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Review

Reprogramming Neuroinflammation After Stroke: A Coupled Network Model of Microglial Control

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Abstract

Ischaemic stroke induces a dynamic neuroimmune response in which microglia act as central regulators of both secondary injury and tissue repair. In the acute phase, microglial activation amplifies neuronal damage through inflammatory signalling and vascular dysfunction; over subsequent days, these cells undergo coordinated transcriptional and metabolic reprogramming toward reparative states. The repeated failure of immunomodulatory therapies in clinical translation, however, suggests that current approaches fundamentally mischaracterise the underlying biology. We propose that microglial state transitions are governed not by discrete linear pathways but by a coupled regulatory network integrating proteostatic clearance, receptor-mediated signalling, inflammasome activation, and intracellular metabolism. Within this network, impaired clearance of cellular debris sustains exposure to damage-associated molecular patterns, perpetuating inflammasome activity and a pro-inflammatory metabolic programme; conversely, restoration of clearance capacity shifts network equilibrium toward resolution and repair. Microglial phenotypes therefore emerge from dynamic shifts in network state rather than progression through fixed activation stages. This framework accounts for the limited efficacy of non-selective or temporally misaligned interventions and identifies the post-acute transitional phase as a window of maximal network plasticity. Aligning therapy with the temporal and functional dynamics of this network—guided by phase-specific biomarkers—provides a mechanistic basis for precision immunomodulation and improved clinical translation in ischaemic stroke.

Keywords: microglia; ischaemic stroke; neuroinflammation; microglial reprogramming; temporal dynamics; precision medicine; biomarker-guided therapy

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1. Introduction

Despite major advances in acute stroke care, including intravenous thrombolysis and mechanical thrombectomy, ischaemic stroke remains a leading cause of death and long-term neurological disability worldwide [1] Even among patients who achieve timely reperfusion, functional outcomes are often poor, reflecting the contribution of secondary injury processes that evolve beyond the initial vascular occlusion. Among these, neuroinflammation has emerged as a central determinant of post-ischaemic injury and recovery [2–4].

Ischaemic stroke induces a neuroimmune response in which microglia—resident innate immune cells of the central nervous system (CNS)—play a decisive role in determining whether tissue progresses toward injury or repair [5]. As dynamic sentinels of brain homeostasis, microglia continuously survey the parenchyma and rapidly respond to metabolic stress and cell death [6]. Following cerebral ischaemia, they are among the first cells to de-

tect injury, initiating inflammatory signalling, shaping blood–brain barrier (BBB) integrity, and orchestrating the recruitment of peripheral immune cells [7,8].

Microglial responses are highly plastic and temporally regulated. Early activation amplifies secondary injury through inflammatory signalling and vascular dysfunction, whereas later programmes are essential for debris clearance, tissue remodelling, and neuroplasticity [9,10]. This functional duality creates a central paradox: the same processes that exacerbate injury in the acute phase become indispensable for recovery.

Immunomodulatory strategies targeting neuroinflammation have repeatedly failed in clinical translation, even when supported by robust preclinical evidence [11]. We argue that these failures do not reflect invalid biological targets, but rather a mis-specification of microglial biology—specifically, the assumption that microglial activation progresses through discrete, targetable stages.

We propose that microglial behaviour is governed by a coupled regulatory network integrating proteostatic clearance, receptor-mediated signalling, inflammasome activation, and intracellular metabolic state. Impaired debris clearance sustains damage-associated signalling and inflammasome activity, reinforcing a pro-inflammatory programme; restoration of clearance shifts the system toward resolution and repair. Microglial phenotypes thus reflect dynamic changes in network equilibrium rather than progression through fixed stages. This framework explains the failure of non-selective or temporally misaligned therapies and reframes microglial modulation as a problem of network reconfiguration rather than pathway inhibition. Integrating these regulatory axes across the temporal phases of ischaemic stroke, and linking network dynamics to therapeutic timing, biomarker-guided stratification, and clinical translation, we advance a systems-level framework for precision immunomodulation following cerebral ischaemia—one in which therapeutic efficacy depends less on suppressing inflammation globally than on aligning intervention with each patient's evolving microglial network state.

2. Microglial Sensing and Initiation of Post-Ischaemic Inflammation

2.1. The Ischaemic Cascade as a Trigger for Microglial Activation and Damage-Associated Molecular Pattern Recognition

Ischaemic stroke results from an abrupt reduction in cerebral blood flow, leading to deprivation of oxygen and glucose within affected brain tissue [8,12]. Rapid depletion of adenosine triphosphate (ATP) disrupts ionic gradients, causes membrane depolarisation, and drives excessive glutamate release with pathological activation of N-methyl-D-aspartate receptors [13]. Sustained excitotoxic stress and energy failure result in rapid necrotic cell death within the infarct core, while cells in the surrounding penumbra undergo delayed forms of regulated cell death, including apoptosis and necroptosis [12]. These cell death pathways generate signals that are rapidly detected by microglia, as necrotic and necroptotic cells release intracellular contents into the extracellular space, providing ligands for microglial pattern-recognition receptors [10].

Dying neurons, astrocytes, and oligodendrocytes release damage-associated molecular patterns (DAMPs)—such as high-mobility group box 1, ATP, heat shock proteins, peroxiredoxins, and nucleic acids—acting as potent endogenous danger signals [14]. Microglia express a broad repertoire of pattern recognition receptors, including Toll-like receptors and the receptor for advanced glycation end products, enabling rapid detection of these signals [5]. Engagement of DAMPs with microglial receptors activates intracellular signalling pathways such as NF-κB and inflammasome cascades, driving transcription of pro-inflammatory mediators including tumour necrosis factor-α (TNF-α), interleukin (IL)-1β and -6 [2,15,16]. Through this process, the ischaemic cascade does not merely precede inflammation but actively programs the magnitude and quality of the microglial response,

defining the initial inflammatory microenvironment of the ischaemic brain. Microglia-derived cytokines and chemokines subsequently activate endothelial cells, alter tight junction integrity, and upregulate adhesion molecules, facilitating leukocyte infiltration across the BBB [2,17]. By the time patients present clinically, microglial activation is often already established, narrowing the window for effective therapeutic intervention [18,19].

2.2. Intracellular Metabolic Reprogramming and Epigenetic Regulation

Beyond surface receptor signalling, emerging evidence indicates that microglial responses to DAMPS are further shaped by intracellular metabolic reprogramming and epigenetic regulation.

2.2.1. Intracellular Metabolic Reprogramming

Activation of glycolytic pathways, often referred to as 'Warburg-like' metabolic shift in innate immune cells, supports the rapid production of pro-inflammatory mediators during the acute phase of ischaemic injury [20–22]. In addition to glycolytic reprogramming, mitochondrial dysfunction and shifts in oxidative phosphorylation play a key role in regulating microglial activation states across different phases of injury and repair [22,23]. Lipid metabolism is also critical during the recovery phase, supporting phagocytic clearance of cellular debris and facilitating tissue remodelling [21,24].

A central but under-integrated driver of this immunometabolic reprogramming is hypoxia itself. Stabilisation of hypoxia-inducible factors (HIF-1α and HIF-2α) within microglia and other neurovascular cells directly reshapes inflammatory signalling following ischaemia. In the acute phase, HIF-1α promotes glycolytic metabolism and amplifies pro-inflammatory gene expression, including interleukin-1β and TNF-α, thereby reinforcing injury-amplifying microglial states [25,26]. Concurrently, hypoxia alters microglial–endothelial crosstalk, contributing to BBB dysfunction and leukocyte recruitment [8,12]. However, as oxygenation and tissue conditions evolve, HIF-dependent signalling can also support angiogenesis, metabolic adaptation, and aspects of tissue repair, reflecting a context-dependent dual role [27,28]. This positions hypoxia not merely as an upstream trigger of injury, but as a dynamic regulator of microglial state transitions across phases of stroke. This dual role of HIF signalling maps directly onto the transition from injury-amplifying to reparative microglial states. Importantly, the temporal and cell-type-specific effects of HIF signalling suggest that therapeutic strategies targeting hypoxia pathways must be precisely aligned with the evolving inflammatory landscape to avoid exacerbating early injury or impairing later recovery.

2.2.2. Epigenetic Regulation

In parallel, chromatin remodelling and histone modifications regulate the transcriptional accessibility of inflammatory genes, enabling sustained or amplified cytokine production in response to persistent danger signals [29,30]. These observations suggest that microglial activation is not solely determined by receptor–ligand interactions but is also governed by integrated metabolic and epigenetic states that modulate the magnitude and duration of the inflammatory response. However, much of the current evidence derives from preclinical models, and the extent to which these mechanisms translate to human stroke remains incompletely defined. Importantly, such regulatory layers may represent additional therapeutic targets, as modulation of microglial metabolism or epigenetic programming could attenuate injurious inflammation while preserving essential immune functions [31].

2.3. Recruitment of Peripheral Immune Cells

While microglia represent the principal resident immune cells of the CNS, the post-ischaemic inflammatory response is further shaped by the recruitment of peripheral immune cells from the systemic circulation. Monocytes and macrophages are among the first to infiltrate the injured tissue, where they contribute to the amplification and perpetuation of neuroinflammation [32,33]. Once within the CNS parenchyma, these infiltrating cells interact dynamically with resident microglia, collectively modulating the magnitude, trajectory, and duration of the post-stroke immune response.

3. Microglial Functional States in Post-Ischaemic Injury and Repair

3.1. Injury-Amplifying Microglial Programmes in the Acute Phase

Within the first 24–48 h following cerebral ischaemia, microglia predominantly adopt pro-inflammatory functional states characterised by the heightened expression of inflammatory cytokines, reactive oxygen and nitrogen species, and proteolytic enzymes [34,35]. Microglial-derived mediators disrupt endothelial tight-junction proteins—including claudin-5 and occludin—increasing BBB permeability and driving vasogenic oedema [2,4]. Through interactions with endothelial cells and platelets, microglia further contribute to microvascular dysfunction, limiting tissue-level reperfusion despite successful upstream recanalisation [8].

Beyond their vascular effects, activated microglia drive neuronal injury in the ischaemic penumbra through several convergent and mutually reinforcing mechanisms. Phenotypic activation is accompanied by upregulation of NADPH oxidase (NOX2) and inducible nitric oxide synthase (iNOS), resulting in sustained overproduction of reactive oxygen and nitrogen species [36,37]. The ensuing oxidative and nitrosative burden overwhelms endogenous antioxidant defences of penumbral neurons—including superoxide dismutase, catalase, and the glutathione system—precipitating widespread lipid peroxidation, protein carbonylation, and oxidative DNA damage that collectively compromise neuronal structural and functional integrity [38,39].

Activated microglia concurrently engage apoptotic signalling cascades in vulnerable penumbral neurons through both contact-dependent and paracrine mechanisms. Pro-inflammatory cytokines—principally TNF-α, IL-1β, and IL-6—activate neuronal death receptors, including TNFR1, triggering caspase-8 activation and initiating the extrinsic apoptotic pathway [40]. Microglial-derived glutamate, released via the system Xc^- cystine-glutamate transporter and connexin hemichannels, further exacerbates excitotoxic calcium influx through NMDA receptors, culminating in mitochondrial membrane depolarisation, cytochrome c release, and activation of the intrinsic apoptotic pathway via caspase-9 and caspase-3 [41].

Critically, these processes are not independent; they form interconnected, self-reinforcing loops that collectively accelerate penumbral deterioration. Microglial-derived reactive oxygen species potentiate NF-κB signalling within microglia themselves, sustaining cytokine production and perpetuating oxidative injury in a feed-forward manner [42]. The net effect is the progressive conversion of metabolically stressed but potentially salvageable penumbral neurons into cells committed to apoptotic or necroptotic death—a process that critically narrows the therapeutic window and drives expansion of the evolving infarct core [43].

3.2. Reparative and Pro-Resolution Microglial Functions

As the post-ischaemic environment evolves, microglia undergo functional reprogramming toward anti-inflammatory and reparative states. These later programmes are characterised by reduced production of pro-inflammatory cytokines and increased expression of

mediators associated with the resolution of inflammation, including IL-10 and transforming growth factor-β [3,9]. Microglia in this phase play a critical role in phagocytosing apoptotic neurons, synaptic remnants, and myelin debris, a prerequisite for restoration of tissue homeostasis [35,44].

Beyond debris clearance, reparative microglia secrete growth factors such as vascular endothelial growth factor and insulin-like growth factor-1, which support angiogenesis, neurogenesis, and synaptic remodelling in peri-infarct regions [3,35]. Microglial interactions with astrocytes and neurons further shape the re-establishment of functional neural networks, highlighting the importance of microglia-driven crosstalk in long-term recovery [7]. These reparative functions are essential for restoring tissue homeostasis and promoting functional recovery following stroke [3].

3.3. Microglial Plasticity Beyond Binary Classifications

Although the M1/M2 framework has been widely used to describe microglial activation states, it represents a considerable oversimplification of a highly complex and continuous functional spectrum [10,45]. Advances in single-cell RNA sequencing and spatial transcriptomics have demonstrated that post-ischaemic microglia occupy multiple transcriptionally distinct states that evolve dynamically over time and across brain regions. Rather than transitioning between two discrete phenotypes, microglia exhibit graded shifts in gene-expression programmes encompassing inflammation, metabolism, phagocytosis, lipid handling, and synaptic remodelling [3,46,47]. The dynamic nature of this reprogramming is summarised schematically in Figure 1.

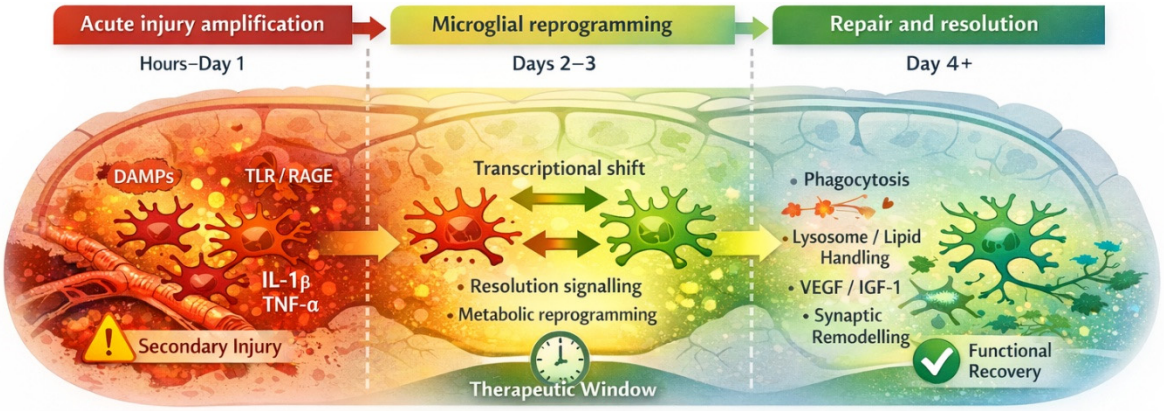


Figure 1. Temporal microglial state transitions after ischaemic stroke. Schematic overview of the dynamic reprogramming of microglia following cerebral ischaemia. In the acute phase (hours–day 1), microglia rapidly sense tissue injury through damage-associated molecular patterns (DAMPs) and pattern-recognition receptors, adopting injury-amplifying transcriptional programmes characterised by pro-inflammatory cytokine release, oxidative stress, and blood–brain barrier disruption, contributing to secondary neuronal injury. During the transition phase (days 2–3), microglial programmes undergo transcriptional and metabolic reorganisation, with partial attenuation of pro-inflammatory signalling and emergence of resolution-associated pathways, defining a narrow therapeutic window for immune modulation. In the recovery phase (day 4 onwards), reparative microglial states predominate, marked by enhanced phagocytosis, lysosomal and lipid-handling pathways, growth factor secretion, and support of angiogenesis, synaptic remodelling, and neuroplasticity. The timing and balance of these microglial states underpin the temporal paradox of post-ischaemic neuroinflammation.

In addition to temporal transcriptional heterogeneity, recent work suggests that microglial functional states are also shaped by spatial context within the ischaemic brain. Microglia located in the infarct core and more distal areas exhibit distinct activation profiles, reflecting differences in metabolic stress, cellular debris burden, and local signalling

environments [48,49]. For example, core-associated microglia are enriched for gene expression signatures linked to phagocytosis, lipid metabolism, and lysosomal activity, whereas microglia in peri-infarct regions display profiles more closely associated with synaptic remodelling and neuroplasticity. This spatial compartmentalisation further complicates attempts to define discrete microglial phenotypes, as functional states are not only time-dependent but also region-specific [50,51].

Single-cell studies in experimental stroke models have identified early microglial states enriched for interferon signalling, inflammasome components, and antigen presentation pathways, consistent with injury-amplifying functions [46,48]. As the post-ischaemic environment evolves, these states give way to transcriptional programmes associated with phagocytosis, lysosomal activity, cholesterol efflux, and growth factor production. These reparative microglial states partially overlap with disease-associated microglial signatures described in other neurological conditions, suggesting conserved mechanisms of adaptation to tissue injury [47,48].

Key marker profiles characterise each of these functional states. Homeostatic microglia are defined by expression of *P2RY12*, *TMEM119*, and *CX3CR1* [52,53], while acute injury-associated programmes are marked by *IL1B*, *TNF*, *CCL2*, *CXCL10*, *CD68*, and *NOS2* [54]. Lipid-handling and disease-associated states are characterised by *APOE*, *TREM2*, *LPL*, *CST7*, *AXL*, and *MERTK* [46]; antigen-presenting and interferon-responsive states by *HLA-DRA*, *CD74*, and interferon-stimulated genes [55]; and repair-associated programmes by *IGF1*, *VEGFA*, *SPP1*, and complement-related genes [56,57]. Critically, these profiles do not represent fixed cellular identities but dynamic, spatially heterogeneous programmes shaped by lesion stage, anatomical compartment, ageing, comorbidity, and interaction with infiltrating myeloid cells.

Taken together, these findings establish that post-ischaemic microglial identity cannot be adequately captured by M1/M2 classification. Landmark transcriptomic studies collectively support this conclusion: Masuda et al. [48] identified spatially distinct microglial clusters in the infarct core versus peri-infarct regions; Hammond et al. [49] demonstrated context-dependent transcriptional heterogeneity that exceeds the M1/M2 dichotomy; and Sankowski et al. [50] and Tan et al. [51] characterised a broad spectrum of human microglial states in neuropathological tissue. Collectively, these studies establish that microglial functional identity is defined by multi-dimensional transcriptional programmes—a view consistent with the coupled network framework advanced in this thesis.

4. Translational Failure of Microglial-Targeted Therapies

4.1. Preclinical Success Under Controlled Conditions

In experimental models of ischaemic stroke, pharmacological modulation of microglial activation frequently reduces infarct volume and improves functional outcomes [4,11]. Agents targeting pro-inflammatory cytokines, inhibiting microglial activation, or broadly modulating immune responses have shown consistent benefit when administered at defined time points relative to stroke onset [7,58]. These studies typically involve young, healthy animals with precisely controlled lesion timing, enabling alignment of therapy with specific microglial activation states [58].

4.2. Clinical Inconsistency and Mistimed Targeting

In contrast, targeting post-stroke inflammation has largely failed to produce meaningful benefit in stroke patients, reflecting a fundamental mismatch between therapeutic intervention and the evolving microglial response [4,59]. Broad, non-selective anti-inflammatory strategies applied across all phases may attenuate early inflammation but disrupt the emergence of reparative microglial programmes, ultimately impairing recovery (Figure 2).

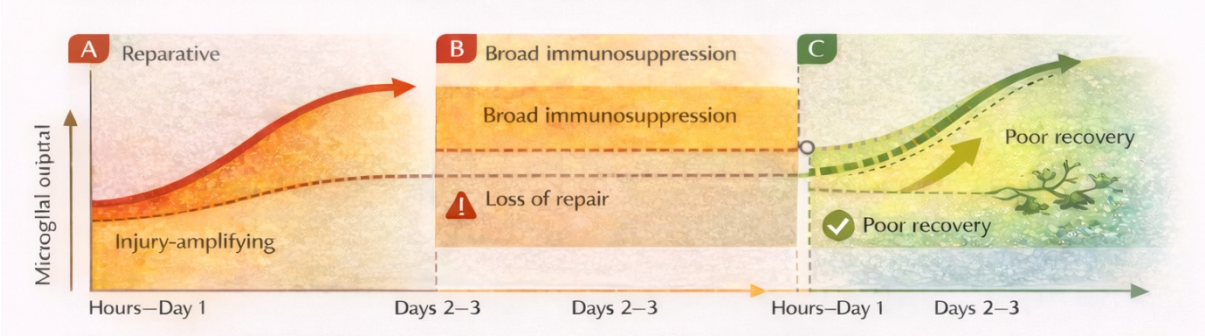


Figure 2. Therapeutic misalignment with microglial temporal dynamics after ischaemic stroke. Conceptual framework illustrating how therapeutic efficacy depends on alignment with microglial functional state over time. (**A**) Under post-ischaemic conditions, microglial output evolves from early pro-inflammatory, injury-amplifying programmes toward later reparative and pro-resolution states. (**B**) Broad, non-selective anti-inflammatory therapy applied across all phases may attenuate early inflammation but disrupts the emergence of reparative microglial programmes, resulting in impaired recovery. (**C**) In contrast, phase-aligned modulation targeted to the transitional period biases microglial reprogramming toward reparative states while preserving essential immune functions, supporting tissue repair and functional recovery.

In human patients, stroke onset is often uncertain, treatment initiation is delayed, and patient populations are highly heterogeneous. As a result, therapies are frequently administered without regard to the prevailing microglial phenotype, leading to mistimed or inappropriate modulation of microglial activity.

Several clinical trials illustrate the consequences of non-selective and temporally misaligned immunomodulation. For example, the Enlimomab trial, which targeted intercellular adhesion molecule-1, not only failed to improve outcomes but was associated with worsened neurological recovery, likely reflecting an exaggerated inflammatory response and inappropriate targeting of early immune mechanisms [60]. Similarly, trials of IL-1 receptor antagonism demonstrated biological activity but failed to produce consistent clinical benefit, in part due to variability in treatment timing and patient heterogeneity [61]. Minocycline, a broad-spectrum anti-inflammatory agent, has shown promise in preclinical models but yielded mixed results in clinical studies, further highlighting the challenges of translating non-specific immunosuppression into effective therapy [62,63].

4.3. From Bench to Bedside: The Translational Gap in Microglial-Targeted Therapies

A key limitation of many tested therapies is their lack of specificity. Broad suppression of microglial activation fails to distinguish between injury-amplifying and reparative programmes; while early intervention may attenuate cytokine production, it risks impairing the phagocytic clearance and trophic support essential for later recovery [3,35]. This indiscriminate targeting offers a compelling explanation for the translational failure of anti-inflammatory strategies in human stroke—a conclusion reinforced by experimental evidence demonstrating that microglial depletion exacerbates injury and impairs functional recovery [35,64]. Recent advances in single-cell transcriptomics have further established that post-ischaemic microglial responses are highly heterogeneous, spatially compartmentalised, and temporally dynamic [48,49]. Collectively, these findings underscore the complexity of the post-ischaemic immune environment and highlight the need for therapeutic strategies sensitive to both the temporal evolution and spatial diversity of microglial states.

Additional factors contributing to translational failure include limited drug penetration across the BBB, suboptimal target engagement, and the intrinsic heterogeneity of stroke pathology—encompassing variability in infarct size, location, and systemic immune state [8,59]. Moreover, it remains debated whether post-ischaemic inflammation

is a primary driver of injury or principally a downstream response to tissue damage [7], raising fundamental questions about the causal leverage of anti-inflammatory interventions. Critically, however, these limitations do not diminish the importance of temporal dynamics; rather, they interact with them. Even agents with adequate pharmacokinetic profiles may fail if administered during an inappropriate phase of the immune response—whether microglial activity is predominantly injurious or essential for recovery. Temporal misalignment should therefore be understood not as an isolated mechanism of failure, but as a central organising constraint that conditions the effectiveness of all other therapeutic variables. Improving translational success will require the integration of this temporal dimension with considerations of drug delivery, target specificity, and patient heterogeneity.

A further limitation of current translational approaches is the reliance on simplified experimental models that fail to capture the complexity of human stroke (Table 1). Most preclinical studies are conducted in young, otherwise healthy animals, whereas clinical populations are typically older and characterised by comorbidities—including hypertension, diabetes, and systemic inflammation—all of which influence microglial priming and immune responsiveness [58,59]. Ageing is associated with a primed microglial phenotype defined by heightened inflammatory responsiveness and diminished capacity for resolution [65,66], a baseline alteration that may contribute to exaggerated post-ischaemic inflammation and poorer functional recovery in older patients [67]. Furthermore, experimental models involve tightly controlled onset times and relatively homogeneous lesions, permitting precise therapeutic timing that is rarely achievable in clinical practice. These structural differences affect both the magnitude and temporal dynamics of the inflammatory response, compounding the difficulty of translating therapeutic strategies developed under idealised conditions. Failure to account for patient heterogeneity and baseline immune state therefore represents an important and underappreciated source of variability in the efficacy of immunomodulatory therapies observed across clinical trials [58,59].

Table 1. Major translational differences between preclinical stroke models and human stroke, and their implications for therapeutic development.

Factor	Preclinical Models	Clinical Stroke	Implication for Translation
Age	Typically young, otherwise healthy animals	Predominantly Older populations [66–68]	Age-related immune changes influence microglial responses and therapeutic efficacy
Comorbidities	Rarely included (e.g., absence of hypertension, diabetes)	Common (e.g., hypertension, diabetes, atherosclerosis) [65,66]	Comorbidities modify inflammatory pathways and treatment responses
Stroke onset and timing	Precisely controlled induction and treatment timing	Variable and often delayed clinical presentation [58,66]	Misalignment of therapeutic windows reduces clinical effectiveness
Lesion characteristics	Relatively homogeneous and reproducible infarcts	Heterogeneous in size, location, and severity [59]	Increased variability complicates treatment standardisation and outcome prediction
Immune state	Controlled and uniform	Highly variable between individuals [8]	Immune heterogeneity affects microglial activation and outcomes
Treatment timing	Defined dosing and timing in controlled settings	Variable adherence, delayed intervention, comorbid influence [58,66]	Reduced reproducibility and efficacy in real-world settings
Sex differences	Often male-only animal models	Both sexes, with historical under-representation of females in preclinical models and additional hormonal variability [69]	Limits generalisability and may affect therapeutic response

Taken together, these observations suggest that translational failure in stroke immunotherapy reflects the convergence of three recurring limitations: first, temporal misalignment between intervention and immune phase; second, non-specific targeting of functionally heterogeneous microglial states; and third, inadequate accounting for patient-level variation in immune response and disease trajectory. Addressing these limitations

necessitates a shift away from indiscriminate immunosuppression toward strategies that selectively modulate microglial function in a temporally and mechanistically informed manner.

5. Future Directions: Microglia-Centric Therapeutic Strategies

Future therapeutic strategies in ischaemic stroke should shift from broad immunosuppression toward precision immunomodulation, in which microglial activity is selectively reprogrammed to favour resolution and repair. The dynamic and heterogeneous nature of post-ischaemic microglial responses indicates that therapeutic benefit is more likely to arise from reshaping cellular state than from indiscriminate inhibition—a conclusion supported by the observation that broadly immunosuppressive approaches have consistently shown limited clinical efficacy despite promising preclinical findings.

Microglial behaviour is governed by a coupled regulatory network integrating proteostatic clearance, receptor-mediated signalling, inflammasome activation, and intracellular metabolism (Figure 3). These processes form an interdependent system that determines whether microglia sustain injury-amplifying inflammation or transition toward resolution. Impaired clearance of cellular debris and protein aggregates prolongs exposure to DAMPs, reinforcing pattern-recognition receptor signalling and downstream inflammasome activation [46,70], and stabilising a pro-inflammatory metabolic programme characterised by glycolytic bias and mitochondrial dysfunction [71,72]. Conversely, restoration of phagocytic and lysosomal function reduces inflammatory ligand burden, attenuates inflammasome activity, and permits a shift toward oxidative metabolism that supports reparative functions [73,74]. Receptor-mediated pathways—including TREM2-associated signalling—act as critical upstream regulators linking extracellular cues to intracellular metabolic and transcriptional states [71,72,75–77]. Within this framework, microglial phenotype emerges from the dynamic coupling of these processes rather than from discrete pathway activation, implying that therapeutic reprogramming requires coordinated network modulation rather than isolated pathway inhibition [78,79].

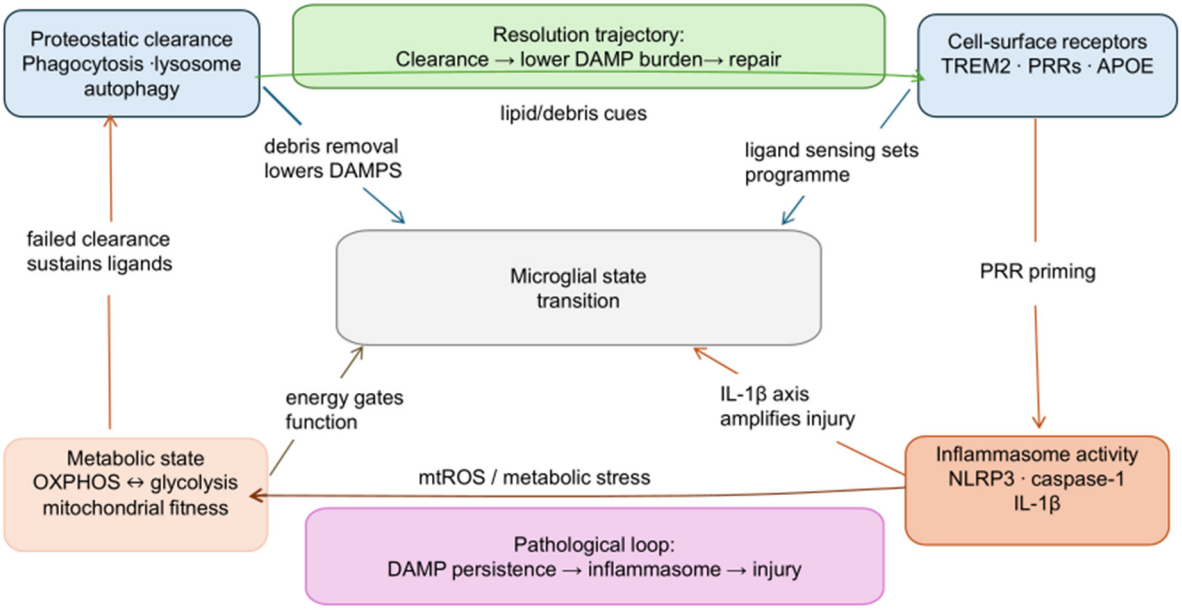


Figure 3. Microglial state transitions following ischaemic stroke are governed by a coupled regulatory network integrating proteostatic clearance, receptor-mediated signalling, inflammasome activation, and metabolic state. Impaired clearance of cellular debris sustains DAMP signalling and pattern-recognition receptor activation [46], which in turn drives pro-inflammatory metabolic reprogramming

through TREM2-associated pathways [71,72]. Mitochondrial dysfunction and glycolytic bias promote NLRP3 inflammasome activation [70], reinforcing inflammatory signalling [73]) and further impairing proteostatic capacity. Lipid accumulation and lysosomal stress provide an additional coupling between clearance and metabolic dysfunction [74,75]. Within this framework, microglial phenotype emerges from the dynamic equilibrium of these interconnected processes rather than discrete pathways. The transitional phase following stroke represents a point of maximal network plasticity, at which coordinated therapeutic modulation may redirect system dynamics toward resolution and repair.

A key priority is the identification of interventions capable of shifting microglial activation toward pro-resolution and reparative phenotypes. Emerging evidence indicates that such reprogramming is achievable through modulation of cellular metabolism and epigenetic regulation. In the acute inflammatory state, microglia exhibit increased reliance on glycolysis to sustain rapid production of pro-inflammatory mediators; therapeutic strategies that promote a shift toward oxidative phosphorylation may attenuate injurious signalling while preserving essential cellular functions. In parallel, targeting chromatin remodelling and histone modification pathways offers a mechanism for durably reshaping the transcriptional programmes governing inflammatory and reparative responses [21,22,31].

Beyond metabolic and epigenetic reprogramming, interventions that enhance phagocytic capacity, lipid handling, and trophic signalling represent an important complementary therapeutic axis. Microglia play a central role in clearing cellular debris and orchestrating tissue remodelling following injury—processes essential for restoring homeostasis and supporting functional recovery [3,35,44]. Selective amplification of these functions may improve outcomes without disrupting the broader immune response.

Microglial responses are, moreover, embedded within a wider neuroimmune network involving astrocytes, endothelial cells, and peripheral immune populations. Bidirectional communication between the CNS and peripheral organs—including the spleen and gut—further shapes post-ischaemic inflammation [80]. Future therapeutic strategies should therefore situate microglial modulation within this integrated system rather than treating microglia as an isolated cellular target.

Recent work by Tsuyama et al. [81] provides direct experimental support for the concept that microglial state transitions can be actively reprogrammed through targeted molecular intervention. Using a ZFP384 antisense oligonucleotide approach, the authors demonstrated sustained reparative microglial gene expression associated with improved post-ischaemic recovery—a mechanistic example of how selective phenotypic regulation may shift the balance between injurious and reparative programmes. These findings reinforce the view that temporally aligned modulation of interconnected microglial networks represents a more promising therapeutic strategy than broad suppression of neuroinflammatory signalling.

6. Clinical Translation Framework for Temporal Precision

This network framework provides a mechanistic basis for the existence of a temporal therapeutic window. The post-acute transition phase likely represents a period of maximal network plasticity during which coordinated modulation can redirect microglial dynamics toward resolution rather than persistent injury amplification. Therapeutic efficacy is therefore likely to depend on time-dependent reconfiguration of network state rather than on the inhibition of individual pathways. Pharmacological strategies currently under investigation include IL-1 receptor antagonists, NLRP3 inflammasome inhibitors, CSF1R modulators, and TREM2-targeting approaches (Table 2), each of which may support phase-specific modulation of microglial activation following stroke.

Table 2. Phase-specific mechanistic pathways underlying microglial reprogramming: biomarkers and therapeutic targets in ischaemic stroke.

Pathway	Key Mediators/Biomarkers	Therapeutic Strategy	Representative Agents	Phase of Action	Key References
Glycolytic-to-oxidative shift	HIF-1α, LDHA, SUCNR1	Promote oxidative phosphorylation	Itaconate analogues, dimethyl fumarate	Acute → subacute	[28,71,82,83]
Epigenetic reprogramming	H3K27ac, HDAC1/2, BRD4	HDAC inhibition; bromodomain targeting	Valproic acid, JQ1	Subacute → chronic	[21,22,31]
NLRP3 inflammasome	NLRP3, caspase-1, IL-1β	Inflammasome inhibition	MCC950, OLT1177	Acute	[70,73]
TLR/NF-κB signalling	TLR2/4, NF-κB, HMGB1	TLR antagonism; NF-κB inhibition	TAK-242, minocycline	Acute	[84,85]
TREM2–APOE axis	TREM2, DAP12, APOE, LPL	TREM2 agonism	AL002c, anti-TREM2 antibodies	Subacute → chronic	[72,75,76]
CSF1R signalling	CSF1R, IL-34, CSF1	CSF1R modulation	PLX5622, PLX3397	Acute/subacute	[86–88]
Phagocytosis/ efferocytosis	MerTK, AXL, LAMP1	Efferocytosis enhancement	GAS6 analogues	Subacute → chronic	[44,74,89]
IL-1 receptor signalling	IL-1β, IL-1RA, IL-1R1	IL-1 receptor antagonism	Anakinra	Acute	[7,40]
Chromatin remodelling	HDAC3, SIRT1, EZH2	Epigenetic editing	GSK-J4, UNC0638	Subacute → chronic	[21,22]

Table note: Phase of action refers to the post-ischaemic temporal window during which each pathway is most therapeutically relevant. Acute = 0–48 h; subacute = 2–7 days; chronic = beyond 7 days. Representative agents include both clinically evaluated and preclinical compounds. References reflect mechanistic evidence for pathway involvement; agent-specific trial data are discussed in the main text.

Interventions delivered during this transitional period have the potential to attenuate injurious microglial signalling while preserving or enhancing reparative functions [7]. By contrast, non-selective suppression of microglial activity administered too early may disrupt signals necessary for later recovery, whereas interventions delivered too late may impair beneficial microglial processes without meaningfully reducing initial injury [3,4]. Importantly, the timing of this window varies between individuals according to factors such as age, comorbidities, infarct size, and reperfusion status—contributing to the heterogeneity in outcomes observed across clinical trials [12,59].

The central implication of this framework is that therapeutic efficacy depends on alignment between intervention and the evolving biological state of the post-ischaemic immune response. Translating this principle into clinical practice requires moving beyond fixed, time-based treatment paradigms toward approaches that account for temporal and inter-individual variability in microglial activation.

6.1. Phase-Specific Therapeutic Windows

Microglial responses to ischaemic stroke can be conceptualised across three overlapping phases: an early injury-amplifying phase (0–48 h), a transitional reprogramming phase (approximately 48–72 h), and a later reparative phase (beyond 72 h) [3,35,48,90]. These phases reflect progressive shifts in transcriptional and metabolic activity rather than discrete temporal boundaries, and are shaped by reperfusion status, infarct size, and baseline immune state.

During the early phase, excessive pro-inflammatory microglial signalling contributes to secondary neuronal injury and BBB disruption [3,35]. Therapeutic strategies in this period should therefore prioritise selective attenuation of injurious pathways—avoiding global immunosuppression that risks impairing downstream recovery processes. The transitional phase represents a critical window of heightened microglial plasticity, during which active reprogramming toward pro-resolution states is most achievable. In the later reparative phase, interventions should focus on supporting debris clearance, synaptic remodelling, and trophic signalling rather than attenuating inflammation that has already begun to resolve [3,35].

Emerging therapeutic strategies increasingly aim to selectively modulate microglial function rather than broadly suppress neuroinflammation. TREM2-targeted approaches

may enhance phagocytic activity and promote resolution-associated microglial phenotypes, whilst inhibition of NLRP3 inflammasome signalling may reduce excessive pro-inflammatory cytokine production and secondary tissue injury [71,91]. Additional strategies targeting microglial metabolism, mitochondrial function, and lysosomal activity are also under investigation as means of supporting reparative microglial reprogramming following ischaemic stroke [74]. Collectively, these approaches reinforce the principle that effective immunomodulatory therapy will require temporally aligned and pathway-specific regulation of microglial states.

6.2. Biomarker-Guided Stratification

A major barrier to implementing phase-specific therapy is the inability to directly measure microglial state in vivo. Current approaches provide partial but incomplete insight. TSPO-PET imaging enables whole-brain assessment of neuroinflammation but lacks the specificity required to distinguish discrete microglial activation states [92]. Blood-based markers—including cytokine profiles and soluble TREM2—are more clinically accessible but are substantially influenced by systemic immune activity, limiting their specificity for CNS microglial dynamics [93].

A pragmatic approach may involve multi-modal biomarker integration, combining systemic, neuroimaging, and emerging transcriptomic signals to approximate the dominant underlying immune state (Table 2). Such strategies could enable patient stratification by inflammatory phase, allowing treatment to be aligned with biological processes rather than relying solely on time from symptom onset.

6.3. Therapeutic Implementation and Patient Heterogeneity

The effectiveness of phase-aligned interventions is likely to vary according to patient-specific factors, including age, comorbidities, and baseline immune priming, all of which influence both the magnitude and temporal trajectory of microglial responses. This biological heterogeneity contributes to variability in treatment response and represents a fundamental challenge for clinical implementation. Therapeutic strategies must therefore be designed to accommodate, rather than assume uniformity across, the range of post-ischaemic immune trajectories encountered in clinical populations.

6.4. Interaction with Reperfusion Therapies

Microglial dynamics are closely linked to vascular events, particularly the timing and completeness of reperfusion [94]. Successful recanalisation may accelerate the transition from injury-amplifying to reparative microglial states, whereas delayed or incomplete reperfusion is likely to prolong pro-inflammatory activity and compress the transitional window. The timing of immunomodulatory intervention must therefore be interpreted in the context of reperfusion status, with therapeutic strategies designed to complement rather than interfere with established vascular interventions.

6.5. Testable Predictions and Future Validation

This phase-specific framework generates a set of mechanistically grounded, testable predictions. Interventions targeting the transitional reprogramming phase are predicted to yield greater functional benefit than early, non-selective suppression of neuroinflammation. Patients with delayed presentation or heightened baseline inflammatory priming may derive disproportionate benefit from phase-specific modulation strategies. Biomarker-guided stratification (Table 2) is predicted to improve trial efficiency by selecting patients whose dominant immune phase aligns with the mechanism of the intervention under investigation.

Prospective validation will require integrated clinical studies combining longitudinal biomarker profiling with targeted intervention strategies, designed to determine whether

aligning therapy with microglial temporal dynamics can overcome the longstanding translational barrier in stroke immunotherapy.

7. Conclusions

Microglial responses to ischaemic stroke are not governed by isolated pathways but by a coupled regulatory network integrating proteostatic clearance, receptor-mediated signalling, inflammasome activation, and metabolic state. Failure to account for this network architecture offers a unifying explanation for the limited clinical success of immunomodulatory therapies, which have typically targeted individual components without reconfiguring the system as a whole.

Within this framework, microglia act as central regulators of the post-ischaemic neuroinflammatory response, linking the initial ischaemic insult to both secondary injury and long-term recovery. Their function is inherently dynamic: early activation amplifies neuronal damage through pro-inflammatory signalling and vascular dysfunction, whereas later programmes are required for debris clearance, tissue remodelling, and restoration of neural circuit function. This functional duality creates a temporal dependency in which the same biological processes exert opposing effects depending on the prevailing network state.

Therapeutic failure, we propose, arises from a fundamental mismatch between intervention and this evolving system. Non-selective suppression of microglial activity disregards functional heterogeneity and risks impairing essential reparative processes, whilst imprecise timing—compounded by patient heterogeneity and uncertain stroke onset—limits efficacy even when targets are biologically valid. Mechanistically, impaired clearance sustains exposure to DAMPs, perpetuating inflammasome activation and a pro-inflammatory metabolic programme; conversely, restoration of clearance capacity shifts network equilibrium toward resolution and repair. Microglial phenotype therefore emerges from dynamic changes in network state rather than progression through discrete activation stages.

Therapeutic efficacy is thus likely to depend on coordinated, time-dependent reconfiguration of this network—particularly during the transitional phase of maximal plasticity in the days following stroke. Future strategies must move beyond indiscriminate immunosuppression toward phase-specific, microglia-centric modulation encompassing early containment of excessive inflammatory signalling, targeted reprogramming of network state during the transitional window, and active support of reparative functions during recovery. Clinical translation will require biomarkers capable of resolving microglial network states *in vivo* to guide both intervention timing and patient stratification.

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