Secondary mechanisms of injury and viable pathophysiological targets in intracerebral hemorrhage

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Abstract: Intracerebral hemorrhage (ICH) can be divided into a primary and secondary phase. In the primary phase, hematoma volume is evaluated and therapies are focused on reducing hematoma expansion. In the secondary, neuroprotective phase, complex systemic inflammatory cascades, direct cellular toxicity, and blood-brain barrier disruption can result in worsening perihematoma edema that can adversely affect functional outcome. To date, all major randomized phase 3 trials for ICH have targeted primary phase hematoma volume and incorporated clot evacuation, intensive blood pressure control, and hemostasis. Reasons for this lack of clinical efficacy in the major ICH trials may be due to the lack of therapeutics involving mitigation of secondary injury and inflexible trial design that favors unilateral mechanisms in a complex pathophysiology. Potential pathophysiological targets for attenuating secondary injury are highlighted in this review and include therapies increasing calcium, antagonizing microglial activation, maintaining macrophage M1 versus M2 balance by decreasing M1 signaling, aquaporin inhibition, NKCC1 inhibition, endothelin receptor inhibition, Sur1-TRPM4 inhibition, matrix metalloproteinase inhibition, and sphingosine-1-phosphate receptor modulation. Future clinical trials in ICH focusing on secondary phase injury and, potentially implementing adaptive trial design approaches with multifocal targets, may improve insight into these mechanisms and provide potential therapies that may improve survival and functional outcome.

Keywords: intracerebral hemorrhage, neuroprotection, perihematoma edema, secondary injury, vasogenic edema

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Introduction

Spontaneous intracerebral hemorrhage (ICH) is one of the most common causes of death and disability in the USA, with over 795,000 new strokes every year of which 10% are ICH (incidence of 24.6 per 100,000 person-years). However, while the incidence remains steady, ICH boasts the highest mortality with rates ranging between 53% and 59% and the highest comorbidities in patients 50–80 years old.

The primary phase in ICH management, calculating initial hematoma volume and rate of expansion, has been aggressively targeted in large multicenter trials over the last 15 years (Table 1). Despite the number of major clinical trials, all of the novel therapeutic strategies targeting primary mechanisms from these clinical trials have been unsuccessful in demonstrating improved functional outcome, suggesting that secondary mechanisms of injury after ICH may represent a complementary and essential pathophysiological target.

In this review, we summarize pathophysiological mechanisms critical in wound repair after ICH and current evidence from important clinical trials that suggest viable therapeutic targets for attenuating secondary injury in patients with ICH.
Table 1. Summary of clinical trials targeting primary phase hematoma volume and expansion.

| Study | Time of intervention (hours) | Patient number | Intervention | Primary Outcome | Secondary Outcome |
|-------|-----------------------------|----------------|--------------|-----------------|-------------------|
| Hematoma evacuation | Phase III | N = 601 | Surgery for hematoma evacuation | Death or disability as per dichotomized modified Rankin scale | No improvement in death or disability at day 90 |
| STICH 2 | < 48 h of ictus | N = 506 | Image-guided, catheter-based removal of hematoma < 13 doses at 1.0 mg via catheter delivery | Functional outcome as per dichotomized modified Rankin scale at 180 days | No improvement in functional outcome at day 90 |
| MISTIE 3 | < 72 h of ictus | N = 500 | Image-guided catheter-based removal of hematoma < 13 doses at 1.0 mg via catheter delivery | Functional outcome as per dichotomized modified Rankin scale at 180 days | No improvement in functional outcome at day 90 |
| CLEAR 3 | < 72 h of ictus | N = 2794 | Administration of alteplase via intraventricular catheter, < 13 doses at 1.0 mg via external ventricular drain | Functional outcome as per dichotomized modified Rankin scale at day 90 | No difference in functional outcome at day 90 |
| INTERACT-2 | < 4 h of ictus | N = 841 | Factor VIIa 20 or 80 mcg/kg | Death or severe disability as per dichotomized modified Rankin score | No difference in functional outcome at day 90 |
| PATCH | < 6 h of ictus | N = 190 | Platelet transfusion for patients on antiplatelet therapy | Death or dependence as measured by modified Rankin score and ordinal analysis at day 90 | No improvement in death or dependence at day 90 |

ATACH-2, Antihypertensive Treatment of Acute Cerebral Hemorrhage Study; CLEAR-3, The Clot Lysis: Evaluating accelerated resolution in Intracerebral Hemorrhage Trial; MISTIE, Minimally invasive surgery plus alteplase in Intracerebral Haemorrhage Evacuation; PATCH, Platelet transfusion versus standard care after acute stroke due to spontaneous cerebral haemorrhage; STICH2, Surgical treatment for Intracerebral Hemorrhage.
also propose a new paradigm to establish novel strategies and targets for future ICH therapy clinical trials that can specifically target secondary mechanisms of injury. The lack of efficacy in therapies targeting primary mechanisms in ICH, emphasizes the need for better prospective clinical trials that both target and provide quick therapies to mitigate secondary mechanisms of injury.

Secondary mechanisms of ICH triggering systemic inflammatory responses

ICH is characterized by a massive cascade of pathophysiologic events amplified by the intrinsic attempts at hemostasis and physiological wound repair to blood-brain barrier (BBB) disruption at the injury site. The pathophysiology of ICH has been divided into two main phases. The primary phase develops within the first 6 h of hemorrhage and is characterized by hematoma formation, mass effect, and volume expansion that leads to increased intracranial pressure (ICP) (Figure 1). Beyond being a single event, hematoma formation and expansion appear to be accompanied by complex inflammatory and vascular processes (Figure 1) characterized by an “avalanche model” where ruptured vessels at the hematoma periphery propagate mechanical shearing of neighboring vessels and lead to hematoma expansion via both microbleeds and large vessel hemorrhage.

Hematoma expansion then transitions into the secondary peri-hematoma edema (PHE) phase where brain tissue reacts to acute hematoma development, and local extravascular blood products and peripheral expansion trigger an inflammatory response to disintegrate the hematoma (Figures 1 and 2). Vasogenic edema results due to increased extravasated fluid accumulation due to BBB disruption of endothelial tight junctions.

The endothelial tight junction, made of claudin and ZO1, is the functional unit of BBB and works with astrocytes and pericytes to modulate permeability and regulate ion channel transporters. Disrupted endothelial tight junctions can cause irreversible damage via release of excess calcium and reactive oxygen species (ROS) molecules.

The migration of inflammatory leukocytes and microglia activation create a pro-inflammatory milieu where cytokines and chemokines further increase BBB disruption and increase the likelihood of circulatory systemic cytokines and other inflammatory circulatory cells into the brain or cerebral circulation.

In addition, vascular endothelial growth factor (VEGF) is released during BBB disruption and leads to proliferation and migration of vascular endothelial cells. VEGF peaks during both primary and secondary injury phases.

Figure 1. Intracerebral hemorrhage secondary mechanisms. (a) Avalanche model demonstrating initial blood vessel rupture and further hematoma expansion causing re-bleeding, intracranial pressure elevation, and vasogenic edema. (b) Peripheral hematoma expansion after vessel rupture and blood extravasation into brain tissue generating (c) systemic inflammatory response.
and occludins are transmembrane proteins that form tight junctions and decrease after exposure to VEGF-A in human endothelial in-vitro cells. In-vivo animal models have also demonstrated that VEGF inhibition using VEGF neutralizing antibody can reduce glioblastoma-induced vasogenic edema.

During secondary brain injury, extravasated blood in the extravascular spaces also releases hemoglobin, hemin, thrombin, and iron that contribute to neuronal damage and inflammation. Secondary brain injury is a leading cause of irreversible neurological deficits and brain death. Patients with ICH exhibit irreversible progressive deterioration due to secondary injury, which include neuroinflammation caused by oxidative stress, neurotoxicity or cell death by ferroptosis, BBB disruption, PHE, and hematoma expansion as well as axonal damage and neuronal loss due to apoptotic cell death.

**Time frame of secondary mechanisms of injury in ICH for acute intervention**

After ICH onset, wound repair immune response occurs within seconds after injury with microglia and macrophage activation and migration occurring in the first hour. The first 6 h are often critical to establish a management plan to mitigate secondary insult and PHE. Early intervention may prevent the development of secondary injuries but are dependent on the mechanisms targeted and timing of intervention. In addition, as wound repair often involves normal physiological mechanisms that are inopportunistically activated and may consequently lead to further injury, this temporal profile between physiological harm and physiological repair must be better delineated.

**Acute intervention targets for attenuating secondary injury after ICH**

**Calcium and PTH as pre-existing conditions impacting ICH prognosis**

The exact pathophysiological role of calcium, including whether it can be actively modulated or is a passive marker of inflammation, requires greater study. However, growing evidence demonstrates that intracellular calcium and hypocalcemia may be critical in determining prognosis and progression after ICH. High serum calcium is associated with smaller hematoma volume and better clinical outcome. Calcium influences physiological homeostasis by playing a critical role in the coagulation cascade and neuronal, skeletal, smooth muscle, and hormone secretion. Serum calcium is normally found ionized and charged (active physiological form), in a free state and protein...
bound, or as an anion bound complex. Calcium is regulated by parathyroid hormone, vitamin D metabolites, and calcitonin. Calcium has numerous potential roles in ICH. First, hypocalcemia potentially promotes the cytokine activation that induces inflammation and PHE within 24–48 h of ICH. Serum calcium also influences blood pressure. Calcium has been shown to reduce blood pressure due to immediate action on calcium-sensing receptors (CaSR). These G-coupled receptors modulate calcium concentrations to maintain vascular tone and are expressed in perivascular nerves, vascular smooth muscle cells, and endothelial cells. Lower serum calcium correlates with increased microbleeds, pre-existing unstable cardiac arrhythmias and rheumatic heart disease, larger hematoma volume, and worse outcome in ICH. Strategies to improve serum calcium and decrease the influx of intracellular calcium may be useful for mitigating secondary injury.

**Microglia role in ICH**

Microglial activation in the brain is critical in secondary injury due to their role in remodeling synapses during adult neurogenesis, recapturing old damaged neuronal debris, and clearing infectious particles. Microglial activation also results in neuroinflammation via infiltration of leukocytes and cytokine release. Hemorrhagic brain injury results in extensive microglial activation in preclinical models of ICH. This is characterized by growth and increased microglial expression of IBA1 CD11b compared to non-injured control brain. IBA1-positive microglia encircle peripheral areas of hematoma. Microglial to macrophage phenotype activation takes place 1–4 h after ICH in animal collagenase models. Peak activation of microglia has been observed at day 3 and 5 after ICH with an eight-fold increase in microglia or macrophages. In mouse models of ICH, macrophages migrate to the injury site within 12 h. Moreover, different stages of activated monocytes are detected through this period in the circulation, and this activation of microglia to macrophage persists for at least 4 weeks in animal models with confirmation in ICH patients.

Activated microglia and macrophages share immune cell markers that are difficult to assess by histopathology. Activated microglia or M1 promotes a self-sustained inflammatory release of cytokines, chemokines, prostaglandins, proteases, ferrous iron, glutamate, and ROS. Activated microglia also activate and recruit more M1 and M2. Macrophage inhibitory factor (MIF) is a small peptide released from CD8 T-cells. MIF injections greatly diminish the volume of hemorrhagic lesions in mouse models. Current potential strategies for clinical trials would target the transformation of microglia to prevent scar formation mechanisms within the brain and reprogram macrophages to act as neuroprotectants or scavengers.

**M1/M2 reprogramming role in secondary insult and cytokines**

M1 and M2 macrophages have been classically defined as pro-inflammatory and anti-inflammatory based on in-vitro conditions. M1 and M2 macrophages mainly differ in metabolic profile and cytokine production. Transformation of M1 to M2 macrophages requires the presence of a specific inflammatory milieu continually signaling for wound repair and blood vessel remodeling. However, it has been difficult to distinguish among biomarkers and other transition activation states. M1 express iNOS/CD86 whereas M2 express Arginase/CD206. Pathological expression has been seen in central nervous system macrophages after acute and chronic brain injury.

Microglia to macrophage activation, mediated by IL-4 and signifying a transition from pro-inflammatory to anti-inflammatory states, has been demonstrated in animal models. It is currently unknown if microglia are polarized to macrophage or recruited from circulation. Some of the current animal models have shown decreased brain damage and neuroprotection by utilizing inhibitors of M1 to M2; this includes administration of IL-4 to decrease edema induced by ICH and macrophage removal from circulation after the insult.

Increased efforts have been made to delineate and clearly separate M1 and M2 from their genetic and mRNA transcriptome signature. However, only metabolic activity provides a realistic insight on the dynamic function of M1 and M2 macrophages. Metabolic reprogramming is one of the features of the innate immune system. This is based on availability of glucose or fatty acid utilization. M1 microglia are characterized by a faster metabolic rate – mainly using glycolysis,
pentose phosphate production and NADPH production as well as fatty acid synthesis – since they reach and degrade bacteria, release prostaglandins and cytokines, and have high turnover for phagocytosis. In contrast, M2 macrophages with an anti-inflammatory and remodeling role require sustained energy activation that enables transcription of tissue repair genes and amino acids and fatty acid breakdown of bacteria and phagocytic activity.

Recently, studies in vivo have demonstrated that M2 anti-inflammatory macrophages can revert to an M1 signature and use autophagy as its main metabolism and function. M2 macrophages also have increased expression of CD206 ligand, which is a C-lectin glycan peptide that confers unique characteristics for M2 to sustain signaling with endothelial and neutrophil glycan and thus guides endothelial remodeling. Furthermore, this increases the complexity of immunometabolism reprogramming since availability of C-lectin and other glycan promotes activation of M2 macrophages and decrease M1 activity. Metabolic reprogramming of M2 include anti-inflammatory signaling with pyruvate dehydrogenase used as a substrate for the tricarboxylic acid (TCA) cycle and mitochondrial oxidative phosphorylation. Increased pyruvate dehydrogenase leads to proinflammatory conditions and increased itaconate. Oxygenation conditions also contribute to this change, promoting oxidative phosphorylation and proinflammation with active glycolytic activity on M1 microglia. TCA cycle and oxidative phosphorylation are critical to maintaining M1/M2 balance. Decreased oxidative phosphorylation impairs the ability of M1 repolarization to anti-inflammatory M2 and promotes an M1 pro-inflammatory phenotype. This phenomenon occurs in the primary phase of ICH due to hypoxia.

Glycolysis and oxidative phosphorylation have been associated with pro-inflammatory microglial activation. Thus, glucose levels during the first hours of ICH ictus in the primary phase may rapidly determine proinflammatory versus anti-inflammatory responses. This has been observed in animal models where exposure of high extracellular concentrations of glucose induced CD11b, iNOS, TNFα, and IL-6 activity and decreased M2 macrophage activity. Possible novel targets to decrease M1 signaling are mTOR-HIF1α, IL-1, and TNFα, which may occur via rapamycin or metformin.

Aquaporins: inhibiting cerebral edema in ICH
Aquaporins (AQP) are proteins that regulate water intake and balance in all cells and act like ion channels. Thirteen different subtypes of aquaporins have been identified. Expression of AQP4 is most abundant in the central nervous system, particularly on astrocyte foot processes surrounding capillaries and modulate water influx into brain parenchyma. Aquaporins are also expressed in the choroid plexus and produce CSF.

AQP4 has been extensively studied as a potential target for brain edema, but AQP4 expression has been shown to be different in TBI, ICH, and liver damage models for brain edema. Particularly relevant in cytotoxic edema, AQP4 null mice have demonstrated severe brain edema compared to wild type. In cytotoxic edema, AQP4 induces excessive influx of extracellular water into the cell as a response to correct ionic imbalance, whereas during vasogenic edema, AQP4 is involved in parenchymal fluid accumulation. AQP4 inhibitors have demonstrated effectiveness in cytotoxic edema by diminishing cell swelling. AQP4 activators or up-regulators might be effective in vasogenic edema by facilitating parenchymal fluid clearance.

NKCC1 Na cotransporter activity on ICH edema
NKCC1 is one of the main ionic co-transporters maintaining cellular and water homeostasis in the brain. NKCC1 is expressed both in astrocytes and neurons and is critical for proper nerve synaptic communication. NKCC1 Na⁺ K⁺ Cl⁻ cotransporter transports Na⁺ and K⁺ and maintains Na concentration and regulates cell volume. NKCC1 is increased after TBI and cerebral ischemia in animal models and facilitates inflow of extracellular Na into cells. Animal models have demonstrated that cytotoxic brain edema was decreased in NKCC1 null mice compared to wildtype. Bumetanide, an NKCC1 inhibitor, has proven to be effective in cerebral edema mouse models. Piroxicam, an NSAID, binds to AQP4 and reduces cerebral edema in animal ischemia models. These two compounds may represent potential novel targets for secondary injury in ICH clinical trials targeting cerebral edema after ICH.
inhibitor, has been used as a loop diuretic and has shown no efficacy in decreasing cytotoxic cerebral edema in animal ICH models.\textsuperscript{42}

**Endothelin receptor ETB-R and ICH repair and remodeling in secondary injury**

Endothelins are a vasoconstrictor family of peptides released by the endothelium and immune cells that are important physiological modulators in the central nervous system. Endothelins act on two main receptors in the brain: ETA-R and ETB-R. ETA mediates vasoconstriction in the smooth muscle, and ETB is expressed in the astrocytes. ETB-R astrocyte activation induces GFAP reactive activity and proliferation and stimulates several pathophysiological responses to injury including MMP-9 and VEGF-A release.\textsuperscript{43} Studies evaluating the mechanisms of endothelin reveal an important pathway that targets ETB-R, matrix metalloproteinase-9 (MMP) and the VEGF axis where ETB-R can be inhibited upstream in the signaling pathway and decrease cerebral edema due to MMP-9 and angiogenesis in ICH.\textsuperscript{44}

Several ETB-R antagonists have been developed to date, including BQ788 and IRL2500. These have been tested in brain edema mouse models. ETB-R antagonists have been locally administered intrathecally after BBB disruption and vasogenic brain edema.\textsuperscript{20,44} In a recent study, BQ788 and IRL2500 reduced the number of reactive astrocytes that were GFAP positive and decreased MMP-9 and VEGF-A in a cerebral edema mouse model. This inhibition decreased BBB permeability and decreased status epilepticus induced by vasogenic edema.\textsuperscript{45} These effects were also reproduced in a cerebral ischemia model indicating the potential of ETB-R antagonists in cerebral anti-edema therapies.\textsuperscript{17,20,43} Much attention has been brought to these secondary mechanisms with a number of clinical trials including: GLP-1 CellBeads\textsuperscript{®} for the Treatment of Stroke Patients With Space-occupying Intracerebral Hemorrhage, Glibenclamide Advantage in Treating Edema After Intracerebral Hemorrhage (GATE-ICH), Interleukin-1 Receptor Antagonist in Intracerebral Hemorrhage (BLOC-ICH), Registration of Idarucizumab for Patients With Intracranial Hemorrhage (RIC-ICH), Efficacy, and Safety and Tolerability of BAF312 Compared to Placebo in Patients With Intracerebral Hemorrhage. These studies are ongoing and the results are not yet available.

**Sur1-TRPM4 channels targeting cerebral edema**

Sur1 is a ligand binding receptor that associates with the Trpm4 channel after neuronal damage and activates a calcium dependent ATP channel. Sur1-NC1\textsubscript{Ca-ATP} channel leads to vasogenic edema by allowing sodium ion dependent chloride entry into the cell and water influx into the extravascular space.\textsuperscript{46} Neuronal depolarization leads to ATP depletion and sodium-dependent water influx into the cell. Sur1-NC1\textsubscript{Ca-ATP} channels are regulated by the Sur1 ligand, and pathological activation results in depolarization and cytotoxic edema and the passive influx of Cl\textsuperscript{−} and water, which results in vasogenic edema.\textsuperscript{46} An exploratory cohort from a pediatric TBI Phase II/III trial demonstrated that Sur1 cerebrospinal fluid levels increased and correlated with increased ICP and worse outcome, revealing Sur1 as a potential biomarker for secondary injury.\textsuperscript{4}

Preclinical and clinical studies identified sulfonylurea glibenclamide as effective in controlling and decreasing cerebral edema following ICH and stroke.\textsuperscript{36} The mechanism of action was blockade of Sur1-KIR channels. The blocking activity of Sur1-TRPM4 channels (also named Sur1) regulated Na-Ca ATP, which is expressed in the neurovascular unit after BBB disruption and trauma. Glibenclamide inhibits the regulatory subunit and decreases efficacy of channel activity. Sur1 belongs to a family of four different types of K\textsubscript{ATP} channels linked to K influx ionic entry – Sur1-KIR 6.2, Sur2A-KIR6.2, Sur2B-KIR6.2, and Sur2B-KIR6.1 – that are expressed in astrocytes and neurons.\textsuperscript{36,47} Activation of K\textsubscript{ATP} channel causes hyperpolarization of the cell, allows outward flow of potassium, and decreases excitability. Activation of K\textsubscript{ATP} channels may lead to depolarization or hyperpolarization.\textsuperscript{47}

Glibenclamide was designed for beta pancreatic cells that have resting membrane potentials above the K equilibrium potential. This leads to depolarization and calcium influx needed for insulin release. Injured astrocytes and neurons have a more depolarized resting membrane potential that leads to an inward sodium current and further efflux of potassium.\textsuperscript{36,47} Blocking Sur1 leads to increased positive influx charges and hyperpolarization, which decreases depolarization and prevents further BBB leakage. Glibenclamide has demonstrated effectiveness when targeting both
vasogenic and cytotoxic edema in multiple models of brain injury including ICH, TBI, ischemic stroke, and subarachnoid hemorrhage.

Glibenclamide’s bioavailability and potential uses for stroke treatment have been extensively studied. Glibenclamide is formulated to be dosed three times a day orally to achieve supratherapeutic plasma levels. However, increased risk of hypoglycemia in non-diabetic patients can induce cerebral damage. The intravenous formulation of glibenclamide (BIIB093) facilitates rapid supra-therapeutic levels and stable maintenance of desired plasma levels of the drug.

GAMES (Glyburide Advantage in Malignant Edema and Stroke) RP was a randomized, multi-center prospective double-blind placebo-controlled trial that evaluated BIIB093 in 86 patients (41 patients in the BIIB093 group and 36 patients with placebo). Its primary objective was to demonstrate increased proportion of patients with malignant anterior circulation strokes achieving modified Rankin score of 0–4 at 90 days without decompressive hemicraniectomy. No significant difference in functional outcome or mortality was noted between the BIIB093 and controls. However, radiological imaging of cerebral edema did show smaller median midline shift, lower total plasma concentration of MMP-9 measured at 24–72 h, and a reduced proportion of deaths attributed to cerebral edema in the BIIB093 group.

Potential applications for Sur1 inhibition by sulfonylureas in ICH have been suggested by retrospective studies that suggest that diabetic patients pretreated with sulfonylureas have better clinical outcomes and smaller initial hematoma volumes.

These studies show efficacy and safety of the Sur1 mechanism for treating cerebral edema in malignant ischemic stroke and ICH. The results of these studies may present a potential therapeutic target for mitigating secondary injury in ICH or severe TBI. Efforts to study this mechanism in ischemic stroke and severe TBI are ongoing with the current phase III multicenter randomized trials CHARM (Cirara in large Hemispheric infarction Analyzing modified Rankin and Mortality) and ASTRAL (antagonizing SUR1 TRPM4 to reduce the progression of intracerebral hematoma and edema surrounding lesions).

Matrix metalloproteinase inhibition
Matrix metalloproteinases (MMP) degrade extracellular matrix components (ECM) such as collagen, laminin, and fibronectin. MMPs are a family of zinc-dependent enzyme endopeptidases with four main classes: collagenase, gelatinase, stromelysin, and membrane bound. 24 MMPs have been identified. These endopeptidases are usually present in the inactive form and are activated by autocalysis or plasmin. MMP-9 degrades basal lamina and brain micro-vessels resulting in increased BBB permeability after ICH. Upon BBB disruption, MMPs are activated resulting in hematoma volume and PHE expansion. Encephalogens activate pro-forms of inflammatory molecules of cell, ECM interaction as well as processing of cell death debris and molecules, and the apoptosis mediated FasL pathway. MMP-9 enzymes stimulate repair and remodeling of damaged nerve tissues promoting angiogenesis and axonal injury.

Minocycline is a semisynthetic derivative of tetracycline. It has a broad spectrum of activity against gram-positive and gram-negative bacteria and is approved by the Food and Drug Administration (FDA) for treatment of Acinetobacter infections as well as experimental use for ICH. A recent study analyzing the pharmacokinetic profile of intravenous minocycline in critically ill patients with Acinetobacter infections noted that volume distribution increased with body size, and circulating plasma proteins such as albumin directly contributed to reducing free fraction of many drugs.

MMP-9 inhibition using repurposed medications, including minocycline, show promising results: decreased circulating MMP-9 and increased overall survival in all patients regardless of the hematoma volume and mean systolic blood pressure. In ICH, MMP-9 levels have positively correlated with PHE volume in subcortical ICH, although MMP-9 levels failed to be a predictor for PHE volume in logistic regression analysis.

The ACUMIN study was a phase IV multicenter open label study to evaluate PK of single dose intravenous minocycline in patients receiving antimicrobial therapy for a known suspected gram-negative infection. A maximum dose of intravenous minocycline 200 mg every 12 h was used but only achieved critical fAUC: (MIC 50) targets in plasma associated with stasis and selectivity for Acinetobacter. This indicated that a
1600 mg intravenous minocycline dosing regimen would be required to ensure > 90% probability of achieving fAUC-MIC. The overall conclusions from both PK models suggest that current intravenous dosing of minocycline used in the intensive care unit might provide only suboptimal dosing and microbiological efficacy, and the higher intravenous minocycline doses used in critically ill patients with infections will require a detailed risk versus benefit assessment for toxicities related to potential kidney damage.49

Immune migration suppression preventing wound repair via S1P-receptor inhibition

Fingolimod was approved for relapsing remitting multiple sclerosis in 2010.56,57 One of the main features of fingolimod is its ability to cross the BBB and act directly on neural and glial cells. It also acts as an analogue of Sphingosine-1-phosphate receptor (S1P-R), a membrane-derived lysophospholipid signaling molecule, and inhibits activated T-lymphocytes from leaving lymph nodes58,59 and migrating into the circulation. This therapeutic strategy has been extensively used in autoimmune disorders to prevent inflammation in autoimmune diseases such as ulcerative colitis and multiple sclerosis.

The S1P -R family comprise G-coupled receptors representing a large family and expressed in many cells throughout the epithelial cells and central nervous system. S1P -R13 are widely expressed whereas S1P -R4 and S1P-R5 are restricted to certain cell types in the central nervous system. These receptors have important functions on cell adhesion, migration, and endocytosis and modulate cell proliferation immune response trafficking of T and B-cells.

Fingolimod was first studied in ICH collagenase and autologous blood rat models. Fingolimod was administered as an intraperitoneal dose versus daily injection and evaluated at different time points.57 These studies demonstrated significant less cerebral edema and circulating lymphocytes in the daily fingolimod injection group.

Pre-clinical studies in the mouse ICH collagenase model showed a significant decrease in cerebral edema and apoptotic cells.57 This study also showed decreased brain atrophy at 2 weeks, improved neurobehavioral tests, and decreased hematoma volume and CD68 + cells. These promising results lead to the development of a phase I clinical trial using Fingolimod as a treatment for cerebral edema after ICH.60 Patients received fingolimod orally every day for three days and had a mean arterial pressure goal < 130 mmHg. Primary outcome was modified Rankin score (mRS) and NIH stroke scale (NIHSS) scores at days 7, 14, 30, and 90. A total of 23 patients with hematoma volumes between 15 and 17 ml were enrolled with a mean time onset time to fingolimod treatment of 19.5 h. Glasgow Coma Scale scores significantly improved by day 7 and 14 compared to controls, NIHSS scores improved from admission to day 7, and mRS scores improved on day 90. In addition, there was radiological evidence of reduction in PHE volumes and decreased lymphocyte proliferation. All CD4, CD8 and CD19 cells were significantly reduced in the fingolimod group.60 No significant adverse events were observed in the fingolimod group, and lymphopenia was present until 7–9 days with no further complications. This trial showed improved clinical outcome in ICH by rapidly targeting the source of inflammatory PHE by preventing migration of adaptive immune infiltrates to the brain after the ICH insult.38,57

Ozanimod is another modulator of S1P1 and S1P5 that has been tested in a Phase 2 clinical trial61 for the treatment of moderate to severe ulcerative colitis. It was designed to treat with two doses, 0.5 mg and 1 mg, by mouth daily for 32 weeks. This trial had positive secondary outcomes and decreased remissions and may be considered for ICH.61 Etrasimod is another S1P modulator developed as a novel small molecule for ulcerative colitis. It selectively targets S1P1, S1P4, and S1P5.61 This drug was tested in the OASIS study as a Phase 2 randomized parallel group induction trial for efficacy and safety of 12-week treatment of Etrasimod on moderate to severe ulcerative colitis. This study demonstrated improvement on primary outcomes and decreased circulating lymphocytes and improved remissions and mucosal healing compared to placebo.61 These encouraging results may allow for clinical evaluation of novel S1P1 modulators in neurological diseases such as ICH.

Adaptive trial design and combination therapy

Recently, novel translational approaches have evolved rapidly with new advances in imaging and clinical data trial design allowing for improved understanding of the natural history and
Figure 3. Evolution of translational therapies for intracerebral hemorrhage secondary mechanisms and potential molecular targets for vasogenic edema.

therapeutic time window to treat ICH. Figure 3 and Table 2 provide an outline summary as to the advances in clinical trials targeting secondary mechanisms for ICH. Although marginal improvement in conventional phase III clinical trials have previously been reported, the current challenge is to treat multiple injury mechanisms in a single trial. This would have to be a complex multi-arm study which would be difficult to justify in patients who do not meet eligibility and would take a long time to obtain statistically significant data for each arm.

Extensive and growing evidence on adaptive clinical trial design has enabled the targeting of complex clinical syndromes using biomarkers and patient responses.62 Targeting ICH mechanisms using combination therapy on patients that can be accrued on an adaptive trial design may also result in better therapeutic combined stratified strategies and algorithms than targeting single mechanisms individually (Figure 4) and less investigator bias with proper statistical Bayesian implementation.26

Knowledge of various mechanisms of ICH as delineated in this review have increased over the last decade. Adaptive trial design will allow for the optimization of the various mechanisms that have shown promise in the last few decades but have not been able to produce a definitive Phase 3 trial demonstrating improved outcome.

Conclusion
The mechanisms of secondary injury after ICH include direct cellular toxicity, inflammatory mediators, BBB disruption, PHE, and free radical activation. BBB disruption results in a signaling cascade of innate immunological and hemostatic responses. Clinical and translational studies using repurposed FDA approved drugs targeting some of these pathways have primarily involved fingolimod, sulfonylureas, and minocycline in the last 10 years. These trials and therapeutic targets individually have shown some promise in attenuating secondary injury after ICH (Table 2), however, have fallen short of achieving definitive success. Several strategies, including improved understanding of pathophysiological mechanisms, appropriate temporal use of therapeutics, and adaptive trial design encompassing multiple mechanisms and trial arms may provide an avenue in the future for better understanding these complex mechanisms and improving clinical outcomes.

Author contributions
Dr. Bautista: manuscript writing, critical revision of manuscript for important intellectual content, and takes responsibility for the paper as a whole. Dr. Adelson: critical revision of manuscript for important intellectual content. Dr. Bicher: critical revision of manuscript for important intellectual content. Dr. Themistocleous: critical revision of manuscript for important intellectual
| Agent         | Trial name                                                                 | Mechanism                                      | Preclinical year | Phase I year | Phase II       |
|---------------|-----------------------------------------------------------------------------|------------------------------------------------|------------------|--------------|----------------|
| Deferoxamine  | Dose Finding and Safety Study of Deferoxamine in Patients with Brain Hemorrhage (DFO in ICH) NCT00598572 | Iron chelator                                  | 2008–2010        | 2013–2014    | 2014–2019      |
|               | High-Dose Deferoxamine in Intracerebral Hemorrhage (HI-DEF) NCT01662895      |                                                | 2008–2018        |              |                |
|               | Intracerebral Hemorrhage Deferoxamine Trial–iDEF Trial NCT02175225          |                                                | 2014–2019        |              |                |
| Pioglitazone  | Safety of Pioglitazone for Hematoma Resolution in ICH [SHRINC] NCT00827892  | PPARγ agonist NF-kB suppression                | 2009–2013        |              |                |
| Erythropoetin | Preclinical                                                                 | Decreases M1 Anti-thrombotic                   | 2017             |              |                |
| Pinocembrin   | Preclinical                                                                 | Decreases M1 inhibition of the TLR-4 signaling | 2016             |              |                |
| Resartoid     | Preclinical                                                                 | Decreases M1 TLR-4 inhibitor                   | 2020             |              |                |
| Rapamycin     | Preclinical                                                                 | Enhances M2 Increase TNF-α and IL-1β           | 2016             |              |                |
| TGN-02        | Preclinical                                                                 | aquaporin-4 inhibitor                          | 2011–2019        |              |                |
| Bumetanide    | Preclinical                                                                 | NKCC1                                          | 2010–2019        |              |                |
| Endothelin BQ 788 BQ 123 | Preclinical                                                                 | ET-1 ET-2 antagonist vasoconstriction | 1994–2018        |              |                |
| Glibenclamide | Glibenclamide Advantage in Treating Edema After Intracerebral Hemorrhage [GATE-ICH] NCT03741530 | Sur1-KIR, Sur1-TRPM4                            | 2018–2021        |              |                |
| Minocycline   | A Pilot Study of Minocycline in Intracerebral Hemorrhage Patients [MACH] NCT01805895 Use of Minocycline in Intracerebral Hemorrhage [MITCH] NCT03040128 | Matrix metalloproteinase-9 inhibitor            | 2011–2019        | 2013–2018    | 2013–2016      |

(Continued)
| Agent          | Trial name                                                                 | Mechanism                                      | Preclinical year | Phase I year | Phase II |
|----------------|-----------------------------------------------------------------------------|------------------------------------------------|------------------|--------------|----------|
| Fingolimod     | Fingolimod for Treatment of ICH (FICH)                                       | S1 P1,3,4,5R agonist                           | 2012             | 2014–2016   | 2014     |
| Ozanimod       | Preclinical                                                                   | S1P5R > S1P5R potent agonist                   | 2010–2013        |              |          |
| Ponesimod      | Preclinical                                                                   | S1 P-R1 modulator                              | 2009             |              |          |
| Ceralifimod    | Preclinical                                                                   | Second-generation S1 P-R modulator selective for S1 P-R1 and S1P-R5 | 2010             |              |          |
| Siponimod      | Efficacy, Safety and Tolerability of BAF312 Compared to Placebo in Patients with Intracerebral Hemorrhage | Selective S1 P-R1,5 modulator                   | 2016             | 2017–2020   |          |
| SEW 2871       | Preclinical                                                                   | S1 P-R1 agonist                                | 2011–2018        |              |          |
| AUJ954         | Preclinical                                                                   | S1 P-1 modulator                               | 2009–2010        |              |          |
| ONO-W061       | Preclinical                                                                   | S1 P-1 agonist,                                |                 |              |          |
| CS-0777        | Preclinical                                                                   | Selective S1 P agonist                         | 2011–2018        |              |          |
| GSK 2028662    | Preclinical                                                                   | S1 P agonist                                   | 2014             |              |          |

ET, endothelin; ICH, Intracerebral hemorrhage; IL, interleukin; M1, macrophage 1; M2, macrophage 2; NF-kB, nuclear factor kappa-light-chain enhancer of activated B-cells; PPAR, peroxisome proliferator-activated receptor; S1 P-R, sphingosine-1-phosphate receptor; TLR-4, toll-like receptor; TNF, tumor necrosis factor; TRPM, transient receptor potential ion channels.
Figure 4. Adaptive trial design targeting intracerebral hemorrhage primary and secondary mechanisms of injury. Increasing knowledge on current biomarkers can include a combination of experimental therapies utilizing [A] no controls or [B] controls and allow for identification and allocation of more patients in each arm depending on the response and endpoint outcome.

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