Review

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Review

Hydralazine as a Repurposed Epigenetic and Metabolic Modulator in Glioblastoma: Promise, Pitfalls, and Future Directions

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Abstract

Glioblastoma multiforme (GBM) remains one of the most challenging malignancies to treat, characterized by extreme heterogeneity, therapeutic resistance, and a dismal prognosis. Current multimodal treatments frequently fail due to the tumor's complex epigenetic landscape, infiltrative nature, and a highly hypoxic microenvironment that fosters immune evasion. This review evaluates the potential of repurposing hydralazine, a long-established FDA-approved vasodilator, as a novel epigenetic and metabolic modulator for GBM. Mechanistically, hydralazine acts as a non-nucleoside inhibitor of DNA methyltransferase (DNMT), facilitating the demethylation and reactivation of silenced tumor suppressor genes, such as p16 and GSTP1. Beyond its epigenetic effects, it influences tumor metabolism by modulating the adenosine signaling axis and inducing vascular dynamics—specifically the "vascular steal" phenomenon—that alter intratumoral oxygenation. These pleiotropic actions provide a compelling rationale for its use in sensitizing glioblastoma cells to conventional radiotherapy and chemotherapy. However, significant pharmacological hurdles, including uncertain blood-brain barrier penetration, a short plasma half-life, and risks of systemic toxicity such as drug-induced lupus, suggest that hydralazine is unlikely to be effective as a stand-alone clinical therapy. In conclusion, while hydralazine faces clear translational pitfalls as a monotherapy, its ability to strategically reshape the GBM biological landscape positions it as a valuable adjunctive agent. Future innovation must focus on optimizing central nervous system delivery and identifying biology-driven combination strategies to overcome the adaptive resistance of this aggressive disease.

Keywords: glioblastoma multiforme; hydralazine; drug repurposing; epigenetic modulation; DNA methyltransferase inhibitor; tumor metabolism; hypoxia; therapeutic resistance

1. Introduction

Glioblastoma multiforme (GBM) is the most common primary brain tumor and is classified as a grade IV astrocytoma. It is widely recognized for its highly aggressive behavior and extremely poor prognosis. Despite advances in multimodal therapeutic approaches, GBM remains difficult to treat and manage, with a median survival of less than one year following diagnosis [1]. Recent epidemiological data indicate that GBM is among the most prevalent and malignant primary brain tumors in terms of both incidence and occurrence. In this regard, GBM alone accounts for approximately 54% of all malignant brain tumors, with an incidence rate of 3.20 cases per 100,000 individuals per year [2].

The clinicopathological features of GBM are highly heterogeneous and are believed to arise from multiple underlying biological mechanisms. Recent research has identified a population of cancer stem cells (CSCs) within GBM, which may account for the marked heterogeneity observed in these

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tumors. These CSCs are thought to play a critical role in tumor recurrence and contribute significantly to therapeutic resistance. Gliomas can also be broadly classified into two major groups based on their pattern of spread within the surrounding brain tissue: diffuse and circumscribed gliomas. Diffuse gliomas are characterized by progressive infiltration into the surrounding brain parenchyma, which makes complete surgical resection extremely challenging [3].

In contrast, circumscribed gliomas exhibit a more localized growth pattern. However, even when gross total resection appears to be achieved, diffuse gliomas frequently recur due to their inherent tendency to infiltrate adjacent normal brain tissue. This infiltrative behavior, combined with the presence of CSCs and the resulting tumor heterogeneity, limits the effectiveness of current therapeutic strategies and contributes to the frequent recurrence of GBM, highlighting the urgent need for innovative treatment approaches, including the repurposing of established drugs with previously unrecognized anticancer properties [4].

Hydralazine, one of the earliest FDA-approved vasodilators, has been used clinically for more than 70 years, primarily for the management of hypertension and preeclampsia [5]. Although its classical pharmacological action involves relaxation of vascular smooth muscle and improved blood flow, its precise molecular mechanisms remain incompletely understood. Beyond its cardiovascular applications, emerging evidence suggests that hydralazine possesses non-canonical biological effects relevant to cancer biology, including epigenetic and metabolic modulation [6,7].

Recent studies have identified cysteamine dioxygenase (ADO), an iron-dependent oxygen-sensing enzyme, as a potential therapeutic target in GBM. Elevated ADO expression and increased levels of its metabolic product, hypotaurine, have been associated with glioblastoma progression and malignancy. Although specific ADO inhibitors have not yet been developed, experimental findings indicate that hydralazine may interfere with ADO-related pathways and influence protein degradation processes linked to cellular oxygen sensing. Importantly, glioblastoma cell lines have demonstrated concentration-dependent growth inhibition when treated with hydralazine, while non-cancerous cells show considerably lower sensitivity [8]. These observations suggest a potential selective anti-glioblastoma effect of hydralazine.

Given its long clinical history and well-characterized safety profile, hydralazine represents an attractive candidate for drug repurposing in glioblastoma therapy. In this context, our review aims to summarise the emerging evidence regarding the epigenetic and metabolic effects of hydralazine, evaluate its potential mechanisms of action in Glioblastoma, and critically discuss the current challenges, limitations, and future research directions for its clinical application in GBM treatment.

2. Biology of High-Grade Gliomas Relevant to Therapeutic Resistance

2.1. Tumor Hypoxia and Metabolic Adaptation

Glioblastoma (GBM), the most common malignant glioma, is characterized by extensive angiogenesis, widespread necrosis, and a highly invasive growth pattern [9]. It is an aggressive tumor that most commonly affects individuals between 45 and 70 years of age and accounts for approximately 40% of all primary tumors of the central nervous system. Due to its infiltrative nature, GBM disrupts normal brain function and carries a very poor prognosis, with median survival rarely exceeding 60 weeks despite multimodal treatments such as surgical resection, radiotherapy, and chemotherapy [10].

The malignant progression of gliomas is closely associated with increased tumor vascularization and cellular density. Early tumor recurrence often occurs locally and is frequently associated with greater hypoxia than the original tumor mass [11]. Hypoxia-driven necrosis is a hallmark feature of GBM. One characteristic histopathological structure is the formation of pseudopalisades, which consist of clusters of densely arranged fusiform glioma cells surrounding multiple small necrotic foci. In many cases, central necrotic regions may account for up to 80% of the total tumor mass [12]. These necrotic zones are typically bordered by tumor cells exhibiting low proliferative activity but elevated expression of matrix metalloproteinases, particularly MMP-2 and MMP-9, reflecting migratory tumor

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cells that have become hypoxic or anoxic due to intravascular thrombosis [13]. Importantly, these pathological alterations are not primarily driven by excessive proliferation or suppression of apoptosis. Histological analyses have demonstrated that intravascular thrombosis is present in approximately 92% of primary GBM resections, although it is less frequently observed in anaplastic astrocytoma (grade III) [14].

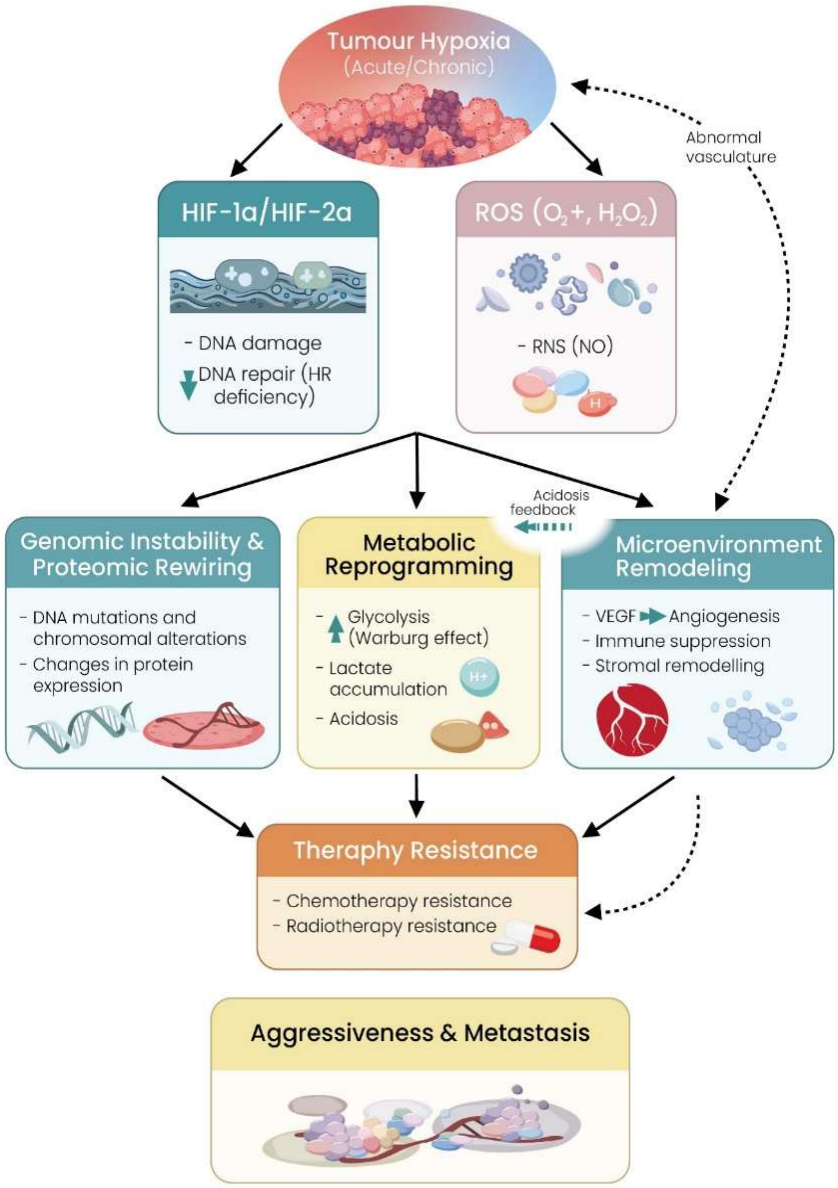

Figure 1. Illustration showing hypoxia-driven tumour progression and therapy resistance, which collectively promote therapy resistance and aggressiveness/metastasis.

2.2. Cellular Senescence and Therapy Resistance

The poor prognosis of GBM, even after standard-of-care treatments such as surgical resection followed by DNA-damaging chemotherapy and radiotherapy, is largely attributed to the inherent resistance of glioblastoma cells to conventional therapies, a phenomenon partly mediated by their robust DNA repair mechanisms and dynamic cellular states [15,16]. A significant proportion of

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glioblastoma cells undergo cellular senescence, an irreversible state of growth arrest, rather than apoptosis following therapeutic interventions such as ionizing radiation and temozolomide [17]. Senescent cells exhibit distinct morphological changes, including cytoplasmic enlargement and flattening, along with characteristic biochemical alterations, such as elevated senescence-associated β-galactosidase activity [18].

Beyond these overt phenotypic markers, senescent glioblastoma cells also secrete a diverse array of pro-inflammatory cytokines, chemokines, and growth factors, collectively known as the Senescence-Associated Secretory Phenotype [19]. This phenotype is crucial as it can profoundly modulate the tumor microenvironment, influencing processes such as immune cell recruitment, extracellular matrix remodeling, and angiogenesis [20]. However, the persistent presence of these senescent cells can paradoxically contribute to tumor recurrence and progression by fostering an immunosuppressive microenvironment and enhancing the self-renewal capacity of surviving tumor cells [21,22]. This dual role of senescence, initially tumor-suppressive but ultimately pro-tumorigenic, presents a complex challenge for therapeutic strategies [23]. Consequently, understanding the intricate mechanisms governing senescence induction and its subsequent impact on glioblastoma progression is pivotal for developing novel therapeutic interventions that specifically target these therapy-resistant cell populations [15].

2.3. Epigenetic Dysregulation in GBM

Despite significant advancements in diagnostic and therapeutic strategies, the median life expectancy for glioblastoma patients remains a dismal 12-18 months [24]. The intrinsic heterogeneity of Glioblastoma, particularly at the epigenetic level, significantly contributes to therapeutic resistance and tumor recurrence [25]. This epigenetic dysregulation encompasses a range of alterations, including DNA methylation patterns, histone modifications, and chromatin remodeling, all of which critically modulate gene expression without altering the primary DNA sequence [26]. These epigenetic modifications are pivotal in driving tumorigenesis and influencing the adaptive mechanisms that underpin therapeutic resistance in Glioblastoma [27]. Specifically, altered DNA methylation, exemplified by O6-methylguanine-DNA methyltransferase promoter methylation, and widespread histone deacetylation, are key epigenetic mechanisms driving therapy resistance, often contributing to a stem-like state in glioblastoma stem cells [28]. These epigenetic changes are integral to tumor progression and to mediating chemotherapy resistance [29].

The nuanced interplay between these epigenetic mechanisms, particularly DNA methylation, histone modifications, and chromatin remodeling, collectively orchestrates the complex regulatory landscape of gene expression, thereby fostering a highly adaptable and treatment-resistant phenotype in glioblastoma cells [30,31]. This adaptive resistance is frequently facilitated through treatment-induced epigenetic modifications, with accumulating evidence suggesting that these alterations are central to the tumor's ability to evade therapeutic efficacy [32]. A deeper understanding of these epigenetic mechanisms is crucial, as they represent promising targets for novel therapeutic interventions to overcome resistance and improve patient outcomes in Glioblastoma [33]. In conclusion, epigenetic dysregulation is a key contributor to glioblastoma progression and therapy resistance, making it an important target for future treatments.

3. Hydralazine: Mechanistic Rationale or Repurposing In GBM

The rationale for repurposing hydralazine in GBM extends beyond its established antihypertensive activity. It lies in its capacity to influence multiple interrelated biological processes that underpin tumor plasticity, therapeutic resistance, and immune evasion. This section provides evidence across epigenetic regulation, hypoxia-driven signaling, adenosine metabolism, and vascular dynamics to frame hydralazine as a context-modifying rather than a cytotoxic agent in GBM.

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Table 1. Summary of the principal mechanistic pathways through which hydralazine may influence glioblastoma biology, integrating epigenetic, vascular, metabolic, and immunologic effects across experimental systems.

Mechanistic Axis	Primary Target of Process	Observed Biological Effects	Evidence Base	Relevance to Glioblastoma
Epigenetic modulation	DNMT1/ DNMT3a inhibition	Promoter demethylation, re-expression of tumor suppressor genes	In vitro, in vivo, early clinical	Epigenetic plasticity, therapy resistance, transcriptional reprogramming
Hypoxia induction (vascular effects)	Tumor perfusion, vascular steal	Reduced tumor pO_2, increased hypoxia	In vivo (solid tumor models)	Hypoxia-driven radioresistance, metabolic adaptation
Adenosine signaling (indirect)	CD39/CD73–A_{2A}/A_{2B} axis	Immune suppression, metabolic reprogramming	Preclinical, translational	Profound immunosuppression in hypoxic GBM microenvironment
Blood–brain/blood–tumor barrier effects	Endothelial integrity, permeability	Altered barrier function, drug penetration	In vitro, translational	CNS drug delivery constraints
Chemo-and radiosensitization (combination effect)	Epigenetic priming	Enhanced response to chemotherapy and radiotherapy	In vitro, in vivo	Overcoming intrinsic and acquired resistance

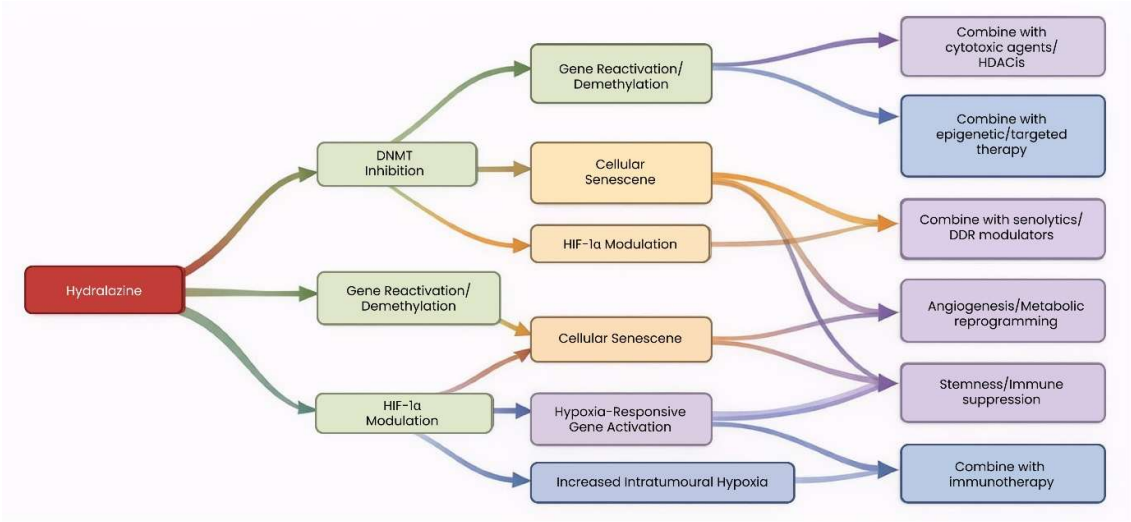

Figure 2. Flowchart illustrating the pleiotropic effects of hydralazine, leading to downstream phenotypes, such as cellular senescence, hypoxia-responsive gene activation, and altered tumor hypoxia. These biological effects provide a rationale for combination strategies with various therapeutic agents. Abbreviation: DNMT, DNA methyltransferase; HIF-1a, hypoxia-inducible factor-1 alpha; HDACis, histone deacetylase inhibitors; DDR, DNA damage response.

3.1. Inhibition of DNA Methyltransferase and Epigenetic Modulation

Hydralazine, historically used as a sedative, has garnered attention for its potential as a DNA methyltransferase (DNMT) inhibitor capable of reversing epigenetic silencing in cancers, including

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gliomas driven by mutations in isocitrate dehydrogenase (IDH), which induce hypermethylation phenotypes [34–36]. This inhibition facilitates the demethylation and reactivation of cancer-related genes, exerting antineoplastic effects that synergize with histone deacetylase inhibitors, such as valproic acid, in solid tumors [37]. Experimental evidence indicates that hydralazine lowers the global methylcytosine content in tumors and reduces the expression of DNMT1 and DNMT3a, thereby promoting the re-expression of tumor suppressor genes without directly inhibiting their enzymatic activity [38,39]. Clinical trials have further substantiated this demethylating capacity, revealing dose-dependent tumor gene demethylation rates of up to 67% for p16, accompanied by re-expression in a majority of informative cases, without altering methylation in peripheral blood cells [39]. This selective action highlights the potential of hydralazine to target GBM-associated epigenetic dysregulation, particularly in wild-type IDH1 tumors that exhibit global DNA hypomethylation and unmethylated methylguanine methyltransferase (MGMT) promoters, which confer temozolomide resistance [40]. Such targeted demethylation aligns with hydralazine's classification as a non-toxic, orally administered agent that effectively suppresses DNMT activity at micromolar concentrations, distinguishing it from more cytotoxic nucleoside analogs, such as 5-azacytidine and decitabine [39,41].

Moreover, the established clinical safety profile of hydralazine, derived from decades of antihypertensive use, has enabled its progression into phase I and II trials for solid tumors, where it demonstrates DNA demethylating activity without the myelosuppression associated with nucleoside analogs [39]. This non-nucleoside mechanism involves stable interactions between hydralazine's nitrogen atoms and the DNMT active site, enabling effective inhibition at clinically achievable concentrations with a favorable safety profile for chronic oral administration [37]. These properties position hydralazine as a promising candidate for epigenetic modulation in GBM, particularly through its capacity to reverse promoter hypermethylation of tumor suppressors such as GSTP1, BCL2, and CCND2, thereby restoring their expression in neoplastic cells [37].

3.2. Adenosine Pathway Inhibition

Beyond its direct epigenetic effects, hydralazine may also exert indirect, yet biologically meaningful, influences on immunometabolic pathways, particularly those regulated by extracellular adenosine accumulation in hypoxic tumor niches. Adenosine signaling has emerged as a central metabolic checkpoint in the tumor microenvironment (TME), particularly under conditions of chronic hypoxia, which is characteristic of GBM and other solid malignancies [42]. In hypoxic regions, cellular stress and ATP release result in rapid extracellular conversion to adenosine via the ectonucleotidases CD39 and CD73. Accumulated extracellular adenosine engages purinergic P1 receptors, most notably the A_{2A} and A_{2B} subtypes, on both immune and tumor cells, triggering downstream G-protein-coupled pathways that elevate cyclic AMP (cAMP) and broad immunosuppressive signaling. This adenosinergic axis effectively dampens effector T-cell responses, skews macrophage polarisation towards pro-tumorigenic phenotypes, and blunts innate and adaptive anti-tumor immunity, thereby facilitating immune escape and tumor progression [43]. Targeted blockade of adenosine production or receptor engagement has been shown to reverse this immunosuppressive milieu in preclinical models, underscoring its therapeutic potential in cancer therapy [44].

Recent publications have consolidated evidence that the adenosine pathway represents both metabolic and immunological vulnerabilities in Glioblastoma. Elevated expression of CD39/CD73 and A_{2A} and A_{2B} receptors in hypoxic tumor niches correlates with increased adenosine accumulation and immune dysfunction. In Glioblastoma specifically, adenosine fosters immunosuppression and reprograms tumor-associated macrophages towards pro-tumor phenotypes, thereby supporting immune evasion and therapeutic resistance. Targeting these components with small-molecule inhibitors or blocking antibodies can mitigate adenosine-mediated suppression while enhancing anti-tumor immunity [45].

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Inhibition of adenosine signaling also intersects with hypoxia-induced metabolic adaptations. Adenosine receptor signaling influences not only immune suppression but also the metabolism of tumor and stromal cells. The activation of A_{2A} and A_{2B} receptors, which regulate the immune response, angiogenesis, and cell growth, has been implicated in modulating glycolytic and oxidative metabolic pathways, driving the expression of glycolytic enzymes, and further supporting metabolic adaptation to hypoxia [43]. Blocking these receptors has been shown to moderate tumor acidosis and hypoxia, alleviate metabolic stress, and normalize elements of the TME that favor tumor growth and immune suppression. These metabolic effects extend beyond immune modulation, indicating that adenosine pathway inhibition may weaken tumor survival strategies rooted in altered energy metabolism [46].

Collectively, the adenosine pathway serves as a nexus between hypoxic adaptation, metabolic signaling, and immune suppression in GBM. Therapeutic strategies that inhibit CD39/CD73 enzymatic activity, block A_{2A}/A_{2B} receptor signaling, or otherwise reduce extracellular adenosine have demonstrated preclinical efficacy and are under active clinical investigation. By mitigating both metabolic and immunologic resistance mechanisms, adenosine pathway inhibition holds promise as part of rational combination regimens to enhance responses to immunotherapy, radiotherapy, and conventional chemotherapy in Glioblastoma and other solid cancers [43]. These effects are particularly relevant in Glioblastoma, where myeloid-dominant immune infiltration, pronounced hypoxia, and high extracellular adenosine concentrations collectively reinforce a profoundly immunosuppressive tumor microenvironment.

3.3. Effects on Tumor Perfusion and Vascular Dynamics

The classical pharmacological action of hydralazine as a direct-acting arteriolar vasodilator has implications for tumor perfusion and vascular dynamics that extend beyond systemic blood pressure lowering. In normal cardiovascular physiology, hydralazine relaxes vascular smooth muscle, decreasing peripheral resistance and increasing cardiac output [47]. However, this systemic vasodilatory effect paradoxically affects tumor blood flow due to the aberrant structure and limited autoregulatory capacity of the tumor vasculature. Tumors often lack functional smooth muscle in their vessels, rendering them unable to dilate in response to systemic vasodilators. Consequently, the reduction in systemic blood pressure by hydralazine precipitates a 'vascular steal' phenomenon, in which blood flow is preferentially diverted to normal tissues at the expense of tumor perfusion. This results in reduced tumor blood flow and oxygen delivery in multiple experimental tumor models [48,49].

Direct measurement of tumor oxygen partial pressure (pO2) after hydralazine administration confirmed a marked decrease in intratumoral oxygenation. In both transplanted and spontaneous murine tumors, intravenous hydralazine significantly reduced the median pO2 values and increased the fraction of severely hypoxic regions, consistent with compromised perfusion [50]. Such reductions in tumor oxygenation are mechanistically meaningful because oxygen tension is a critical determinant of cellular radiosensitivity; well-oxygenated cells fix radiation-induced DNA damage more effectively than hypoxic cells. Hypoxia, therefore, can attenuate the cytotoxicity of radiotherapy and select for radioresistant clones [51].

The relationship between changes in perfusion caused by hydralazine and radiotherapy outcomes is complex. Early preclinical studies have explored hydralazine in combination with hypoxia-targeted cytotoxins, demonstrating enhanced cytotoxicity by increasing hypoxic fractions within tumors. For instance, hydralazine enhanced the effectiveness of nitroimidazole radiosensitizers when combined with radiation, suggesting that hypoxia-induction could potentiate hypoxic cell-specific therapies [52]. Nonetheless, the increase in hypoxic tumor regions may also limit the effectiveness of conventional radiotherapy, which relies on oxygen-mediated fixation of DNA damage, highlighting the dualistic nature of hydralazine's impact on vascular dynamics in a therapeutic context [53]. Furthermore, hydralazine's effect on perfusion also affects the delivery of the chemotherapeutic drug. Reduced tumor blood flow and the resultant hypoxic microenvironment

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can constrain the delivery and distribution of systemically administered cytotoxic agents, potentially diminishing intratumoral drug concentrations. In cases where bioreductive or hypoxia-activated prodrugs are employed, hydralazine-induced hypoxia may potentiate selective drug activation within poorly perfused tumor zones [54].

Collectively, these observations underscore that hydralazine's vasodilatory properties produce nuanced effects on tumor perfusion and oxygen dynamics. While the resultant reduction in blood flow and increased hypoxia may be therapeutically leveraged with hypoxia-targeted agents, they also pose challenges for therapies reliant on adequate perfusion and oxygenation. The integration of hydralazine into combination regimens should therefore be guided by a mechanistic understanding of tumor vascular biology and the specific modalities involved.

3.4. Pharmacokinetics and BBB Considerations

Hydralazine exhibits a pharmacokinetic profile characterized by rapid oral absorption, extensive first-pass metabolism, and significant interindividual variability, primarily driven by genetically determined acetylator phenotypes. Peak plasma concentrations are generally reached within 1–2 hours of oral ingestion; however, systemic bioavailability is highly variable owing to substantial presystemic metabolism, with slow acetylators demonstrating higher systemic exposure and prolonged drug levels relative to rapid acetylators [55]. The elimination half-life of hydralazine itself is approximately 1–3 hours. Still, the duration of pharmacodynamic effects often exceeds the plasma half-life, likely reflecting contributions from active metabolites and sustained vascular actions. These metabolic characteristics influence both dose requirements and systemic exposure, with consequences for tolerability and toxicity at higher doses [56].

Regarding central nervous system (CNS) penetration, direct evidence of hydralazine crossing the intact BBB remains limited. The physicochemical properties of hydralazine, including moderate lipophilicity and relatively high plasma protein binding, do not strongly favor passive diffusion across an intact BBB, and there is a paucity of in vivo studies quantifying its unbound brain concentrations. In vitro models have demonstrated that hydralazine can induce hypoxia-like stress in endothelial cells of the BBB and increase paracellular permeability, characterized by decreased tight junction integrity and enhanced efflux transporter activity. This suggests that CNS exposure may be influenced by BBB modulation rather than intrinsic permeability [57,58]. While these findings imply the potential for altered BBB dynamics under stress conditions, they do not establish robust and predictable CNS penetration under physiological conditions.

These pharmacokinetic and BBB considerations must be juxtaposed with the clinical dose limitations and systemic toxicity profiles. The vasodilatory effects of hydralazine, primarily mediated by direct arteriolar smooth muscle relaxation, can precipitate dose-dependent adverse events, including reflex tachycardia, headache, flushing, and hypotension. Chronic use carries the risk of immunologically mediated sequelae, most notably a lupus-like syndrome, which appears to correlate with the cumulative dose and slow acetylator status. Other reported toxicities include gastrointestinal discomfort and, rarely, hepatic injury with variable clinical presentations. The requirement for frequent dosing due to the short plasma half-life also poses challenges for sustained exposure, particularly at doses approaching toxicity thresholds [47].

In the context of Glioblastoma and other CNS malignancies, these pharmacokinetic constraints highlight the challenge of achieving consistent, therapeutically effective brain concentrations of hydralazine without inducing systemic side effects. To fully exploit the epigenetic and microenvironmental mechanisms of hydralazine in the brain, it may be necessary to develop strategies that enhance local delivery, utilize transient BBB disruption, or employ analogous agents with improved BBB permeability. Nonetheless, a comprehensive understanding of the systemic disposition of hydralazine and its interactions with the BBB is crucial for rational dose selection and safety monitoring in repurposing clinical investigations.

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4. Evidence Synthesis: Effects of Hydralazine in Preclinical and Clinical Contexts

Given the mechanistic nature of hydralazine, a traditional linear review of individual studies risks fragmenting the interpretation of the biological effects. Instead, this section adopts a systematic narrative synthesis, integrating data across experimental systems and clinical contexts according to shared biological outcomes. This approach enables reconciliation of heterogeneous findings while highlighting convergent patterns relevant to glioblastoma biology and therapeutic resistance.

4.1. Outcomes Assessed Across Experimental Contexts

4.1.1. Cell Proliferation and Viability

Across multiple in vitro cancer models, hydralazine consistently demonstrates anti-proliferative effects, although the magnitude and mechanism vary by cell type and molecular properties. Studies in prostate, breast, and hematologic malignancies report dose-dependent reductions in cell viability, clonogenic survival, and proliferative capacity following hydralazine exposure at micromolar concentrations (38, 59, 60). These effects are frequently accompanied by cell-cycle arrest, most commonly at the G1/S transition, rather than overt cytotoxicity, suggesting a predominantly cytostatic mechanism. In several systems, growth suppression has been associated with re-expression of epigenetically silenced tumor suppressor genes and altered expression of key cell-cycle regulators following hydralazine-mediated DNA demethylation, consistent with restoration of transcriptional control over proliferation checkpoints [61,62].

In glioma-relevant systems, direct evidence remains limited; however, the observed suppression of proliferation in epigenetically dysregulated tumors aligns with Glioblastoma's reliance on transcriptional plasticity, aberrant DNA methylation states, and dynamic cell-cycle regulation, features increasingly implicated in therapeutic resistance and tumor recurrence. Importantly, the growth-inhibitory effects of hydralazine are often potentiated when combined with histone deacetylase inhibitors or cytotoxic agents, supporting its role as a biological sensitizer rather than a stand-alone cytotoxic drug [39,63]. Together, these findings suggest that hydralazine's primary contribution to proliferative control is context-dependent and most pronounced when integrated into combination regimens that exploit epigenetic vulnerabilities.

4.1.2. Senescence Modulation as a Therapeutic Mechanism

A recurring theme across preclinical studies is hydralazine's ability to induce or reinforce cellular senescence, particularly in contexts where epigenetic repression of tumor suppressor pathways is prominent. Inhibition of DNMTs by hydralazine reactivates previously silenced genes, including cyclin-dependent kinase inhibitors such as $p16^{Ink4a}$ and $p21^{Cip1/Waf1}$, which are central mediators of senescence. Consequent phenotypes include permanent growth arrest, altered chromatin architecture characterized by senescence-associated heterochromatic foci, and increased expression of senescence-associated β-galactosidase, all hallmarks of the senescent state [34,64].

This mechanistic capacity is particularly relevant in GBM, where therapy-induced senescence contributes to both short-term tumor control and paradoxically to long-term recurrence through the secretion of pro-inflammatory factors known collectively as the senescence-associated secretory phenotype (SASP) [65]. SASP factors can promote tumor cell proliferation, immune evasion, and remodeling of the tumor microenvironment, underscoring the dual-edged nature of senescence in GBM. Although hydralazine-induced senescence has not been systematically studied in glioma models, its epigenetic mode of action suggests a capacity to modulate pre-existing senescence programs rather than trigger apoptotic cell death. This distinction is critical when considering combination strategies, such as pairing hydralazine with agents that selectively target senescent cells ("senolytics") or exploiting vulnerabilities in the DNA damage response, to maximize anti-tumor efficacy while mitigating potential pro-tumorigenic SASP effects [65,66].

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Expanding on these mechanistic and therapeutic implications provides a framework for understanding how hydralazine could be integrated into GBM treatment strategies that leverage epigenetic reprogramming and senescence modulation. Further preclinical investigation is warranted to elucidate the precise signaling pathways involved, the timing of senescence induction, and the interplay with conventional therapies.

4.1.3. Changes in Gene Expression and DNA Methylation

The most consistent and well-characterized biological effect of hydralazine across experimental systems is global and locus-specific DNA demethylation, accompanied by transcriptional reactivation of epigenetically silenced genes. Hydralazine reduces global 5-methylcytosine content and downregulates expression of DNMT1 and DNMT3A in both in vitro and in vivo models, despite lacking direct enzymatic inhibition of DNMTs [67–69]. The precise mechanism appears to involve transcriptional repression of DNMTs and interference with maintenance methylation during DNA replication, leading to gradual hypomethylation across successive cell cycles.

Genes reactivated by hydralazine frequently include tumor suppressors, cell-cycle regulators, and pro-differentiation factors, such as $p16^{Ink4a}$/$p21^{Cip1/Waf1}$, glutathione S-transferase P1 (GSTP1), and cyclin D2 (CCND2), indicating a broad reversal of oncogenic epigenetic programs [64,67]. These transcriptional changes can restore cell-cycle checkpoints, induce differentiation, and potentially enhance sensitivity to DNA-damaging therapies. Clinical correlative studies support these preclinical findings, demonstrating promoter demethylation and gene re-expression in tumor tissue with minimal effects in peripheral blood cells, highlighting a degree of tumor-selective epigenetic modulation [68].

In GBM, where epigenetic heterogeneity underpins intratumoral diversity and contributes to treatment resistance, hydralazine-mediated demethylation may facilitate the reprogramming of tumor cell transcriptional states rather than inhibiting a single oncogenic pathway. By reshaping gene expression patterns, hydralazine can alter tumor cell identity, sensitize subpopulations to conventional therapies, and mitigate adaptive resistance. These observations provide a rationale for combination strategies, such as pairing hydralazine with histone deacetylase inhibitors, DNA-damaging agents, or targeted therapies, to exploit its epigenetic remodeling capacity in a clinically meaningful way.

4.1.4. Effects on Hypoxia Response Pathways

Hydralazine's influence on hypoxia signaling is multifaceted and highly context-dependent. Preclinical vascular studies demonstrate that hydralazine reliably increases intratumoral hypoxia through perfusion redistribution, a consequence of systemic vasodilation that disproportionately reduces blood flow to tumor microvasculature while sparing normal tissue [50,70]. The resulting decline in tissue oxygenation amplifies hypoxia-responsive transcriptional programs mediated by HIF-1α, including genes involved in angiogenesis, glycolytic metabolism, and cell survival. In addition to these vascular effects, hydralazine modulates HIF-1α stability and downstream gene expression through non-vascular, cell-intrinsic mechanisms. These include interference with prolyl hydroxylase domain (PHD) enzymes that degrade HIF-1α, as well as epigenetic regulation of hypoxia-responsive genes via DNA demethylation [71]. These apparently divergent effects (vascular versus intracellular) highlight hydralazine's role as a context-dependent hypoxia modifier rather than a unidirectional inhibitor or activator. The net impact on HIF signaling likely reflects the balance between perfusion-mediated hypoxia and direct epigenetic or enzymatic modulation of HIF activity.

In GBM, where hypoxia drives immune suppression, metabolic reprogramming, angiogenesis, and maintenance of glioma stem-like cells, this duality underscores the need for careful therapeutic scheduling. Hydralazine may sensitize tumors to radiation or hypoxia-activated prodrugs by increasing tumor hypoxia, while simultaneous epigenetic modulation could enhance the efficacy of immunotherapy or stemness-targeting strategies. Rational combination approaches, rather than

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monotherapy, are therefore critical to exploiting hydralazine's multifaceted impact on hypoxia response pathways while minimizing potential tumor-protective adaptations.

5. Why Hydralazine May Fail as a Stand-Alone Clinical Therapy

Although hydralazine demonstrates biologically relevant activity across multiple cancer-associated pathways, several factors strongly argue against its effectiveness as a stand-alone clinical therapy in Glioblastoma. These limitations are not unique to hydralazine, but rather reflect broader translational challenges encountered when repositioning systemically active, pleiotropic agents for a disease defined by extreme heterogeneity, adaptive resistance, and anatomical constraints. These constraints can be broadly categorized into pharmacological limitations, systemic toxicity, tumor-intrinsic resistance mechanisms, and the broader failure of monotherapy paradigms in Glioblastoma.

Table 2. Principal biological and pharmacological barriers limiting the efficacy of hydralazine as a stand-alone therapeutic agent in Glioblastoma.

Limitation Category	Underlying Cause	Clinical Implication
Pharmacokinetics	Short half-life, acetylator variability	Inconsistent target engagement
CNS delivery	BBB penetration uncertainty	Subtherapeutic intratumoral levels
Toxicity	Vasodilation, lupus risk	Limited dose escalation
Tumor biology	Heterogeneity, plasticity	Rapid adaptive resistance

A primary concern is insufficient target engagement at doses that remain clinically tolerable. While hydralazine has been shown to induce DNA demethylation and transcriptional reprogramming in both preclinical models and clinical tumor samples, these effects are dose-dependent. They are often observed at concentrations that approach or exceed human tolerability thresholds. Hydralazine's short plasma half-life, extensive first-pass metabolism, and marked inter-individual variability driven by acetylator status further complicate achieving sustained target modulation [55,72]. In a Phase I study of cervical cancer patients, hydralazine doses of 50-150 mg/day were well tolerated and induced demethylation and re-expression of some tumor suppressor genes. Still, global DNA methylation remained unchanged, and the demethylation rates varied considerably by gene and dose level, suggesting limited and heterogeneous target engagement in vivo [69]. In the context of Glioblastoma, where continuous and durable pathway modulation is typically required to influence tumor behavior, fluctuating systemic exposure is unlikely to translate into consistent intratumoral effects. Moreover, uncertainties surrounding BBB penetration raise additional doubts regarding whether adequate concentrations can be achieved within the tumor core and infiltrative margins without provoking unacceptable systemic toxicity [58].

Closely related to these pharmacological constraints are off-target effects and cumulative toxicity, which limit dose escalation and long-term administration. The vasodilatory effects produced by hydralazine predispose patients to hypotension, reflex tachycardia, headaches, and flushing, adverse effects that become increasingly problematic at higher doses or with chronic use [47]. In the same clinical investigation described above, adverse effects, while generally mild, were common, and prolonged or high-dose administration would likely exacerbate these problems, especially in patients with compromised functional status [69]. More importantly, prolonged exposure is associated with immune-mediated complications, most notably drug-induced lupus-like syndromes, which correlate with cumulative dose and slow acetylator phenotype [73]. In patients with Glioblastoma, who often require corticosteroids, antiepileptics, and multimodal oncologic therapy, additional systemic toxicity may compromise adherence, functional status, and overall treatment feasibility. Although the anticancer effects of hydralazine appear largely modulatory rather than cytotoxic, the requirement for sustained exposure further amplifies these concerns.

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Beyond pharmacology, **tumor heterogeneity and adaptive resistance mechanisms** present formidable biological obstacles. Glioblastoma is characterized by pronounced intratumoral diversity at the genetic, epigenetic, metabolic, and phenotypic levels, with coexisting cell populations exhibiting distinct sensitivities to therapeutic pressure patel [74,75]. While hydralazine may reverse DNA methylation or alter hypoxia-responsive transcriptional programs in specific cellular subsets, other populations may remain unaffected or rapidly compensate through alternative signaling pathways. Epigenetic plasticity, particularly within glioma stem-like cells, enables dynamic reconfiguration of chromatin states and transcriptional networks in response to environmental or therapeutic stress, thereby limiting the durability of epigenetic interventions when applied in isolation [76]. Consequently, any growth suppression or phenotypic shift induced by hydralazine alone is likely to be transient and vulnerable to adaptive escape. In this setting, the capacity of glioblastoma cells, particularly stem-like subpopulations, to dynamically rewire epigenetic and transcriptional programs in response to selective pressure renders isolated epigenetic modulation insufficient, as adaptive resistance emerges not from a single pathway but from the tumor's intrinsic plasticity.

The well-documented failure of monotherapy approaches in Glioblastoma further contextualizes these limitations. With few exceptions, agents targeting single molecular processes have failed to produce durable clinical benefit in GBM, reflecting the disease's reliance on overlapping, redundant survival mechanisms rather than a dominant oncogenic driver [10,77]. Glioblastoma progression is sustained by the convergence of epigenetic deregulation, metabolic flexibility, immune suppression, and aberrant vascular biology—processes that can compensate for one another when perturbed individually. Although hydralazine engages several of these axes simultaneously, its effects are modest. Without concurrent cytotoxic, immunologic, or DNA-damaging pressure, such perturbations are unlikely to translate into meaningful tumor regression.

Taken together, these considerations indicate that hydralazine's limitations as a monotherapy stem not from a lack of biological relevance but from the mismatch between its pharmacological profile and the therapeutic demands of Glioblastoma. Its effects are diffuse, context-dependent, and primarily modulatory, features that diminish its stand-alone efficacy but simultaneously position it as a potentially valuable adjunctive agent. Recognizing that hydralazine is unlikely to succeed alone, therefore, provides a critical foundation for defining its optimal role within rational combination strategies, rather than undermining its translational relevance.

6. Future Directions and Opportunities for Innovation

If hydralazine is to have a meaningful future in glioblastoma research, its development must move beyond whether it works in isolation and instead focus on how its biological effects can be strategically leveraged. The evidence reviewed thus far suggests that hydralazine's true potential lies not in monotherapy but in its ability to reshape tumor biology, thereby making other treatments more effective. This perspective naturally shifts attention toward rational combination strategies, optimization of pharmacologic properties, and more refined approaches to patient selection and experimental design. Rather than proposing hydralazine as a definitive therapeutic agent, these future directions outline how its mechanistic properties could be integrated into more sophisticated, biology-driven treatment strategies (Figure 3).

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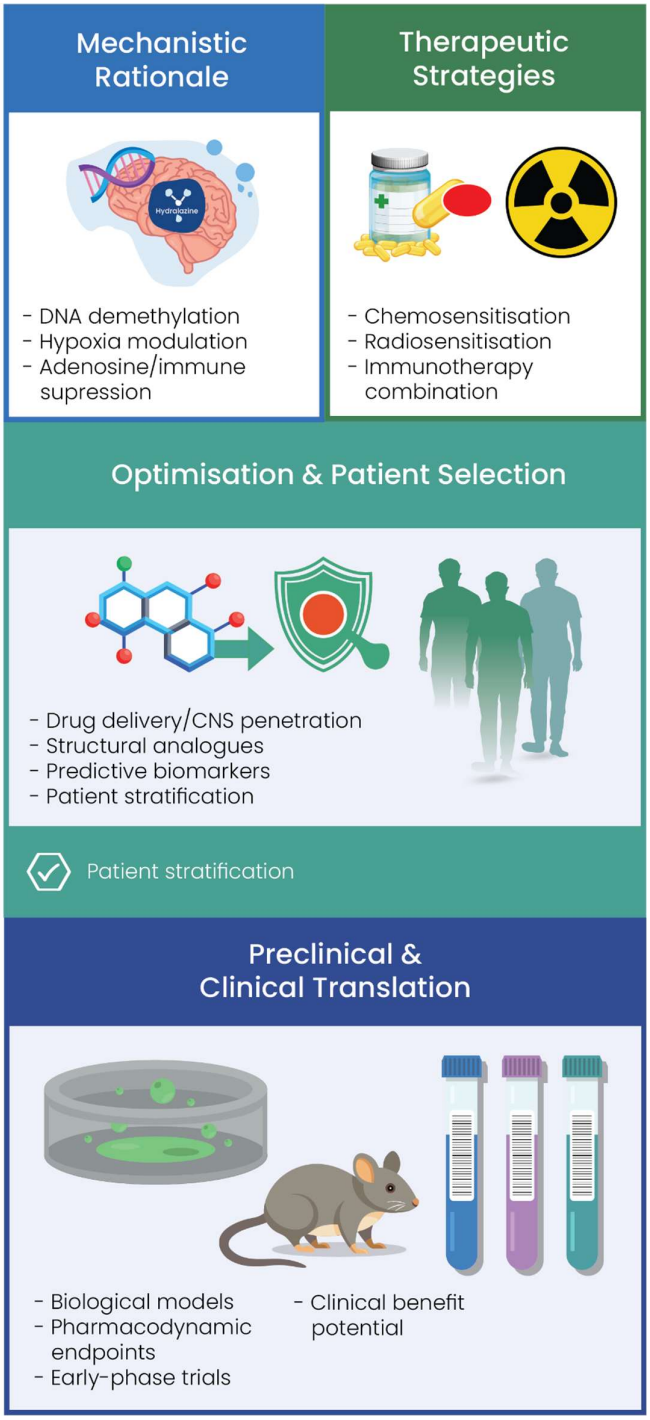

Figure 3. Overview of the mechanistic rationale and translational framework supporting hydralazine in Glioblastoma, and key optimization strategies with progression from preclinical validation to early-phase clinical evaluation.

6.1. Combination Strategies

One of the most immediate opportunities lies in combination therapy. As previously discussed, hydralazine modulates DNA methylation, hypoxia responses, and elements of the tumor microenvironment, suggesting that it may be most effective not as a stand-alone agent but as a sensitizing agent in combination therapies. For example, combinations of hydralazine with histone deacetylase inhibitors such as valproate have shown synergistic anti-tumor activity in various cancer

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models, increasing cytotoxicity when paired with conventional chemotherapy agents in vitro and in animal studies. In a panel of cancer cell lines, including glioma, hydralazine alone did not inhibit growth. Still, when combined with valproic acid, it enhanced gene expression changes and potentiated chemotherapy effects in vivo, indicating a potential role in overcoming resistance to standard cytotoxic agents [63].

Epigenetic combinations have also demonstrated radiosensitization; hydralazine, together with valproic acid, increased the sensitivity of cervical cancer cells to radiation and further enhanced the effect of chemoradiation in vitro, an approach that may be translatable to other tumors characterized by radioresistance [78].

More broadly, the integration of hydralazine with immunotherapy or radiotherapy holds theoretical appeal. Modulating tumor hypoxia and adenosine-dominant immunosuppressive pathways could improve immune infiltration and activation, as tumor hypoxia is a known barrier to effective immune checkpoint blockade. Preclinical models have shown that altering tumor stroma and perfusion can increase intratumoral delivery and enhance immunotherapy potency, suggesting a potential framework for future studies that pair hydralazine-based modulation with immunotherapeutic approaches such as checkpoint inhibitors [79]. Together, these observations support the concept that hydralazine's primary utility lies in lowering biological resistance thresholds rather than exerting direct anti-tumor pressure.

6.2. Structural/Pharmacologic Optimization

Hydralazine was developed for cardiovascular use and was not optimized for oncologic applications; this limits its clinical utility due to short half-life, systemic toxicity, and variable central nervous system exposure [80]. Future innovation could focus on developing hydralazine derivatives with improved pharmacokinetic properties, reduced cardiovascular side effects, and better penetration across the blood–brain and blood–tumor barriers. Such analogs might retain epigenetic or microenvironment-modulating activity while minimizing off-target effects.

Alternatively, structurally distinct compounds that mimic hydralazine's epigenetic effects or selectively target adenosine signaling pathways may offer improved safety and efficacy profiles tailored to CNS malignancies. There is also growing interest in advanced drug delivery systems, including nanoparticles, liposomes, or tumor-targeted carriers, that could enhance hydralazine's local concentration in tumor tissues while limiting systemic exposure. Preclinical work with hydralazine-loaded nanoparticles has demonstrated enhanced drug accumulation and modulation of the microenvironment in desmoplastic tumor models, highlighting the potential of delivery optimization to overcome structural and pharmacologic constraints [79]. Among these approaches, improvements in CNS delivery and pharmacokinetic stability are likely to yield the most significant immediate translational impact.

6.3. Biomarker-Driven Patient Selection

Given the heterogeneity of Glioblastoma and the variable biological effects of hydralazine, biomarker-based patient selection will be crucial for future translational success. Molecular features such as baseline DNA methylation patterns, transcriptional states indicative of epigenetic plasticity, and hypoxia-related pathway signatures could help identify patient subgroups most likely to benefit from hydralazine-based interventions [39].

For instance, tumors with a high burden of promoter hypermethylation or evidence of epigenetic transcriptional repression might be more susceptible to epigenetic modulation [81]. Likewise, hypoxia markers or adenosine pathway activity could be explored as predictive biomarkers for combination strategies aimed at alleviating microenvironment-driven immunosuppression [82,83]. Embedding such biomarkers into early-phase clinical trials would refine signal detection and align these approaches with current trends in precision oncology. Importantly, such biomarkers would ideally function as predictive rather than merely prognostic indicators, enabling enrichment of patient populations most likely to derive benefit from hydralazine-based strategies.

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6.4. Experimental and Translational Priorities

Progress will also depend on experimental models and trial designs that better reflect the complexity of glioblastoma biology. Three-dimensional (3D) cell culture systems can capture hypoxia gradients, tumor–stroma interactions, and epigenetic heterogeneity, providing significant advantages over standard two-dimensional (2D) cell culture systems [84].

Advanced preclinical systems, such as organoids, patient-derived xenografts (PDXs), and 3D co-culture models, are needed to study how hydralazine combinations affect hypoxia–epigenetic interactions and immune infiltration in environments that more closely mimic human tumors (85--87).

On the clinical side, early-phase trials should prioritize pharmacodynamic endpoints, such as changes in DNA methylation profiles, gene expression signatures, or hypoxia-related markers, rather than focusing solely on tumor shrinkage [88]. Such designs would allow the inherent biological effects of hydralazine and its combinations to be evaluated even in the absence of immediate radiographic responses, offering clearer insights into mechanisms of action and opportunities for iterative optimization.

7. Limitations of the Review

Several limitations inherent to this review should be acknowledged:

- As a narrative rather than a systematic review, the synthesis presented here relies on the selection and interpretation of available literature rather than exhaustive, protocol-driven evidence capture. While care was taken to include relevant preclinical, translational, and clinical studies across epigenetic, metabolic, vascular, and immunologic domains, the possibility of publication bias and incomplete coverage cannot be excluded. This is particularly relevant in an emerging field where negative or inconclusive findings may be underreported.
- Much of the evidence supporting hydralazine's anti-tumor relevance is derived from non-glioblastoma models or experimental systems that do not fully recapitulate the biological and anatomical complexity of human GBM. In vitro studies often employ supraphysiologic drug concentrations or simplified cellular environments, while in vivo data frequently focus on vascular or hypoxia endpoints rather than integrated tumor control. As such, extrapolation to GBM biology, particularly within the constraints of the human central nervous system, must be undertaken with caution.
- The heterogeneity of experimental designs, endpoints, and outcome measures across studies limits direct comparison and quantitative synthesis. Variability in dosing regimens, exposure duration, model systems, and assessed biomarkers complicates efforts to draw unified conclusions regarding efficacy, optimal combinations, or therapeutic windows. This heterogeneity is compounded by inconsistent reporting of pharmacokinetic and pharmacodynamic parameters, particularly regarding intratumoral or intracranial drug exposure.
- The review necessarily integrates mechanistic inference where direct evidence is lacking. Connections between hydralazine's epigenetic effects, hypoxia modulation, adenosine signaling, and immune suppression are biologically plausible and supported by parallel lines of evidence. Still, they have not been comprehensively validated within a single experimental framework or in GBM-specific clinical studies. These inferred links should therefore be viewed as hypothesis-generating rather than definitive.
- Clinical data directly evaluating hydralazine in Glioblastoma are absent, and conclusions regarding translational relevance are based on indirect evidence from other solid tumors and

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preclinical systems. Pharmacokinetic limitations, blood–brain barrier considerations, and toxicity profiles further constrain clinical extrapolation. Consequently, the therapeutic potential discussed here should be interpreted as conditional and exploratory, pending rigorous GBM-specific experimental and clinical investigation.

Despite these limitations, this review provides a structured synthesis of disparate evidence streams and highlights biologically grounded opportunities for future research. By explicitly acknowledging uncertainties and knowledge gaps, it aims to inform rational experimental design rather than overstate clinical readiness.

8. Conclusion

Hydralazine demonstrates multiple mechanistic properties, spanning epigenetic modulation, vascular effects, hypoxia regulation, and immunomodulation, that could, in principle, be leveraged against Glioblastoma. However, the current evidence remains largely preclinical, indirect, and heterogeneous, with limited relevance to the complex biology of human GBM. The majority of studies rely on non-glioblastoma models, supraphysiologic dosing, or simplified experimental systems, and no clinical data currently exist to validate translational efficacy. As such, any therapeutic potential should be regarded as exploratory and hypothesis-generating.

Future investigations should prioritize GBM-specific preclinical models, rigorous pharmacokinetic and pharmacodynamic characterization, and integrated assessment of tumor, vascular, and immune endpoints. By systematically addressing these gaps, subsequent work can more reliably evaluate whether hydralazine or related mechanistically informed strategies offer meaningful clinical benefit in this challenging disease. In the interim, this review consolidates existing evidence, highlights mechanistic plausibility, and provides a framework for rational experimental design without overstating clinical readiness.

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