded nanoferrites was associated with marked recovery in hepatic enzymes, antioxidant systems, and tissue integrity. These results do not directly prove glycolysis inhibition or pH normalization, but they are highly consistent with a biological scenario in which reduction of

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metabolic stress is accompanied by rebalancing of redox and signaling systems. The restoration of GSH, SOD, and CAT, together with the reduction of MDA, suggests that glucosodiene-containing treatment was associated with a transition away from severe oxidative burden. That shift is likely not incidental. Tumor-driven glycolytic excess, inflammatory signaling, mitochondrial dysfunction, and acid stress are deeply interconnected sources of redox imbalance. If glucosodiene disrupts the metabolic configuration that sustains this state, then recovery of antioxidant capacity becomes mechanistically plausible. In this sense, the redox findings in the GLONF model may represent a downstream signature of deeper metabolic and microenvironmental modulation. The modulation of PCNA and p53 is similarly informative. PCNA is a marker of DNA replication-associated proliferative activity and its reduction is consistent with decreased proliferative drive. p53, by contrast, is more context-dependent. In the murine hepatic setting, strong p53 immunoreactivity in diseased tissue may reflect pathological stress signaling rather than efficient tumor suppression per se. Nevertheless, the broader implication remains important: glucosodiene-containing nanotherapy altered one of the central molecular interfaces between metabolic injury, growth regulation, and stress response. Within the framework of this review, that observation reinforces the idea that glucosodiene's biological relevance may lie not only in direct metabolic restriction, but in reorganization of the intracellular signaling environment that links metabolism to proliferation and injury response [34].

The glycocalyx dimension may represent the most conceptually distinctive aspect of glucosodiene.

Most discussions of cancer metabolism focus on ATP generation, biosynthesis, or lactate production; far fewer explicitly connect glucose utilization to construction of the tumor cell surface. Yet the glycocalyx is one of the most functionally important products of malignant metabolic rewiring [56]. Tumors depend on glucose-derived activated sugars to maintain glycoproteins, glycolipids, mucins, and proteoglycans that stabilize receptor clustering, amplify signaling, regulate mechanotransduction, and impede immune access [57]. In many tumors, increased flux through the hexosamine biosynthetic pathway and related glycosylation systems contributes directly to malignant fitness. Under this logic, a glucose-derived glycosidic structure such as glucosodiene becomes especially interesting because it may influence this system at two levels [58]. Quantitatively, it may reduce the efficient use of glucose for glycan precursor generation. Qualitatively, it may introduce a structurally discordant substrate into glycosylation-related processing. Even if full biochemical incorporation remains unproven, either mechanism could disturb glycoconjugate maturation, glycan branching, or membrane glycoprotein organization.

The predicted consequence would not necessarily be simple depletion of the glycocalyx, but rather destabilization of glycan-dependent membrane architecture. Such destabilization has major implications for tumor immunobiology. A dense, highly glycosylated tumor surface can interfere with immune synapse formation, reduce antigen accessibility, and promote inhibitory interactions with glycan-sensing receptors such as galectins and siglecs [59]. If glucosodiene weakens glycocalyx integrity or alters glycan composition, it could render tumor cells less sterically protected and more immunologically visible. Importantly, this effect would synergize with reduced extracellular acidosis, since acidity and glycocalyx density are both major contributors to immune dysfunction. In other words, glucosodiene may theoretically act on the tumor–immune interface from both directions: by reducing chemical suppression and by reducing structural concealment [20,60].

The early human case observations, while still limited and non-definitive, are striking in this regard. Across the available reports, clinically meaningful changes tended to appear rapidly, often by approximately day 5, whereas imaging-based reassessment after 15–20 days frequently demonstrated favorable metabolic changes. This temporal pattern is difficult to reconcile with a narrow, slow, purely cytotoxic mechanism alone. It is, however, compatible with a model in which early improvements reflect metabolic decompression, reduced inflammatory-acidic burden, altered tissue chemistry, and reorganization of tumor-associated surface and stromal interactions, followed by later imaging manifestations.

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The breast case with visible morphological improvement and PET-supported disappearance of metabolically active disease, the metastatic skeletal case with improved mobility and scintigraphic stabilization, and the disseminated triple-positive case with PET response plus biomarker decline all fit this broader systems interpretation. These cases do not establish efficacy, especially because concomitant interventions were present in some instances, but the repeated alignment between symptom timing, imaging response, and biomarker movement suggests that the observed effects are not biologically random. The nanotechnology component strengthens this interpretation substantially. Carbohydrate-derived molecules often face translational limitations because of rapid dilution, poor retention, and insufficient local interaction time [49]. The GLONF platform appears to address these constraints by incorporating glucosodiene into a ferrite–chitosan system that improves stability, local availability, membrane interaction, and likely cellular uptake. This is not a trivial formulation detail; it may be central to the biological expression of glucosodiene's mechanism [34].

If the compound acts partly through membrane-proximal processes such as modulation of glycan handling, lactate-associated chemistry, or cell-surface organization, then prolonged residence and close membrane interaction become biologically decisive. The stronger performance of the post-treatment arm in the preclinical model is particularly interesting in this respect, because it suggests that glucosodiene may be especially relevant once the malignant metabolic and microenvironmental state is already established. That observation aligns closely with the human case reports, which mostly involved active, progressive, or advanced disease.

A further point of significance is the in-vitro BJ1 safety profile. The absence of detectable cytotoxicity toward normal fibroblasts across the tested concentration range suggests that glucosodiene does not behave as a broadly indiscriminate toxicant under these conditions. This is consistent with the mechanistic model proposed here. If glucosodiene's biological action depends on metabolic context—especially excessive glucose dependence, glycolytic dominance, acid stress, or glycosylation dysregulation—then normal cells might be expected to tolerate it better than metabolically distorted malignant cells. This notion remains preliminary and must not be overextended, but it provides an important conceptual distinction between context-dependent incompatibility and general cytotoxicity [20,34].

Taken together, the currently available evidence supports a unified interpretation in which glucosodiene influences tumor biology through three mutually reinforcing routes. First, it may interfere with glucose-dependent metabolic processing, reducing effective glycolytic throughput and lactate burden. Second, by reducing the acidic pressure of the tumor microenvironment, it may create conditions more compatible with stress signaling, apoptosis-related pathways, and immune function.

Third, by perturbing glucose-dependent glycosylation and the maintenance of the glycocalyx, it may weaken tumor surface protection and receptor stabilization. These changes could converge on redox homeostasis, proliferation control, tissue integrity, and tumor–immune engagement. The central implication is therefore conceptual as much as therapeutic: glucosodiene invites a shift away from viewing cancer metabolism as an isolated intracellular phenomenon and toward viewing it as a coupled metabolism–pH–glycocalyx system that may be disrupted by structurally altered glucose-derived compounds.

6. Limitations

Several limitations must be acknowledged when interpreting the current evidence base for glucosodiene. First, the structural assignment of glucosodiene, although strongly supported by available ^{1}H NMR, ^{13}C NMR, and LC–MS data, remains provisional.

Definitive confirmation of linkage position, stereochemical configuration, and possible oligomeric heterogeneity requires two-dimensional NMR methods such as HSQC and HMBC, together with high-resolution fragmentation-based mass spectrometry. Second, the available biological evidence is heterogeneous in type and strength, spanning an in-vitro safety assay, a single preclinical nanotechnology-enabled murine study, and a small number of human case observations rather than a unified prospective experimental program.

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Third, the current mechanistic model remains biologically plausible but not yet directly proven at the level of tracer-based metabolism, transporter handling, intracellular carbon routing, glycan incorporation, or membrane glycome remodeling. In particular, the proposed effects on glycolytic throughput, extracellular pH normalization, and glycocalyx destabilization are inferred from chemical logic and biological coherence rather than demonstrated through dedicated metabolomic, glycoproteomic, fluxomic, or tumor-immune interface studies. Fourth, the available clinical observations occurred in complex therapeutic contexts that included dietary restriction and, in some cases, concomitant conventional treatment, making it impossible at present to isolate the independent contribution of glucosodiene with certainty.

Fifth, the preclinical evidence available from the GLONF model reflects the behavior of a nanostructured formulation rather than unequivocally the free compound alone. While this is scientifically valuable from a translational perspective, it complicates strict attribution of biological effects to the chemical entity independent of its delivery platform.

Finally, pharmacokinetics, biodistribution, dose standardization, tissue selectivity, long-term safety, and immunological consequences remain insufficiently defined. For these reasons, glucosodiene should presently be considered an emerging mechanistic and translational hypothesis supported by convergent preliminary evidence, rather than a clinically validated anticancer therapy.

7. Conclusion

Glucosodiene emerges as a chemically defined, glucose-derived glycosidic system with the capacity to perturb core biochemical dependencies of malignant cells. Rather than acting through direct cytotoxicity, the available evidence supports a model in which glucosodiene interferes with glucose handling, alters glycolytic throughput, and indirectly reshapes extracellular pH dynamics and redox balance. These effects appear to extend to higher-order biological structures, including modulation of proliferation-associated signaling and disruption of glycan-dependent membrane organization.

The convergence of in vitro safety, in vivo functional improvement, and early clinical observations characterized by measurable changes in tumor burden, biochemical markers, and imaging responses within a limited timeframe suggests a coherent, though still preliminary, translational signal. Importantly, this framework positions glucosodiene within an integrated metabolic–microenvironmental axis rather than as a single-target agent. Further mechanistic validation and controlled clinical investigation are essential to determine its reproducibility, specificity, and therapeutic potential in oncology.

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References

1. Schiliro, C., & Firestein, B. L. (2021). Mechanisms of Metabolic Reprogramming in Cancer Cells Supporting Enhanced Growth and Proliferation. *Cells*, *10*(5), 1056. https://doi.org/10.3390/cells10051056
2. Iessi, E., Vona, R., Cittadini, C., & Matarrese, P. (2021). Targeting the Interplay between Cancer Metabolic Reprogramming and Cell Death Pathways as a Viable Therapeutic Path. *Biomedicines*, *9*(12), 1942. https://doi.org/10.3390/biomedicines9121942
3. Schiliro, C., & Firestein, B. L. (2021). Mechanisms of Metabolic Reprogramming in Cancer Cells Supporting Enhanced Growth and Proliferation. *Cells*, *10*(5), 1056. https://doi.org/10.3390/cells10051056
4. Clay, R., Li, K., & Jin, L. (2025). Metabolic Signaling in the Tumor Microenvironment. *Cancers*, *17*(1), 155. https://doi.org/10.3390/cancers17010155
5. Zhang, S., Wang, J., Xu, Z., Cheng, Z., Shao, B., & Yu, J. (2026). Lactate: elucidating its indispensable role in human health. *Molecular cancer*, *25*(1), 2. https://doi.org/10.1186/s12943-025-02519-z

 doi:10.20944/preprints202604.1565.v1

6. Estrella, V., Chen, T., Lloyd, M., Wojtkowiak, J., Cornnell, H. H., Ibrahim-Hashim, A., Bailey, K., Balagurunathan, Y., Rothberg, J. M., Sloane, B. F., Johnson, J., Gatenby, R. A., & Gillies, R. J. (2013). Acidity generated by the tumor microenvironment drives local invasion. *Cancer research*, *73*(5), 1524–1535. https://doi.org/10.1158/0008-5472.CAN-12-2796
7. Czowski, B. J., Romero-Moreno, R., Trull, K. J., & White, K. A. (2020). Cancer and pH Dynamics: Transcriptional Regulation, Proteostasis, and the Need for New Molecular Tools. *Cancers*, *12*(10), 2760. https://doi.org/10.3390/cancers12102760
8. Ai, J., Du, Y., Xue, Q., Peng, W., & Zhou, Q. (2026). Metabolic Checkpoints in CD8⁺ T Cells within the Tumor Microenvironment: A Comprehensive Review and Emerging Insights. *International journal of biological sciences*, *22*(4), 1950–1973. https://doi.org/10.7150/ijbs.125206
9. Sabit, H., Arneth, B., Abdel-Ghany, S., Madyan, E. F., Ghaleb, A. H., Selvaraj, P., Shin, D. M., Bommireddy, R., & Elhashash, A. (2024). Beyond Cancer Cells: How the Tumor Microenvironment Drives Cancer Progression. *Cells*, *13*(19), 1666. https://doi.org/10.3390/cells13191666
10. Adhikari, S., Guha, D., Mohan, C., Mukherjee, S., Tyler, J. K., & Das, C. (2022). Reprogramming Carbohydrate Metabolism in Cancer and Its Role in Regulating the Tumor Microenvironment. *Sub-cellular biochemistry*, *100*, 3–65. https://doi.org/10.1007/978-3-031-07634-3_1
11. Paneque, A., Fortus, H., Zheng, J., Werlen, G., & Jacinto, E. (2023). The Hexosamine Biosynthesis Pathway: Regulation and Function. *Genes*, *14*(4), 933. https://doi.org/10.3390/genes14040933
12. Chiaradonna, F., Ricciardiello, F., & Palorini, R. (2018). The Nutrient-Sensing Hexosamine Biosynthetic Pathway as the Hub of Cancer Metabolic Rewiring. Cells, 7(6), 53. https://doi.org/10.3390/cells7060053
13. Kuo, J. C., & Paszek, M. J. (2021). Glycocalyx Curving the Membrane: Forces Emerging from the Cell Exterior. *Annual review of cell and developmental biology*, *37*, 257–283. https://doi.org/10.1146/annurev-cellbio-120219-054401
14. Möckl L. (2020). The Emerging Role of the Mammalian Glycocalyx in Functional Membrane Organization and Immune System Regulation. *Frontiers in cell and developmental biology*, *8*, 253. https://doi.org/10.3389/fcell.2020.00253
15. Johnson, J. L., Jones, M. B., Ryan, S. O., & Cobb, B. A. (2013). The regulatory power of glycans and their binding partners in immunity. *Trends in immunology*, *34*(6), 290–298. https://doi.org/10.1016/j.it.2013.01.006
16. Stark, J.C., Gray, M.A., Ibarlucea-Benitez, I. et al. Antibody-lectin chimeras for glyco-immune checkpoint blockade. Nat Biotechnol (2025). https://doi.org/10.1038/s41587-025-02884-6
17. Li, S., Gong, J., Kang, B., Wang, Z., Ma, Y., Xia, X., & Yan, H. (2026). Targeting Glycolytic Metabolism in Cancer Therapy: Current Approaches and Future Perspectives. *Cells*, *15*(4), 362. https://doi.org/10.3390/cells15040362
18. Geltink, R. I. K., Kyle, R. L., & Pearce, E. L. (2018). Unraveling the Complex Interplay Between T Cell Metabolism and Function. *Annual review of immunology*, *36*, 461–488. https://doi.org/10.1146/annurev-immunol-042617-053019
19. Liu, S., Zhang, X., Wang, W., Li, X., Sun, X., Zhao, Y., Wang, Q., Li, Y., Hu, F., & Ren, H. (2024). Metabolic reprogramming and therapeutic resistance in primary and metastatic breast cancer. *Molecular cancer*, *23*(1), 261. https://doi.org/10.1186/s12943-024-02165-x
20. Akl, M. M. & Ahmed, A. Developing the theory of toxic chemotherapeutic nutrition for cancer cells and targeting tumors via glucose mutation: Medical guidance and integrated therapeutic approach. *Onkologia i Radioterapia*, **18**(2) (2024).
21. Kocdor, M. A., Kocdor, H., Pereira, J. S., Vanegas, J. E., Russo, I. H., & Russo, J. (2013). Progressive increase of glucose transporter-3 (GLUT-3) expression in estrogen-induced breast carcinogenesis. *Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico*, *15*(1), 55–64. https://doi.org/10.1007/s12094-012-0882-3
22. Wang, M., Flaswinkel, H., Joshi, A., Napoli, M., Masgrau-Alsina, S., Kamper, J. M., Henne, A., Heinz, A., Berouti, M., Schmacke, N. A., Hiller, K., Kremmer, E., Wefers, B., Wurst, W., Sperandio, M., Ruland, J., Fröhlich, T., & Hornung, V. (2024). Phosphorylation of PFKL regulates metabolic reprogramming in macrophages following pattern recognition receptor activation. *Nature communications*, *15*(1), 6438. https://doi.org/10.1038/s41467-024-50104-7

 doi:10.20944/preprints202604.1565.v1

23. Hassan, B., Akcakanat, A., Holder, A. M., & Meric-Bernstam, F. (2013). Targeting the PI3-kinase/Akt/mTOR signaling pathway. *Surgical oncology clinics of North America*, *22*(4), 641–664. https://doi.org/10.1016/j.soc.2013.06.008
24. Cifuente, J. O., Colleoni, C., Kalscheuer, R., & Guerin, M. E. (2024). Architecture, Function, Regulation, and Evolution of α-Glucans Metabolic Enzymes in Prokaryotes. *Chemical reviews*, *124*(8), 4863–4934. https://doi.org/10.1021/acs.chemrev.3c00811
25. Brescia, F., Titilas, I., Cacciapuoti, S., & Ronconi, L. (2025). Recent Advances in the Development of Metal-Glycoconjugates for Medicinal Applications. Molecules, 30(17), 3537. https://doi.org/10.3390/molecules30173537
26. Polet, F., Martherus, R., Corbet, C., Pinto, A., & Feron, O. (2016). Inhibition of glucose metabolism prevents glycosylation of the glutamine transporter ASCT2 and promotes compensatory LAT1 upregulation in leukemia cells. *Oncotarget*, *7*(29), 46371–46383. https://doi.org/10.18632/oncotarget.10131
27. Carpenter, K. L., Jalloh, I., & Hutchinson, P. J. (2015). Glycolysis and the significance of lactate in traumatic brain injury. *Frontiers in neuroscience*, *9*, 112. https://doi.org/10.3389/fnins.2015.00112
28. Sedlakova, O., Svastova, E., Takacova, M., Kopacek, J., Pastorek, J., & Pastorekova, S. (2014). Carbonic anhydrase IX, a hypoxia-induced catalytic component of the pH regulating machinery in tumors. *Frontiers in physiology*, *4*, 400. https://doi.org/10.3389/fphys.2013.00400
29. Chao, Z., Mei, Q., Yang, C., Luo, J., Liu, P., Peng, H., Guo, X., Yin, Z., Li, L., & Wang, Z. (2025). Immunological synapse: structures, molecular mechanisms and therapeutic implications in disease. *Signal transduction and targeted therapy*, *10*(1), 254. https://doi.org/10.1038/s41392-025-02332-6
30. Erra Díaz, F., Dantas, E., & Geffner, J. (2018). Unravelling the Interplay between Extracellular Acidosis and Immune Cells. *Mediators of inflammation*, *2018*, 1218297. https://doi.org/10.1155/2018/1218297
31. Xiao, K., Liu, C., Tu, Z., Xu, Q., Chen, S., Zhang, Y., Wang, X., Zhang, J., Hu, C. A., & Liu, Y. (2020). Activation of the NF-κB and MAPK Signaling Pathways Contributes to the Inflammatory Responses, but Not Cell Injury, in IPEC-1 Cells Challenged with Hydrogen Peroxide. *Oxidative medicine and cellular longevity*, *2020*, 5803639. https://doi.org/10.1155/2020/5803639
32. Wang, W., Liu, X., Liu, H., Abolhassani, H., Yan, H., Zhang, H., & Wang, X. (2026). p53: from understanding its structure to advances in therapeutic targeting. *Signal transduction and targeted therapy*, *11*(1), 121. https://doi.org/10.1038/s41392-025-02549-5
33. Tafech, A., & Stéphanou, A. (2024). On the Importance of Acidity in Cancer Cells and Therapy. *Biology*, *13*(4), 225. https://doi.org/10.3390/biology13040225
34. Tousson, E., Atrash, A. E., Abdelrasol, M. Y., & Ghoneim, A. I. (2026). Modulation of oxidative stress and P53/PCNA signaling by glucosodiene-loaded nanoferrites (GLONF) in ehrlich solid tumor-induced hepatotoxicity. *Scientific reports*, *16*(1), 12351. https://doi.org/10.1038/s41598-026-45548-4
35. Foote, C.A., Soares, R.N., Ramirez-Perez, F.I., Ghiarone, T., Aroor, A., Manrique-Acevedo, C., Padilla, J. and Martinez-Lemus, L. (2022), Endothelial Glycocalyx. Comprehensive Physiology, 12: 3781-3811. https://doi.org/10.1002/j.2040-4603.2022.tb00232.x
36. Jasek-Gajda, E., Jurkowska, H., Jasińska, M., & Lis, G. J. (2020). Targeting the MAPK/ERK and PI3K/AKT Signaling Pathways Affects NRF2, Trx and GSH Antioxidant Systems in Leukemia Cells. *Antioxidants (Basel, Switzerland)*, *9*(7), 633. https://doi.org/10.3390/antiox9070633
37. Hu, Q., Zhu, Y., Mei, J. et al. Extracellular matrix dynamics in tumor immunoregulation: from tumor microenvironment to immunotherapy. J Hematol Oncol 18, 65 (2025). https://doi.org/10.1186/s13045-025-01717-y
38. Li, Y., Song, Q., Guo, R., Qian, Y., Jiang, Y., & Song, Z. (2025). Glucose metabolism through the hexosamine biosynthetic pathway drives hepatic de novo lipogenesis via promoting *N*-linked protein glycosylation. *American journal of physiology. Gastrointestinal and liver physiology*, *328*(6), G746–G759. https://doi.org/10.1152/ajpgi.00056.2025
39. Cagnoni, A. J., Pérez Sáez, J. M., Rabinovich, G. A., & Mariño, K. V. (2016). Turning-Off Signaling by Siglecs, Selectins, and Galectins: Chemical Inhibition of Glycan-Dependent Interactions in Cancer. *Frontiers in oncology*, *6*, 109. https://doi.org/10.3389/fonc.2016.00109

 doi:10.20944/preprints202604.1565.v1

40. Yin, S., Li, C., Shen, X., Yu, G., Cui, L., Wu, Y., He, Y., Yu, S., Chen, J., Lu, S., Qiu, G., Song, M., Qian, C., Zou, Z., Yu, Y., & Xu, S. (2024). Siglec-G Suppresses CD8⁺ T Cells Responses through Metabolic Rewiring and Can be Targeted to Enhance Tumor Immunotherapy. *Advanced science (Weinheim, Baden-Wurttemberg, Germany)*, *11*(45), e2403438. https://doi.org/10.1002/advs.202403438
41. Masola, V., Greco, N., Gambaro, G., Franchi, M., & Onisto, M. (2021). Heparanase as active player in endothelial glycocalyx remodeling. *Matrix biology plus*, *13*, 100097. https://doi.org/10.1016/j.mbplus.2021.100097
42. Milusev, A., Rieben, R., & Sorvillo, N. (2022). The Endothelial Glycocalyx: A Possible Therapeutic Target in Cardiovascular Disorders. *Frontiers in cardiovascular medicine*, *9*, 897087. https://doi.org/10.3389/fcvm.2022.897087
43. Jiang, M., Wang, Y., Zhao, X., & Yu, J. (2024). From metabolic byproduct to immune modulator: the role of lactate in tumor immune escape. *Frontiers in immunology*, *15*, 1492050. https://doi.org/10.3389/fimmu.2024.1492050
44. Tufail, M., Jiang, CH. & Li, N. Immune evasion in cancer: mechanisms and cutting-edge therapeutic approaches. Sig Transduct Target Ther 10, 227 (2025). https://doi.org/10.1038/s41392-025-02280-1
45. Giurini, E. F., Pappas, S. G., & Gupta, K. H. (2026). Sweet Surprises: Decoding Tumor-Associated Glycosylation in Cancer Progression and Therapeutic Potential. *Cells*, *15*(3), 233. https://doi.org/10.3390/cells15030233
46. Pajak, B., Siwiak, E., Sołtyka, M., Priebe, A., Zieliński, R., Fokt, I., Ziemniak, M., Jaśkiewicz, A., Borowski, R., Domoradzki, T., & Priebe, W. (2019). 2-Deoxy-d-Glucose and Its Analogs: From Diagnostic to Therapeutic Agents. *International journal of molecular sciences*, *21*(1), 234. https://doi.org/10.3390/ijms21010234
47. Aft, R., Zhang, F. & Gius, D. Evaluation of 2-deoxy-D-glucose as a chemotherapeutic agent: mechanism of cell death. Br J Cancer 87, 805–812 (2002). https://doi.org/10.1038/sj.bjc.6600547
48. Ahmed, A. (2023). Targeting the warburg effect with glucosodiene: a case report of a 43-year-old female after mastectomy of the right breast and axillary clearance with successful first case treatment for metastatic Triple Negative Breast Cancer (TNBC) of bone. Oncology and Radiotherapy, 17(10), 751-757.
49. Ahmed, A. & Akl, M. M. Targeting the warburg effect with glucose mutation theory: a two-case study on the efficacy of glucosodiene in treating metastatic triple-positive breast cancer in stage II and IV patients. *Onkologia i Radioterapia*, **18**(3), (2024).
50. Zhou, D., Duan, Z., Li, Z., Ge, F., Wei, R., & Kong, L. (2022). The significance of glycolysis in tumor progression and its relationship with the tumor microenvironment. *Frontiers in pharmacology*, *13*, 1091779. https://doi.org/10.3389/fphar.2022.1091779
51. Párraga Solórzano, P. K., Yao, J., Rock, C. O., & Kehl-Fie, T. E. (2019). Disruption of Glycolysis by Nutritional Immunity Activates a Two-Component System That Coordinates a Metabolic and Antihost Response by Staphylococcus aureus. *mBio*, *10*(4), e01321-19. https://doi.org/10.1128/mBio.01321-19
52. Zhao, J., Jin, D., Huang, M., Ji, J., Xu, X., Wang, F., Zhou, L., Bao, B., Jiang, F., Xu, W., Lu, X., & Xiao, M. (2024). Glycolysis in the tumor microenvironment: a driver of cancer progression and a promising therapeutic target. *Frontiers in cell and developmental biology*, *12*, 1416472. https://doi.org/10.3389/fcell.2024.1416472
53. Pan, B. S., Hsu, C. C., Wu, H. E., Chen, Y. R., Zhou, X., Wang, S. C., Li, C. Y., & Lin, H. K. (2025). Glucose metabolism and its direct action in cancer and immune regulation: opportunities and challenges for metabolic targeting. *Journal of biomedical science*, *32*(1), 71. https://doi.org/10.1186/s12929-025-01167-1
54. de la Cruz-López, K. G., Castro-Muñoz, L. J., Reyes-Hernández, D. O., García-Carrancá, A., & Manzo-Merino, J. (2019). Lactate in the Regulation of Tumor Microenvironment and Therapeutic Approaches. *Frontiers in oncology*, *9*, 1143. https://doi.org/10.3389/fonc.2019.01143
55. Müller, B., Fischer, B., & Kreutz, W. (2000). An acidic microenvironment impairs the generation of non-major histocompatibility complex-restricted killer cells. *Immunology*, *99*(3), 375–384. https://doi.org/10.1046/j.1365-2567.2000.00975.x

 doi:10.20944/preprints202604.1565.v1

56. Ricciardiello, F., Votta, G., Palorini, R. et al. Inhibition of the Hexosamine Biosynthetic Pathway by targeting PGM3 causes breast cancer growth arrest and apoptosis. Cell Death Dis 9, 377 (2018). https://doi.org/10.1038/s41419-018-0405-4
57. Purushothaman, A., Mohajeri, M., & Lele, T. P. (2023). The role of glycans in the mechanobiology of cancer. *The Journal of biological chemistry*, *299*(3), 102935. https://doi.org/10.1016/j.jbc.2023.102935
58. Paneque, A., Fortus, H., Zheng, J., Werlen, G., & Jacinto, E. (2023). The Hexosamine Biosynthesis Pathway: Regulation and Function. Genes, 14(4), 933. https://doi.org/10.3390/genes14040933
59. Cao, Y., Yi, W., & Zhu, Q. (2024). Glycosylation in the tumor immune response: the bitter side of sweetness. *Acta biochimica et biophysica Sinica*, *56*(8), 1184–1198. https://doi.org/10.3724/abbs.2024107
60. Kellum, J. A., Song, M., & Li, J. (2004). Science review: extracellular acidosis and the immune response: clinical and physiologic implications. *Critical care (London, England)*, *8*(5), 331–336. https://doi.org/10.1186/cc2900