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Emerging therapeutic targets for cerebral edema

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Abstract

Introduction: Cerebral edema is a key contributor to death and disability in several forms of brain injury. Current treatment options are limited, reactive, and associated with significant morbidity. Targeted therapies are emerging based on a growing understanding of the molecular underpinnings of cerebral edema.

Areas Covered: We review the pathophysiology and relationships between different cerebral edema subtypes to provide a foundation for emerging therapies. Mechanisms for promising molecular targets are discussed, with an emphasis on those advancing in clinical trials, including ion and water channels (AQP4, SUR1-TRPM4) and other proteins/lipids involved in edema signaling pathways (AVP, COX2, VEGF, and SIP). Research on novel treatment modalities for cerebral edema [including recombinant proteins and gene therapies] is presented and finally, insights on reducing secondary injury and improving clinical outcome are offered.

Expert Opinion: Targeted molecular strategies to minimize or prevent cerebral edema are promising. Inhibition of SUR1-TRPM4 (glyburide/glibenclamide) and VEGF (bevacizumab) are currently closest to translation based on advances in clinical trials. However, the latter, tested in

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glioblastoma multiforme, has not demonstrated survival benefit. Research on recombinant proteins and gene therapies for cerebral edema is in its infancy, but early results are encouraging. These newer modalities may facilitate our understanding of the pathobiology underlying cerebral edema.

Keywords

Cerebral edema; SUR1-TRPM4 (sulfonylurea receptor 1-transient receptor potential cation channel subfamily M member 4); Glyburide / Glibenclamide; VEGF (Vascular Endothelial Growth Factor); AQP4 (Aquaporin4); bevacizumab; S1P (sphingosine 1 phosphate); Fingolimod; Conivaptan; AVP (Arginine Vasopressin); COX2 (Cyclooxygenase-2); Celecoxib

1. Introduction

Cerebral edema is a pathological accumulation of fluid in the brain that increases the net brain-tissue water mass [1–3]. It affects almost all types of acute brain injuries (ABI) commonly seen in neurocritical care units including ischemic stroke, intracerebral hemorrhage (ICH), subarachnoid hemorrhage (SAH), cardiac arrest (CA), traumatic brain injury (TBI), meningitis/encephalitis, central nervous system (CNS) abscesses, acute liver failure, status-epilepticus, primary brain tumors, and metastases, as well as systemic diseases such as diabetic ketoacidosis and sepsis. In many of these primary pathologies, cerebral edema is an independent risk factor for unfavorable prognosis with increased morbidity and mortality, in some cases by more than 80% [1,2,4–8]. There is some degree of molecular overlap in these diseases, with several of the same components, pathways, and networks identified as contributors to cerebral edema. However, there are also important distinctions depending on predominance of specific pathways, spatial location, timing, individual host response, and genetic predispositions. Substantial advances in identifying the underlying molecular machinery of cerebral edema have yielded exciting therapeutic targets to prevent or reduce edema generation. In isolation, these targeted anti-edema agents may not reach their full potential. However, edema therapy based on precision medicine could be optimized by early recognition and serial monitoring of key pathways that are activated/suppressed in individual patients using complementary tools like biomarkers, imaging, multimodal monitoring, and genetic predispositions.

Current clinical management of cerebral edema remains limited with non-specific temporizing strategies utilized largely to prevent the consequences of raised intracranial pressure (ICP) and impending herniation rather than targeting specific molecular pathways. The mechanism by which raised ICP causes herniation and death is explained by the Monro-Kellie doctrine (Section II-A) whereby expanding edema (or other causes of intracranial volume) is limited by the rigid skull, and after compensatory mechanisms are compensated, increased pressure on the brain tissue causes herniation. The current therapeutic armamentarium to address this devastating clinical problem commonly consists of hyperosmolar therapies, cerebrospinal fluid (CSF) drainage, steroids (primarily for peritumoral edema), therapeutic hypothermia, deep sedation with anesthetic agents, paralysis, and decompressive craniectomy. Many of these agents have been used in some form for centuries. For example, hyperosmolar therapy was described in the 1920s, trephination to relieve intracranial pressure was used in Peru (8000 BCE), ancient Egypt

(1700 BCE) and China (Xinjiang province, 1000 BCE), and was formally described by Hippocrates in 400 BCE [9]. While some of the more extreme therapies, e.g. mannitol, 23.4% NaCl, and craniectomy, can acutely salvage a herniating brain, these agents carry high risks of morbidity. In recent trials, craniectomy for certain conditions causing cerebral edema (like ischemic stroke/large hemispheric infarction), has improved both morbidity and mortality, whereas in other disease-types (like TBI) the benefit has predominantly been life-saving without clear benefit on functional outcome or disability [10–16].

The next generation of anti-edema therapies will leverage molecular pathophysiology to prevent or minimize edema formation [1–3,8,17]. Advances in bioinformatics, unbiased transcriptomics and proteomics, biomarker development, NextGen sequencing, gene and stem-cell therapies have already valuably informed research into cerebral edema therapies, and likely will continue to exponentially grow (Figure 1). As outlined (Box 1), this article discusses physical drivers and molecular contributors to cerebral edema (Section II), and focuses on the mechanisms and therapeutic potential of emerging clinically promising targets (Section III). Newer therapeutic modalities such as recombinant proteins (Section IV) and gene therapies for cerebral edema are also briefly presented (Section V).

2. Pathogenesis

Understanding the physical forces, physiological networks, and molecular drivers of cerebral edema is key to recognizing the rationale underlying currently available therapies. This also enables an appreciation for their limitations, and informs the development of promising targets. In this section, we summarize physical principles that drive cerebral edema, different networks/systems involved in fluid influx and efflux from the CNS, and we provide an overview of key mechanisms and molecular contributors to different cerebral edema subtypes (Figure 2A–B). Specific promising targets are subsequently discussed in detail in Section III on emerging therapies.

2.1. Physical Drivers of Cerebral Edema

Basic Forces—The Monro-Kellie doctrine, described over two centuries ago, outlines the principle that a rigid skull necessitates a fixed sum of intracranial components (blood, CSF/interstitial fluid, brain tissue) whereby an increase in one causes a reciprocal decrease in one (or more) of the others. Once these compensatory reserves are exhausted, ICP rises and can cause herniation and death. The threshold at which this occurs varies between individuals and is a function of intracranial elastance and compliance [8]. Conceptually, this has provided the basis for several current approaches to edema including CSF diversion (external ventricular drain, EVD), reduction in metabolic demand and CBF (sedation, hypothermia), and hyperosmotic agents (mannitol, hypertonic saline). At a local level, the modified Starling equation [quantifies the net flow (J_v) of water across membranes by leveraging differences in capillary (c) vs. interstitial (i) hydrostatic (P) and oncotic (p) pressures [8,18]. This principle explains the action/benefit of hyperosmotic therapies whereby water moves from the brain interstitium and intracellular compartments into the vasculature. The phenomenon of rebound edema can be observed with repeated administration of mannitol or hypertonic saline; it is related to breakdown of the blood–brain

barrier (BBB) and accumulation of the hyperosmolar agent within the interstitium/outside an intact vasculature creating a reverse-osmolar gradient.

2.2. Cerebral Edema Pathophysiology and Systems Facilitating Fluid Movement in the Brain

The taxonomy of cerebral edema has historically resulted in separating cytotoxic/cellular edema from vasogenic edema (BBB disruption). While these distinctions are clinically informative to distinguish intracellular energy failure and cell swelling from a leaky BBB, they are also somewhat artificial in light of increasing recognition that these processes are molecularly interconnected and likely represent a spectrum of edema evolution. Although the primary sources of cerebral edema are putatively the cerebrovascular system and CSF circulation as discussed below (particularly re: ionic, cytotoxic/cellular and vasogenic edema), we also present emerging evidence of the contributions of the glymphatic system and meningeal lymphatic vessels to this process. Cerebral edema is also highly linked with neuroinflammation (reviewed elsewhere)[19–22]. This subsection provides an overview of the underlying pathophysiology and key reported molecular contributors to cerebral edema subtypes.

Importantly, cerebral edema manifests differently in different disease states. For example, edema after cerebral ischemia involves early cellular/cytotoxic swelling followed by delayed opening of the BBB. This is different than manifestation in TBI where mechanical disruption of the BBB occurs almost immediately and is subsequently accompanied by cellular, vasogenic, and inflammatory contributors to edema. Furthermore, edema related to acute brain injury varies significantly from that occurring in more chronic disorders including neurodegenerative diseases like Alzheimer’s disease, or even multiple sclerosis. An exhaustive disease-specific delineation of edema mechanisms is beyond the scope of this review, however we present mechanisms that have been found to be relevant and common across the disease spectrum and in Section III (NextGen Emerging Targets), and outline promising molecular targets as well as the preclinical and clinical research on which they are based.

- **Cellular/Cytotoxic** edema refers to intracellular fluid accumulation resulting in cell swelling after several forms of ABI [1,8,17]. Although historically referred to as ‘edema,’ cellular edema is a redistribution of water from the interstitium to the parenchyma. In isolation, it therefore does not increase total brain water content but in the setting of an external fluid source (e.g. perfusion or the glymphatic system), it can serve as a precursor to ionic and vasogenic edema that do cause brain swelling/increase in water content.

Cellular edema has predominantly been described in perineuronal and perivascular astrocytes, immediately after the insult (hypoxia, injury) but can occur in multiple cell-types [8,17]. It has multiple potential triggers including energy failure and endogenous/exogenous toxins (ammonia, glutamate, arachidonic acid, cyanide, H⁺, K⁺) resulting in maladaptive ion influx. Osmolyte/ionic uptake into the cell can occur via constitutively expressed drivers (NKCC1, EAAT1/2, NHE, NBC, and mGluR4), or *de novo* upregulated channels (SUR1-TRPM4) [23–40]. Ionic influx creates an osmotic gradient for water to follow via diffusion,

water channels (AQP4, GLUT1, and SGLT1), or ion channels that co-transport water (NKCC1, EAAT1/2). When oncotic cell death from cellular swelling affects perivascular astrocyte podocytes, or endothelial cells, it contributes to vasogenic edema. Clinically, magnetic resonance imaging (MRI)-based apparent diffusion coefficient (ADC) sequences are used to identify cellular swelling [41].

- **Ionic edema** is mechanistically similar to cellular edema in that it utilizes many of the same ion transporters, but it occurs primarily at the capillary level and results in transcapillary flux of ions and water based on the spatial distribution of ion channels [8,17]. Water and ions are transported inward through the luminal membrane of the capillary endothelial cell, and, in equivalent amounts, outward through the abluminal membrane. With cellular swelling, the interstitial ionic gradient (e.g. Na⁺) is depleted. The osmotic driving force outlined by the Starling principle () results in an influx of Na⁺ into the interstitium from the actively perfused capillary lumen, followed by water and negatively charged ions (Cl⁻) for osmotic and electrical neutrality [17].
- **Vasogenic edema** is a complex interplay of several mechanisms that results in the loss of BBB integrity and extravasation of water and plasma proteins into the CNS interstitium. It overlaps with neuroinflammation. Release of damage associated molecular patterns (DAMPs) after primary and secondary injury elicits an inflammatory immune response including the recruitment, adherence/migration and activation of inflammatory cells facilitated by various endothelial cell surface receptors (VCAM-1, ICAM-1, LFA, and VLA-4) as well as second messenger cascades involving proinflammatory cytokines (TNF- α , IL-1 β , IL-6, IL-8, and TGF- β) and chemokines (CXCL-1/2)[8,17,19,42–48]. The mechanisms through which these pathways influence BBB integrity are still being elucidated. From what is known, they include increased MMP expression, downregulation of tight junction proteins, expression of permeability-increasing molecules (substance-P, bradykinin) and increasing intercellular gaps by promoting cytoskeletal reorganization [1]. Cellular retraction of endothelial cells via cytoskeletal contraction by actin and myosin light chain kinase, further increases BBB permeability [17].

Other pathways involved in vasogenic edema include degradation and downregulation of tight junction proteins (ZO-1, occludin, claudin 5) and extracellular matrix (laminin) by factors such as MMP9, vascular endothelial growth factor-A (VEGF), angiopoietin-2, CCL2, and NO [8,17,46–55]. The Hedgehog pathway may also play a key role in BBB maintenance. Astrocytic sonic hedgehog (SHH) downregulation has been reported to induce BBB disruption and vasogenic edema in *in vivo* and *in vitro* models of ICH, TBI, ischemic stroke and CNS tuberculosis[56–62]. Reported mechanisms in these studies include increased MMP-9 activity as well as degradation of endothelial tight junctions (ZO-1, occludin) via pathways involving Patched1 (a SHH receptor on endothelial cells), Smoothed (transmembrane receptor inhibited by Patched), and Gli-1 (transcription factor).

Arginine vasopressin (AVP, V1a receptor) and NLR family pyrin domain containing-3 (NLRP3) have also been identified as contributors to vasogenic edema via mechanisms

that include AQP4, peroxisome proliferator-activated receptor (PPAR-g), and tight junction degradation [63–65]. Channels such as SUR1-TRPM4 are expressed on endothelial cells and perivascular astrocytes, and cellular edema and oncotic cell death in these cell-types involved in BBB maintenance may further contribute to vasogenic edema [1,17].

Glymphatic System—First discovered less than a decade ago, the glymphatic system provides a route for CSF influx into the brain (via periarterial space), and interstitial fluid clearance out of the brain (perivenous spaces) with a key role of aquaporin-4 (AQP4) channels on astrocyte vascular endfeet [66,67]. Although this system has been extensively studied in the context of neurodegenerative disease (amyloid- β and tau clearance) and sleep pathways, recent efforts have also identified its involvement in driving cerebral edema development after ischemic stroke [68,69]. Using calcium imaging in transgenic mice expressing GCaMP7 in cortical neurons and astrocytes, spreading depolarizations (triggered by cessation of blood flow) were found to initiate rapid CSF influx along perivascular spaces - coinciding with edema onset and increased brain water content. In the same study, magnetic resonance and multimodal imaging with radiolabeled tracers identified CSF as a hyperacute (within minutes) contributor of both fluid and ions – inflow and spreading edema were dependent upon astrocytic AQP4 channel expression [68].

Subsequent studies in SAH and TBI have also invoked glymphatic system impairment as a contributor to cerebral edema, secondary injury, and BBB dysfunction [70–74]. Exogenous Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP38) reduced edema, BBB disruption, and glymphatic dysregulation after SAH via reduced expression of several recognized (and interconnected) molecular contributors to edema discussed in subsequent sections: SUR1, AQP4, and matrix metalloproteinase-9 (MMP9) [74]. In this study, SUR1 expression was reduced via the negative phosphorylation effect of p-PKA, which reportedly facilitates SUR1 degradation. Taken together, the evidence suggests that the glymphatic system is a promising emerging target for cerebral edema treatment across a range of ABIs.

Meningeal Lymphatic Vessels (mLV)—The concept of CNS immune privilege has continued to evolve. Previously thought to be devoid of conventional lymphatic vasculature, the role of mLVs in cerebral edema clearance was initially described in 2015 [75,76]. Work in mouse models identified a lymphatic vessel network in the dura matter that absorbs CSF from subarachnoid and interstitial spaces via the glymphatic system [75]. This network ultimately transports this fluid into deep cervical lymph nodes, potentially assisting in the clearance of cerebral edema [75]. Recent zebrafish models suggest that cerebrovascular injury induces rapid ingrowth of mLVs into the injured parenchyma where they become lumenized and drain interstitial fluid (alleviating cerebral edema), and additionally serve as growing tracks for nascent blood vessels [77]. These findings were not corroborated in rodent/primate models: transgenic mice with mLV absence had attenuated clearance of macromolecules and a compromised peripheral immune response, but no difference in brain water content; however, these mice did not have cerebral edema pathology [75,78]. Murine models of glioblastoma multiforme (GBM) and SAH demonstrated impaired lymphatic outflow, increased edema and unfavorable markers of neuroinflammation and apoptosis, suggesting that mLVs may indeed play a role in both cerebral edema and secondary injury

after ABI[72,79,80]. There is emerging evidence that meningeal lymphatic drainage is functionally connected with glymphatic flow.

3. NextGen Emerging Targets

This section discusses emerging targets for cerebral edema treatment based on supporting molecular data. Although the literature is rife with reports of strategies targeting several of the molecular components described above (Section II), few have successfully translated to clinical practice. Our focus is on candidates (listed alphabetically) with promising preclinical and clinical data that are currently advancing toward/actively in clinical trials (Table 1).

3.1. AQP4 – AER 271

Mechanism: The role of AQP4 in cerebral edema (both cellular and vasogenic) is complex since it is related to both water influx as well as efflux and edema clearance [8,81]. Water transport via this channel is passive, bidirectional, and is determined by the transmembrane osmotic gradient [17]. AQP4 expression has been demonstrated in several cell-types involved in brain-fluid interfaces including perivascular astrocyte endfeet, glia limitans, ependymal cells (basolateral membranes), subependymal processes [1,82]. It has also been shown to be upregulated in activated/reactive microglia [83]. The glymphatic system discussed above, is dependent on AQP4, likely since this is a major mechanism for rapid neutralization of osmotic gradients during ion transport [17]. The AQP4 M23 isoform associates in membranes and forms supramolecular assemblies by aggregating into orthogonal arrays that facilitate rapid water flux [17].

AQP4 interfaces with several other known contributors to cerebral edema. Arginine vasopressin, via V1a receptor modulation of AQP4, contributes to BBB disruption [63,84]. AQP4 forms a hetero-multimeric complex with SUR1-TRPM4 and drives astrocyte swelling after cold injury via amplifying ion/water coupling [85]. AQP4 is linked with the NLRP3 inflammasome that has been identified as a facilitator of vasogenic edema via a variety of mechanisms including regulation of AQP4 expression and distribution in ischemic stroke, tight junction degradation in TBI, and PPAR-g in TBI [64,65,86]. In preclinical models of cerebral ischemia, water intoxication, TBI, and cardiac arrest, effects of AQP4 inhibition have been mixed, with reports of decreased cellular edema vs. no-effect/potential worsening [1,87–97]. This is possibly due to the divergent role of these channels in both edema generation and edema clearance, suggesting that modulation and timing may be more valuable than consistent/early inhibition.

Human Studies: AQP4 has demonstrated limited but promising potential as a clinical biomarker in patients with brain injury. Levels in CSF have been reported as being elevated after injury, and genetic polymorphisms have been associated with outcome after TBI [8,98–100]. Clinical agents targeting/inhibiting AQP4 include AER-271 (a selective AQP4 antagonist), and Aquaporumab (a monoclonal AQP4-specific antibody). A double-blind, placebo-controlled, sequential group, phase 1 study of AER-271 has recently been completed (NCT03804476), with results pending. There are no currently registered studies evaluating aquaporumab on clinicaltrials.gov. Nonetheless, given the strategic expression

of CNS AQP4, involvement of this channel with several mechanisms of edema, and multiple relationships with other key contributors to cerebral edema, further clinical studies are warranted. Although harnessing the potential of AQP4 channels in cerebral edema management will be complex, and could be complicated by renal toxicity, it may prove to be important.

3.2. AVP – Conivaptan

Mechanism: AVP, also known as antidiuretic hormone (ADH), is a nonapeptide produced as preproAVP by the hypothalamus, in the paraventricular and supraoptic nuclei [101]. After synthesis, it is packaged into neurosecretory granules, processed (cleaved into AVP), and transported to the posterior pituitary where it is released into the systemic circulation. Additional routes of distribution (especially to the brain parenchyma) have been described, including hypothalamo-extrahypophyseal pathways with direct release into ventricular CSF, central secretion by neurons and the choroid plexus epithelium, and transport across the BBB via a carrier mediated system [2,102–105]. Indeed after injury, several sources of AVP synthesis and release have been identified, including microglia and the cerebrovascular endothelium [104].

The V_{1a} receptor (for AVP) is widely distributed in the mammalian CNS in neurons, astrocytes, and microvascular endothelium, and is thought to be the primary mediator of cerebral edema via G protein receptor signaling [1,2]. Several pathways have been implicated, including BBB permeability and vasogenic edema (AQP4 regulation, PPAR-g, and tight junction degradation), modulating astrocyte volume, influencing cerebral blood flow, and affecting CSF production/absorption [1,2,8,63–65,106–111]. The V_2 receptor (renal duct principal cells) are involved in controlling intravascular body fluid volume. AVP signaling is involved in the syndrome of inappropriate antidiuretic hormone, and may also exacerbate cerebral edema via euvolemic hyponatremia.

In several preclinical models (ischemic stroke, cryoinjury, TBI), V_1 receptors are upregulated, AVP exacerbates cerebral edema, and inhibition (genetic or pharmacological) is beneficial [104,112–117]. Pharmacological inhibitors include vaptans (conivaptan, tolvaptan), a small molecule selective V_{1a} antagonist (SR 49059), and a peptide V_1 receptor antagonist (V1880). Both SR 49059 and V1880 have been shown to reduce cytotoxic edema and ICP in preclinical models, but unlike vaptans, have not been tested in humans [116,118,119].

Human Studies: Vaptans, a class of small-molecule AVP receptor inhibitors currently approved to treat hyponatremia, are now being clinically investigated as potential anti-cerebral edema agents. Specificity for different receptors subtypes varies based on the drug. Conivaptan inhibits both V_{1A} and V_2 receptors, whereas tolvaptan is V_2 specific. There are case reports/series of conivaptan improving hyponatremia in the neurocritical care unit (19 patients), reducing intracranial pressure in TBI (1 patient), and decreasing cerebral edema in ischemic stroke (1 patient)[120–122]. A recent single-center open-label phase 1 study of intravenous conivaptan (20 mg, every 12 hours for 2 days) was reported in 7 patients with primary spontaneous intracranial hemorrhage (NCT03000283) [123]. Safe administration

with the expected increase in serum sodium levels was observed ($p = 0.01$). None of the patients required rescue therapy with mannitol, hypertonic saline, or an external ventricular drain. There was no significant change in mean arterial pressure or heart rate to suggest significant intravascular volume depletion. The effect of conivaptan on the V2 receptor and the potential for resultant diuresis did not impact clinical hemodynamics. All patients survived until follow up.

3.3. Cyclooxygenase 2 (COX2) – Celecoxib

Mechanism: COX enzymes catalyze the formation of prostanoids (thromboxane A1, prostacyclin) from arachidonic acid, and thereby contribute to post-injury neuroinflammation (via leukocyte invasion and BBB breakdown) in ICH, ischemic stroke, TBI, SAH [124–134]. Upregulated COX2 expression in these models has been reported primarily in the CNS endothelium, and in other cell types including neurons, astrocytes, microglia, and invading leukocytes [126,129,133–135]. Inhibition in preclinical models, via COX2 antagonists (e.g. celecoxib, diclofenac, CAY10404), have demonstrated decreased lesion volume, decreased apoptosis, decreased cerebral edema, and improved neurologic outcome in ICH, ischemic stroke, SAH, and TBI [124,136–141]. Of note, progesterone has been reported to reduce COX2 expression, particularly in models of TBI [142–145].

Human Studies: Although there may be clinical reluctance to use cyclooxygenase inhibitors, particularly in hemorrhagic CNS disease, due to the potential for decreased platelet aggregation, COX2 inhibitors (particularly celecoxib) have minimal effects on platelet function even at supratherapeutic doses (1200 mg per day) [146]. Human studies on COX2 inhibitors on cerebral edema/in brain injury have primarily been in ICH. In a retrospective study (34 patients), celecoxib treatment resulted in lower edema volumes vs. controls (30.2 ± 17.7 vs. 55.5 ± 40.6 mL, $p = 0.027$) [147]. A subsequent multicenter randomized controlled trial of ICH in Korea (44 patients) demonstrated that acute administration of celecoxib (400 mg twice a day \times 14 days) reduced expansion of both perihematoma edema ($p = 0.005$) as well as hemorrhage progression ($p = 0.046$; [NCT00526214](#)) [148]. No additional studies evaluating COX2 inhibition CNS disorders are currently listed on [clinicaltrials.gov](#).

3.4. Sphingosine-1 Phosphate (S1P) – Fingolimod

Mechanism: S1P is a metabolic product of sphingolipids that can act as an intracellular second messenger, as well as an extracellular signaling ligand at G protein-coupled S1P receptors, S1P1 – S1P5[149]. S1P receptors are widely expressed on neurons (S1P1, S1P3) and endothelial cells (S1P1). S1P5 receptors are expressed predominantly on oligodendrocytes (activation protects against apoptosis)[149]. Currently, S1P4 receptors are not known to be expressed in the brain [149]. After injury, proinflammatory cytokines affect astrocytes, which strongly increase S1P1 and S1P3 expression, which modulate astrocyte proliferation, migration of leukocytes (particularly lymphocytes) and proapoptotic proteins. S1P1 signaling facilitates egress of lymphocytes from secondary lymphoid tissues, including infiltration into cerebral blood vessels after injuries such as ischemic stroke, which can be blocked by inhibition/receptor modulation [150–152]. In cerebral ischemia, S1P1, S1P2, and S1P3 affect microglial polarization toward M1 activation (proinflammatory) [153–155].

S1P signaling through these various receptors is complex, as it involves immunomodulation, cell migration, vascular tone, BBB integrity, vascular cell proliferation, and direct neuroprotection. Effects may be beneficial or detrimental depending on the receptor subtype and specific cell type. For example, via S1P1, S1P reduces leukocyte adhesion (alters endothelial adhesion molecule expression), prevents endothelial apoptosis (Bcl2 activation), and stimulates tight-junction protein ZO1 thereby promoting BBB integrity. S1P1 signaling has also been reported to have direct cytoprotective effects via pathways including PI3K/Akt/FOXO3a, or suppression of neuronal autophagy via mammalian target of rapamycin (mTOR), among others [156,157]. S1P1 activation can also be harmful/pro-inflammatory in microglia [153]. S1P2, like S1P1, is also expressed in endothelial cells. However, in contrast to the protective effects of S1P1, S1P2 contributes to pro-inflammatory phenotypes and increases permeability of vascular beds by disrupting adherens junctions [158]. This has predominantly been demonstrated in lung and spleen vascular beds, but is increasingly recognized as also increasing cerebrovascular inflammation, BBB injury and permeability [158].

CNS S1P receptors and inhibition have long been studied for their role in multiple sclerosis (MS). Fingolimod (previously FTY720), the only approved oral therapy for MS, modulates S1P receptors. The mechanism is complex and incompletely understood, particularly in the setting of multifaceted roles of S1P signaling in the CNS and immune system, different receptor subtypes, and different cell types. In the CNS, fingolimod is phosphorylated to FTY720-p/Fingolimod-p by sphingosine-kinase-2 (Sphk2). Sphk2 is highly expressed in the brain parenchyma, including the microvasculature [159]. Fingolimod-p is a high-affinity, non-specific S1P-receptor modulator at S1P1, 2, 4, and 5 (strongly at S1P1) with high oral bioavailability (93%) [160]. Although fingolimod-p initially acts as an agonist, it is more a modulator since stimulation induced internalization of receptors also results in functional antagonism of S1P signaling [149,151]. Overall, preclinical data suggest a benefit of fingolimod (for edema, infarct volume, and neuroprotection) largely in models of ischemic stroke (reviewed elsewhere [149]), but also in ICH, TBI, and SAH [161–169].

Human Studies: Aside from MS, S1P signaling and inhibition with fingolimod has been studied in patients with ICH, ischemic stroke, and SAH. Although it is generally well tolerated, side effects include bradycardia, and decreased pulmonary function. Importantly, fingolimod has been associated with increased odds of progressive multifocal leukoencephalopathy (although less than natalizumab) [170].

In a proof-of-concept study (NCT02002390; 23 subjects) of primary deep supratentorial ICH, patients treated with fingolimod within 72 hours (0.5 mg daily × 3 days) vs. clinical and imaging-matched patients had reduced perihematomal edema, neurological deficits, and improved recovery [171]. In acute ischemic stroke, the same investigators demonstrated that patients treated within 72 hours (0.5 mg daily × 3 days) had lower circulating lymphocyte counts, milder neurological deficits, and better functional recovery including reduction of the NIHSS ($p = 0.0001$)[172]. Day-7 rT1% (a marker of vascular permeability) was almost 50% lower in treated patients ($p = 0.005$)[172]. No safety concerns were reported in either study. When co-administered with alteplase (in the delayed time window, 4.5–6 hours), a double blind randomized trial in 46 patients, fingolimod improved 24-hour NIHSS

score (by 4 vs. 0 points; $p = 0.004$), and shifted the 90-day mRS scores towards more favorable outcome ($p = 0.037$) [173]. Secondary endpoints were also improved with regards to decreasing the perfusion lesion (0.001), and inhibiting infarct expansion ($p < 0.001$), with a persistent benefit through day-7. Although fingolimod has not been studied in SAH, a prospective study identified higher S1P CSF levels in SAH patients vs. controls (36 patients; $p = 0.0005$), and significant correlations with hemorrhage volume, as well as 12-month mRS (sliding dichotomy; $p = 0.007$) [174].

Several newer agents modulating S1P1 (ozanimod, ponesimod) and S1P1+S1P5 (siponimod, ceralifimod, amiselimod) are being tested, predominantly in MS. Recruitment for [NCT03338998](#), a trial evaluating Siponimod (BAF312) in ICH, is currently paused due to the COVID19 pandemic.

3.5. SUR1-TRPM4 – Glibenclamide

Mechanism: First discovered by patch-clamp experiments in adult rat astrocytes, the SUR1-TRPM4 channel is increasingly recognized as a central contributor to cerebral edema in several neurological conditions including ischemic stroke, TBI, SAH, ICH, spinal cord injury, cardiac arrest, encephalitis, and others [1,17,175–193]. Unique to this pathway, SUR1-TRPM4 is an octameric monovalent cation channel that is not normally expressed in the CNS, but upregulated *de novo* after injury [1,17,191]. It is therefore exceptionally positioned as a potentially important biomarker, as well as target for therapeutic intervention. SUR1 is an ATP-binding cassette family regulatory protein that co-assembles with pore forming subunits, in this case (after CNS injury) with TRPM4 [188]. TRPM4 constitutes the pore-forming subunits of the channel, and is a constitutively expressed monovalent calcium-sensitive cation channel [194,195]. Post-injury co-association of SUR1-TRPM4 doubles TRPM4 calcium sensitivity, and sensitizes this inner pore to intracellular ATP depletion [2,188,194]. Channel opening after injury results in cell depolarization from influx of Na^+ (which raises the osmotic pressure gradient), oncotic swelling/blebbing and cell death [1,17,85,175,176,191,196]. Water influx may be via associated channels like AQP4 [85]. The SUR1 pathway is also linked with other contributors to cerebral edema and BBB integrity including, MMP9, NOS2, and tissue plasminogen activator [197–199]. SUR1-TRPM4 expression has been demonstrated in several cell types including neurons, astrocytes, microglia, and endothelial cells in multiple preclinical models of CNS injury as well as in human tissue [177–179,188,191,196,200]. In addition to cellular edema, SUR1-TRPM4 mediated oncotic cell death of structures involved in BBB maintenance (endothelial cells, astrocytes) results in capillary fragmentation, worsening vasogenic edema, and ultimately can result in secondary hemorrhage progression [1,2,18].

A large and growing body of preclinical literature suggests SUR1-TRPM4 inhibition, both via gene silencing as well as pharmacologic (glibenclamide, a.k.a. glyburide) is beneficial. It has been shown to reduce cerebral edema, hemorrhage progression/BBB disruption and to improve neurological outcome in several preclinical studies of ischemic stroke, TBI, ICH, SAH, spinal cord injury, cardiac arrest, and CNS malignancies [179,180,183,184,187,193,201–215]. Nanoparticle-mediated delivery of glibenclamide for targeted delivery is an area of active research [216].

Human Studies: SUR1 expression has been reported in human contusions, infarcted brain tissue, and brain tumors [178,194,200,217–219]. SUR1 levels in human CSF (both adult and pediatric) and possibly serum may also be informative biomarkers for secondary injury based on exploratory studies in TBI and SAH [220–222]. SUR1 (encoded by *ABCC8*) and TRPM4 genetic variation may also impact the extent of cerebral edema, ICP, hemorrhage progression, and outcome [8,223–225]. The biological mechanism by which this occurs is currently unknown but may be related to transcriptional regulation and mRNA levels (Jha et al., in press) and/or channel function/efficiency.

A retrospective study of oral glibenclamide (61 patients) demonstrated greater improvement in NIHSS ($p = 0.007$) and mRS < 2 ($p = 0.035$) at discharge in patients taking oral glibenclamide [226]. Benefit occurred only in patients with non-lacunar strokes. Another retrospective evaluation of diabetics with acute ischemic stroke revealed that continued use of their sulfonylurea medications was associated with reduced hemorrhagic transformation [227]. Small randomized trials (40–66 subjects) of oral glibenclamide have demonstrated slower contusion expansion rate, and improved outcome after contusional TBI and diffuse axonal injury [228,229]. In a study of patients with ischemic stroke (NIHSS ≥ 8 ; 213 patients), those treated with oral glibenclamide had lower cerebral edema and a trend toward less severe disability and death vs. controls, without increased risk of hypoglycemia [230]. The utility of oral glibenclamide is being evaluated in clinical trials of ischemic stroke ([NCT03284463](#), SE-GRACE) and spinal cord injury ([NCT02524379](#), SCING) [231,232]. In a retrospective review of oral glyburide (2.5 mg twice daily, 71 patients) hypoglycemia occurred in 23.9% of patients, with acute kidney injury and low body mass index being significant risk factors [233].

Intravenous glibenclamide (BIIB093) has emerged as a promising anti-edema/anti-secondary injury agent, particularly in large hemispheric infarction (LHI) but also in TBI [176,197,234–238]. In the first pilot trial (GAMES-pilot), feasibility of IV glibenclamide was reported in 10 patients with anterior circulation LHI [239]. Secondary analyses of these patients demonstrated successful reduction of vasogenic edema on T2 FLAIR MRI with intravenous glyburide [238]. After this initial safety/feasibility study, a phase 2 randomized controlled trial in anterior circulation LHI ($n = 86$, age 18–80) was completed in 2016 [197]. Although there was no difference in the primary endpoint (90-day mRS 0–4 without decompressive craniectomy), there was a trend toward mortality benefit ($p = 0.06$), and significant reduction in MMP-9 levels ($p = 0.006$; marker of BBB integrity) and midline shift (from 8.5 to 4.6 mm; $p = 0.0006$) in the treatment group [197]. The inter-center variability in surgical decompression was thought to contribute to the failure to meet primary end point. Subsequent analyses of patients in this phase 2 study have yielded favorable long-term outcomes in patients < 70 years old, as well as a reduction in neurological and edema-related deaths [235,240]. Two studies of BIIB093 (phase 3 study in LHI [CHARM, [NCT02864953](#)]; phase 2 study in contusional TBI [ASTRAL, [NCT03954041](#)]) are actively recruiting.

3.6. VEGF – Bevacizumab

Mechanism: VEGF, a secreted glycoprotein, has long been known for its role in angiogenesis/vasculogenesis and stimulating endothelial cell proliferation/increasing vascular permeability [241,242]. The role of VEGF in the CNS is expansive and, as reviewed elsewhere, it has been implicated in neurodegenerative disease, neuroinflammatory/demolinating disease, peripheral nerve diseases, neuromuscular disease (amyotrophic lateral sclerosis), epilepsy, stroke, TBI, ICH, SAH and both primary and metastatic CNS malignancies [241,242]. There are several subclasses of VEGF (A–E), and VEGF receptors (1–3). VEGF-B is less angiogenic than VEGF-A [241]. VEGF-A is upregulated in astrocytes, endothelium, and macrophages after both *in vitro* and *in vivo* injury [8,243,244]. Many effects of VEGF are related to VEGF binding to tyrosine kinases cell surface receptors and downstream signaling pathways, including mitogen activated protein kinase (MAPK; proliferation including neural stem cells and neurogenesis, migration), PI3K-Akt (cell survival), and eNOS (vascular permeability). Several molecular mechanisms of VEGF-related increased microvascular permeability have been reported, including downregulation/degradation/ubiquitination of tight junction proteins (occludin, claudin), angiogenesis via endothelial progenitor cell recruitment, activation by MMP-9, and capillary endothelial transcytosis [8,50,242–247].

As is a common theme, effects of VEGF can be both beneficial (collateral formation, reparative angiogenesis, neuroprotection) as well as deleterious (BBB breakdown, vasogenic edema) depending on the specific pathways activated, timing, and cell-types affected. At low levels, VEGF-A is necessary for BBB integrity and endothelial cell survival; however, at high levels, it increases vascular permeability and cerebral edema [241,248,249]. Therefore, the safety/efficacy of VEGF modulation (inhibition or activation) will depend on the dose, timing, indication, and specificity.

In mouse models of glioblastoma multiforme (GBM) VEGF-A is overexpressed and is associated with unfavorable prognosis. Inhibition with bevacizumab (a VEGF antibody) normalized the vasculature and reduced vascular permeability, reducing edema [250]. Preclinical studies in ischemic stroke have demonstrated that VEGF administration worsened vascular edema and infarct size, and that blockade is beneficial [248,249]. However, via vasodilation, perfusion augmentation/penumbra rescue, and collateral induction, delayed VEGF treatment may also stimulate angiogenesis, neuroprotection and improve neurological function without worsening edema in both ischemic stroke and TBI models [241,251–253]. Delayed VEGF inhibition (bevacizumab) has been shown to exacerbate necrosis and neurological deficits in TBI, with no reduction in either vasogenic edema or BBB permeability [254].

Human Studies: Bevacizumab received accelerated approval in 2009 for recurrent GBM after several trials in patients demonstrated radiographic benefit on the BBB and on vasogenic edema [250,255,256]. However, several subsequent trials failed to show overall survival benefit in either recurrent or newly diagnosed GBM [250,257–262]. Other receptor tyrosine kinase inhibitors have shown similar results without improvement in overall

survival (cediranib, enzastaurin) [263,264]. This is possibly related to the divergent effects of VEGF on neuroprotection vs. vasogenic edema.

In ischemic stroke, TBI, and SAH patients, VEGF levels have been elevated in serum as well as CSF [265–267]. No clinical trials of inhibition in these populations have been reported or are currently listed on clinicaltrials.gov.

4. Recombinant Proteins

Over the last decade, hundreds of protein-based therapeutics have been approved in the US – including antibody-based drugs (‘biologics’), fusion proteins, growth factors, hormones, enzymes, and a variety of other molecular types [268]. The majority are recombinant proteins and are utilized in cancers and immune disorders. Based on known signaling pathways of cerebral edema as discussed above, several recombinant proteins have been tested in preclinical models with promising results. Preclinical research on recombinant proteins that impact tight/adherens junctions (fibroblast growth factors; FGF21, FGF20), chemokines (CCL₁₇-CCR4), and VEGF are discussed below.

FGF21 has been reported to have neuroprotective effects including enhancing angiogenesis; it is downregulated in the brain post-TBI and in cerebral ischemia [269]. Recombinant human FGF21 (rhFGF21) treatment post-TBI reduced cerebral edema, BBB breakdown and improved functional recovery by forming an FGF21/FGFR1/ β -klotho complex, likely via PPAR γ and upregulation of tight junction proteins ZO-1, occludin, VE-cadherin, and claudin-5 [270]. Recombinant human FGF20 (rhFGF20) also helped preserve BBB integrity by increasing tight and adherens junction proteins, and reducing IL-1 β , IL-6 and iNOS levels via AKT/GSK β and JNK/NF κ B pathways *in vitro*, and after *in vivo* TBI [271]. In this study, rhFGF20 also improved behavioral outcomes.

As discussed earlier, chemokines influence vasogenic edema, neuroinflammation, as well as cell migration for survival/recovery post-injury. CC chemokine ligand 17 (CCL₁₇) a specific ligand for the high affinity CC chemokine receptor 4 (CCR₄), is expressed in hippocampal neurons. CCR4 is expressed on microglia, neurons, astrocytes, monocytes and natural killer cells [272]. CCR4 activation by CCL₁₇ activates the downstream PI3K-Akt pathway associated with cell survival and proliferation; neuroinflammation and neuronal apoptosis were inhibited via suppression of Foxo1 (which increases pro-inflammatory cytokine production) [272]. In an ICH model, intranasal recombinant CCL₁₇ (rCCL₁₇) dependent activation of CCR4 reduced cerebral edema, neuronal apoptosis, and neuroinflammation and improved functional outcome [272]. The same group demonstrated that intranasal rCCL₁₇ facilitated hematoma resolution and improved long-term neurobehavioral outcomes post-ICH associated with increased CD163 expression through CCR4/ERK/Nrf2 signaling pathway [273].

A recent study explored the therapeutic potential of recombinant mouse VEGF-D (rmVEGF-D) in a murine model of ischemic stroke (middle cerebral artery occlusion) [274]. Intranasal rmVEGF-D administration reduced infarct volume, dendrite loss, and improved functional recovery. In this study, changes on cerebral edema or BBB were not reported.

Although rmVEGF-D therapy is in its infancy, it warrants further investigation. Modulation and timing may be important, particularly given the dual role of VEGF in both advancing neurogenesis/neuroprotection and worsening cerebral edema.

5. Gene Therapies

Gene therapy delivery has classically been divided into viral (adeno-, adeno associated-, lenti- retro- viral vectors) vs. non-viral approaches (CRISPR-based approaches, mesenchymal stem cell delivery, physical mediators like microbubbles/microparticles/nanoparticles)[275–278]. DNA- as well as RNA-based targets have been described (including RNA interference, microRNA); the strategies have predominantly included gene augmentation, modification, blockage (of defective gene) or replacement (of a deficient gene)[279]. The first clinical trial using gene therapy was reported by Rosenberg et al., in 1990 in patients with advanced melanoma [280]. Since then, the role of gene therapy has exponentially grown, and although historically the majority of studies have addressed cancer and monogenic disorders, indications have now widely expanded to include cardiovascular disease, infectious disease, and neurological disease [281]. This section summarizes recent advances in gene therapy pertaining to cerebral edema including microRNA targets as well as delivery mechanisms, currently these are all at the preclinical stage and not currently ready for clinical translation.

5.1. Micro RNA

MicroRNAs are endogenous small (~22nt, 19–25nt) non-coding RNAs discovered in the 1990s that bind to complementary target mRNAs via sequence complementarity, and either inhibit mRNA translation inhibition or facilitate their degradation [282–284]. The latest version of miRbase (v22) contains microRNA sequences from 271 organisms, >38,589 hairpin precursors and >48,860 mature microRNAs [285]. Multiple clinical trials using microRNA-based therapeutics are ongoing worldwide [286–288].

Current studies suggest that circulating microRNAs can be successfully used as noninvasive surrogate biomarkers for various CNS diseases including TBI, stroke, SAH and multiple neurodevelopmental and neurodegenerative disorders [289–292]. Several microRNAs (including miR-1228–5p, miR-1268a, miR-1268b, miR-4433b–3p, miR-6090, miR-6752–5p, and miR-6803–5p) have been identified as potential biomarkers predicting risk of cerebrovascular disorder before the clinical onset of ischemic stroke in human patients [293]. Others have been reported as influencing (increasing/decreasing) secondary injury after TBI and ischemic stroke. Increased expression of some microRNAs (miR-93, miR-126, miR-210, and miR-130a) is associated with cerebral edema and BBB disruption in preclinical models [294–297]. Downregulation of others (miR-374, miR-29b) increases infarct volume and neuronal apoptosis [298–300]. Moreover, loss of miR-29b post-acute ischemic stroke contributes to neural cell death and infarct size [300]. *In vitro* and *in vivo* models demonstrate that miR-126–3p and 5p are anti-inflammatory; miR-126–3p is thought to promote angiogenesis and attenuate BBB disruption, and –5p promotes endothelial proliferation and survival. A clinical study in 197 patients with ischemic stroke

demonstrated markedly reduced miR-126 (and miR-30a) levels vs. 50 healthy volunteers [301].

MicroRNAs have also been evaluated for their therapeutic potential in CNS disease. For example, miR-21 and miR-21-5p have been demonstrated to confer neuroprotection, improved cognitive function and alleviate BBB disruption via MAPK and PTEN/Akt signaling pathways [302,303]. Similarly, miR-223 has been shown to be neuroprotective by targeting glutamate receptors [304]. Exosomes derived from mesenchymal stromal cells express miR-17-92 and miR-410 or miR-206 knockdown and confer neuroprotection [305–307].

In addition to microRNAs, the long noncoding RNA (lncRNA) MALAT1 has been demonstrated to ameliorate TBI-induced brain edema by inhibiting AQP4 and NF- κ B/IL-6 [308,309]. Intravenous delivery of the exosomes derived from adipose-derived stem cells containing MALAT1 lncRNA revealed that the exosomes migrated into the spleen ~1 hour after injury in a contusional model of TBI, and entered the brain several hours later. Treatment with MALAT1 containing exosomes improved motor impairment and reduced lesion volume [309]. Systemic delivery of bone marrow stem cell-derived exosomes nucleofected with miR-193b-3p has been shown to attenuate neuroinflammation, suppress the activity of HDAC3, upregulate acetylation of NF- κ B p65, mitigate neurological behavioral impairment, reduce brain edema and BBB injury as well as neurodegeneration after SAH [310]. While there are no data in humans, such studies highlight the therapeutic potential for microRNAs in the treatment of secondary injury processes such as cerebral edema after several forms of ABI.

5.2. Viral Vectors

Studies of adenoviral and lentiviral vector delivery of different therapeutic molecules have successfully demonstrated the ability to reduce secondary injury (cerebral edema, BBB disruption, proinflammatory cytokine secretion) and improve functional outcome in preclinical models of stroke, ICH, SAH and TBI [292,311–317]. Not surprisingly, several of the downstream targets in these studies are familiar players, including AQP4, MMP9, NOS, inflammatory cytokines (IL1b, TNF-a), tight-junction proteins (E-selectin), and adhesion molecules (VCAM-1).

Examples of preclinical studies evaluating viral-vector-based treatment for cerebral edema are presented here. As discussed in Section V-A, miR-126-3p/5p is important for maintaining BBB integrity, angiogenesis and reducing cerebral edema, with levels being reduced after ischemic stroke. Lentiviral-mediated overexpression of miR-126-3p/5p in a mouse middle cerebral artery occlusion (MCAO) model reduced brain infarct and cerebral edema volumes, and improved behavioral outcome [292]. Further analyses demonstrated that injection of lentiviral-miR-126-3p or -5p downregulated the expression of proinflammatory cytokines (IL-1b, TNF α), VCAM1, and attenuated the ischemia-induced decrease in ZO-1. In subarachnoid hemorrhage, mammalian-sterile 20 like kinase 1 (MST1) was knocked down via intraventricular injection of AAV packaged with MST1 short hairpin. This successfully reduced BBB disruption, edema and white matter injury via downstream NF- κ B and MMP-9 signaling [315]. N-myc downstream regulated gene 2 (NDRG2),

expressed in mammalian astrocytes, can worsen cerebral edema when deficient via changing the distribution of AQP4 and the Na⁺/K⁺/ATPase pump [316]. In a mouse MCAO model of transient cerebral ischemia and reperfusion, 5 days after intraventricular injection with an NDRG2 overexpressing lentivirus, brain water content was significantly decreased, as was BBB permeability [316].

One disadvantage of viral vectors (particularly adeno-associated viral vectors, AAV) is the potential for eliciting a potent immune response which may limit benefit in acute CNS injury [318]. It is also important to note that AAV clearance may be affected in aged and diseased brains via the glymphatic system and function of AQP4[319]. Advantages include the availability of multiple serotypes, vector modification to limit tropism to a specific cell-type, lack of integration into the host genome in certain types of viral vectors, and machine learning-based creation of numerous diverse AAV capsid variants [279,320–322]. Although there are several ongoing clinical trials using AAV gene transfer in the CNS (Alzheimer's, Parkinson's), viral vector-based gene therapy is not yet being evaluated clinically in acute brain injuries or for cerebral edema [323].

5.3. CRISPR

Attempts to harness the 2020 Doudna-Charpentier Nobel prize winning discovery (in chemistry) of CRISPR to treat human disease are ongoing, with phase 1 reports emerging in cancer [324–327]. However, unlike cancer or monogenic disorders, cerebral edema in many cases is a reactive and acute condition. Thus, permanent genetic modification may not be desirable. Off target effects and limited efficiency may further limit current clinical utility, although ongoing research has demonstrated improved editing efficiency in several cell types *in vitro* and *in vivo* by using different Cas9 proteins. Moreover, off-target effects in both mouse and human cell lines have been improved by several techniques including optimizing single guide RNA sequence/design rules [328–331].

Despite the unclear role of CRISPR/Cas9 technology in clinical treatment of cerebral edema, given the limitations above, it is being used to further understand edema pathobiology in preclinical models. Recently, AQP4ex (the extended isoform of AQP4a containing a 29 amino-acid C terminal extension), has been identified *in vitro* as the potential modulator of supramolecular organization and regulation of water channel activity [332]. CRISPR/Cas9 technology was used to generate an AQP4ex^{-/-} mouse. In these mice, although total AQP4 protein expression was unchanged, removal of AQP4ex completely suppressed the location of AQP4 at the astrocyte endfeet [333]. Similarly, although the number of AQP4 orthogonal arrays of particles (OAP) remained unchanged, the OAP size was markedly reduced.

This has important implications for cerebral edema generation and may valuably inform future targeted therapies. Both homozygous and heterozygous *ABCC8* (encoding SUR1) mutant cell lines have been generated using CRISPR/Cas9 technology[334,335]. These were generated to evaluate effects of *ABCC8* genetic mutations on congenital hyperinsulinism and revealed defective K_{ATP} channels unresponsive to diazoxide. Nonetheless, this technique may be valuable for understanding the biological consequences of important *ABCC8* genetic variation contributing to cerebral edema and hemorrhage progression (described in Section III-E).

6. Conclusions

We have reviewed fundamental principles underlying the clinically relevant and molecularly complex phenomenon of cerebral edema. Unchecked, it causes morbidity and mortality across several different types of acute and chronic brain injury. Basic physical principles such as the Monro-Kellie doctrine and Starling-forces have long been recognized as the drivers of edema across intracranial compartments – the former having particular bearing on the development of intracranial hypertension and herniation. While the cerebrovascular and CSF circulation systems are putatively major contributors to edema development, there is emerging evidence of key involvement from the glymphatic system and meningeal lymphatic vessels. The spectrum of cerebral edema can be classified as cellular/cytotoxic edema, ionic edema, and vasogenic edema – with overlapping molecular mechanisms. The clinical manifestation of cerebral edema however, varies by disease type and acuity – e.g., the timeline, extent, and predominant components of edema in stroke may vary from TBI despite having some shared molecular channels and mechanisms. Preclinical and clinical research has identified several promising candidates for targeted inhibition including AQP4, AVP, COX2, S1P, SUR1-TRPM4, and VEGF- these targets have demonstrated importance in edema pathogenesis across several different types of brain injury. Other emerging therapies include recombinant proteins (e.g. FGF21 and CCL17) as well as gene therapies (micro-RNA, viral vectors, and CRISPR). Ultimately, a nuanced and precision-medicine approach that endophenotypes the constellation of molecular drivers of cerebral edema in different patients may guide targeted modulation of the complex underlying pathophysiology and thereby improve outcomes for patients across a wide range of affected diseases.

7. Expert Opinion

Cerebral edema is a complex pathobiological phenomenon, and a key determinant of secondary injury that occurs in almost all types of acute neurological insults. Untreated, cerebral edema can be fatal. The landscape of cerebral edema diagnosis and management is evolving. Current practices are reactive; they address existing edema and attempt to minimize the downstream deleterious consequences such as intracranial hypertension and herniation. Recent key developments in this area include emerging therapies that focus primarily on molecularly derived approaches to prevent or minimize edema formation by targeting key pathways/contributors discussed in this review including AQP4, AVP, COX2, S1P, SUR1-TRPM4, and VEGF. These targets were identified based on data from *in vitro*, *in vivo* preclinical and clinical studies. Inhibition of SUR1-TRPM4 and VEGF are currently the most advanced in terms of clinical trials and translation to humans. However, VEGF inhibition with bevacizumab, despite encouraging anti-edema effects radiographically, has not demonstrated improved survival in GBM. Alternative modalities such as recombinant proteins and various gene-based therapies are being developed (many with similar/known molecular targets involved in edema pathophysiology); these newer modalities are currently in their infancy with regards to clinical translation, but early preclinical reports are encouraging. However, despite this exponential growth in research, several gaps remain including a comprehensive understanding of how these mechanisms interact with and influence each other, disease heterogeneity, translation across species, molecularly endophenotyping patients using biomarkers (fluid, imaging, and genetics) to

identify key pathways activated in individuals. It is therefore crucial, for researchers in this space, to continue rigorous, reproducible and blinded preclinical studies of therapeutics in different models of brain injury, and across species. Similar to the field of oncology, it is likely that combination therapies may be required depending on individual profiles/signatures of cerebral edema. Therefore, in addition to drug development, a parallel focus on biomarkers and deep endophenotyping of cerebral edema is likely crucial for successful translation. Over the next few years, the advent of technologies such as single cell gene expression and spatial transcriptomics may further inform identification of key/novel contributors to cerebral edema and important differences based on variables such as age, sex, race, and types of primary brain injury. Given the potential teleological role of edema, it may also be important to harness components that are necessary for repair/regeneration while minimizing the harmful processes and impact on intracranial pressure. Given the complex interplay between harm and benefit/neuroprotection of many of the discussed molecular targets involved in edema generation, a nuanced modulation of dose and timing based on both the individual and the specific disease/pathways will likely be critical to reducing this form of secondary injury and improving clinical outcome.

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R.M.J. provides consulting services for Biogen and is on the advisory board for ASTRAL. J.M.S. holds a US patent (7,285,574), 'A novel non-selective cation channel in neural cells and methods for treating brain swelling.' J. M.S. is a member of the Board of Directors and holds shares in Remedy Pharmaceuticals and is a paid consultant for Biogen. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Article Highlights

- Cerebral edema is a complex and heterogeneous process that contributes to morbidity and mortality in several different types of acute and chronic brain injury.
- The relationship of cerebral edema with intracranial pressure is nuanced and related via the Monro-Kellie doctrine as well as principles of intracranial compliance/elastance and autoregulation.
- Current treatment modalities while potentially life-saving (like decompressive craniectomy), are reactive, non-specific, invasive, and carry significant risks to patients.
- The cerebrovascular system and CSF are major sources of cerebral edema contributing to the spectrum ionic, cellular/cytotoxic and vasogenic edema – with overlapping contributory molecular mechanisms. Emerging evidence suggests key involvement of the glymphatic system and meningeal lymphatic vessels to the development of cerebral edema.
- Recent research has identified promising molecular targets to treat and potentially prevent cerebral edema – those that are advancing in clinical trials are discussed here, including AQP4, AVP, COX2, S1P, SUR1-TRPM4, VEGF. These targets include both ion and water channels as well as proteins/lipids involved in edema signaling pathways. Of these, SUR1-TRPM4 inhibition is actively in phase-2 and-3 clinical trials for TBI and large hemispheric infarction respectively.
- Several treatment modalities being developed include recombinant proteins (e.g. FGF21, CCL17) and different types of gene therapies (micro-RNA, viral vectors, CRISPR). These are currently in their infancy with regards to clinical translation but are gaining momentum in preclinical studies.
- Ongoing challenges for successful translation include identifying different molecular ‘signatures’ of cerebral edema to inform combination therapies, rigor/reproducibility across species, parallel development of biomarkers (fluid, imaging, physiologic neuromonitoring, genetics), and identifying differences in pathophysiology based on important variables like age, sex, race, and disease type.
- A granular precision-medicine and combination-therapy approach may be important for mitigating the deleterious effects of cerebral edema and improving clinical outcomes. This strategy includes molecularly endophenotyping drivers of cerebral edema to inform individualized targeted modulation of this complex process – reducing the harmful effects while maintaining components important for neuro-regeneration and repair.

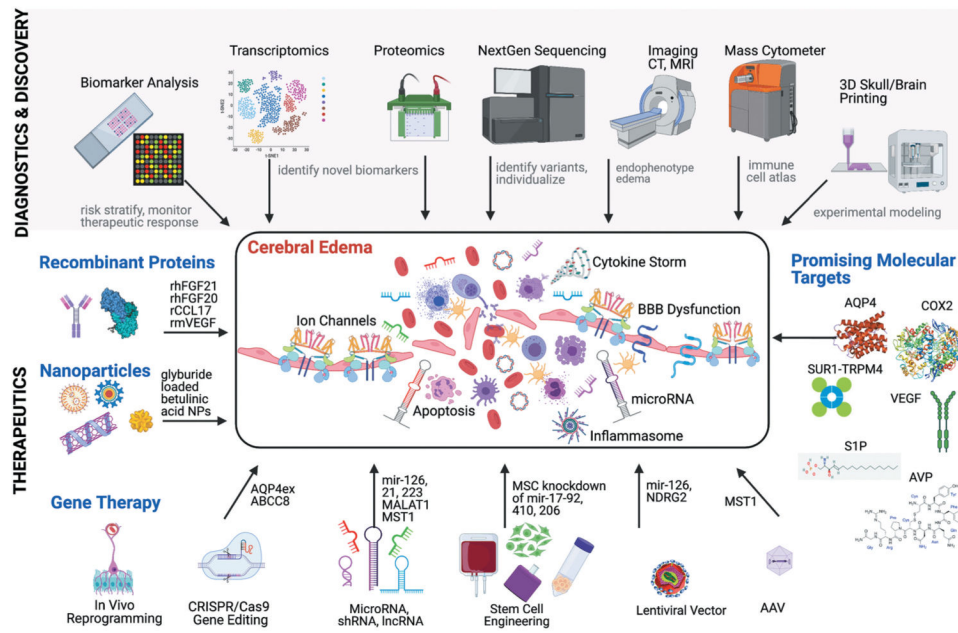
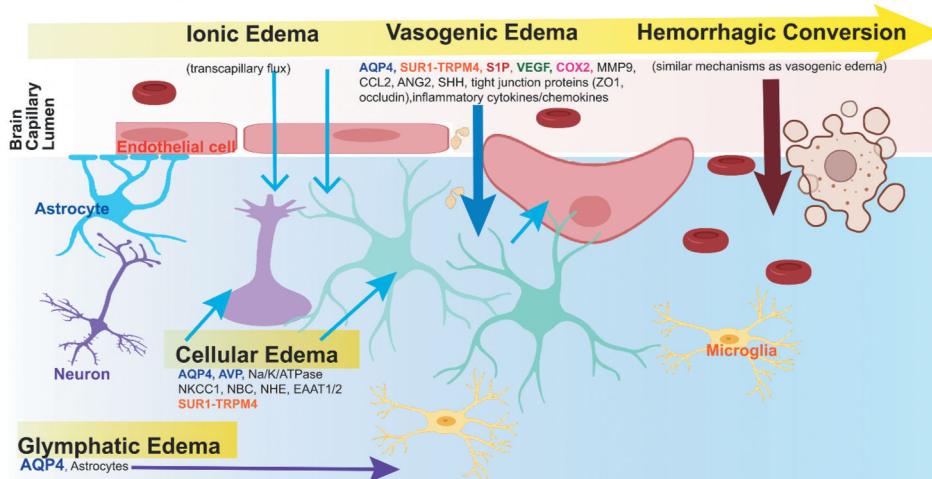


Figure 1. Emerging Therapeutic Strategies for Cerebral Edema.

Schematic showing advances in diagnostics and discovery technologies (shaded pink) that have the potential to inform and identify key molecular contributors to cerebral edema. These include biomarker analysis (for risk stratification, theragnostics, and monitoring the therapeutic response), transcriptomics and proteomics (for unbiased identification of novel RNA and protein-based biomarkers and therapeutic targets), NextGen sequencing (to identify important variants that may alter host response to injury and inform individualized targets), advanced imaging (for edema endophenotyping), mass cytometry (immune cell atlas to determine cellular properties), and 3D printing (to facilitate experimental modeling). The bottom panel highlights some novel anti-cerebral edema therapies under development including recombinant proteins (rhFGF21, rhFGF20, rCCL17, rmVEGF), nanoparticles (loaded with glyburide/glibenclamide), gene therapies (including CRISPR/Cas9 gene editing, microRNA, shRNA and lncRNA targets, AAV and lentiviral vectors), and current promising molecular targets with therapeutic agents in clinical trials (AQP4, AVP, COX2, S1P, SUR1-TRPM4 and VEGF). The inset rectangle depicts various contributors to cerebral edema (ion channels, inflammasome, BBB dysfunction, and cytokine storms) as well as potential detrimental after-effects (apoptosis). Figure created with the assistance of BioRender.

Abbreviations: AAV = adeno associated virus; ABCC8 = ATP Binding Cassette Subfamily C Member 8; AQP4 = aquaporin4; AVP = arginine vasopressin; BBB = blood–brain barrier; COX2 = cyclooxygenase 2; CRISPR = clustered regularly interspaced short palindromic repeats; rhFGF = recombinant human fibroblast growth factor; lncRNA = long non-coding RNA; MALAT1 = Metastasis Associated Lung Adenocarcinoma Transcript 1; MST1 =mammalian-sterile 20 like kinase 1; NDRG2 = N-myc downstream regulated gene 2; rCCL17 = recombinant CC chemokine ligand 17; rmVEGF = recombinant mouse vascular endothelial growth factor; RNA = ribonucleic acid; shRNA = short hairpin RNA; S1P = sphingosine 1 phosphate; SUR1 = sulfonylurea receptor 1; TRPM4 = transient receptor potential cation channel subfamily M member 4; VEGF = vascular endothelial growth factor

A: Molecularly Related Spectrum of Cerebral Edema



B: Promising Molecular Targets for Cerebral Edema Treatment

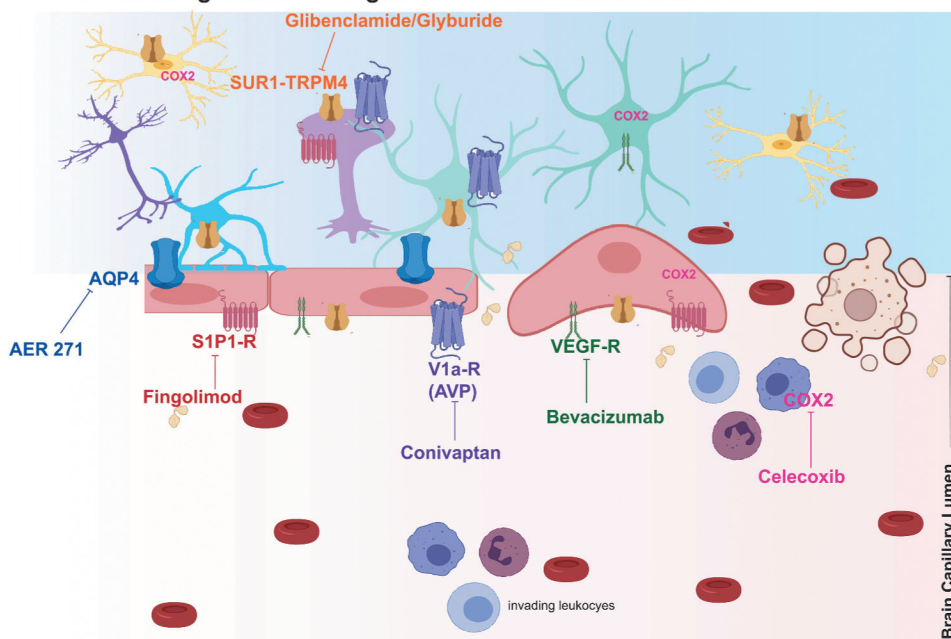


Figure 2. Promising Molecular Targets and Pathophysiologic Evolution of Cerebral Edema. (A) One side of the capillary endothelium is used to demonstrate the molecularly related spectrum of cerebral edema as it progresses from cellular/ionic to vasogenic to hemorrhage progression. Key mediators for each step are listed under the edema subtype, with evidence of molecular overlap. Mediators involved with ionic and cytotoxic edema are listed under the respective subheadings with the direction of water movement shown with light blue arrows. Cellular edema affects neurons (purple) astrocytes (blue-green), microglia (yellow), and endothelial capillary cells (red). Ionic edema results in transcapillary flux of water (light blue open head arrows) due to ion channels present on the luminal and abluminal surfaces, whereas cellular/cytotoxic edema results in water influx into the cell without equivalent efflux (light blue closed head arrows). Vasogenic edema, results in paracellular protein extravasation along with water (dark blue arrow) into the interstitial

space due to mechanisms that result in blood–brain barrier disruption (e.g. disruption of tight junctions, inflammation, endothelial cell retraction, mechanisms that cause cellular swelling and oncotic cell death in endothelial cells or astrocyte podocytes such as via SUR1-TRPM4). Ultimately, complete breakdown of the blood–brain barrier (phase-3) results in red blood cell extravasation along with water and plasma proteins, resulting in hemorrhagic progression. The relatively more recent contribution of the glymphatic system to cerebral edema (purple arrow) involves CSF contribution to water and ions entering the interstitium and perivascular spaces, possibly mediated by AQP4.(B) Schematic showing key molecular targets that contribute to cerebral edema and the corresponding pharmacological agents being evaluated in clinical trials. These targets are distributed on multiple cell types and include ion channels (SUR1-TRPM4, yellow; AQP4, blue), G-protein receptors for S1P (S1P1-R, red) and AVP (V1a-R, purple), tyrosine kinase receptors for VEGF (VEGF-R, green), and COX2 (enzyme that catalyzes conversion of arachidonic acid to prostaglandins).

I. INTRODUCTION

II. CEREBRAL EDEMA PATHOGENESIS

A. BASIC FORCES

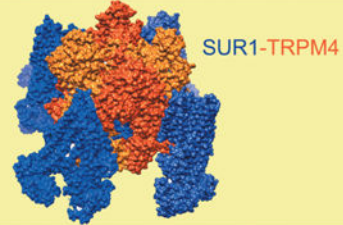
MONRO KELLIE
STARLING FORCES

B. PATHOPHYSIOLOGY & SYSTEMS FACILITATING FLUID MOVEMENT

CEREBROVASCULAR AND CSF CIRCULATION
CELLULAR (CYTOTOXIC)
IONIC
VASOGENIC
GLYMPHATIC SYSTEM
MENINGEAL LYMPHATIC VESSELS

III. NEXTGEN EMERGING TARGETS

- A. AQP4-AER 271
- B. AVP-CONIVAPTAN
- C. COX2- CELECOXIB
- D. S1P- FINGOLIMOD
- E. SUR1-TRPM4 - GLIBENCLAMIDE
- F. VEGF- BEVACIZUMAB



IV. RECOMBINANT PROTEINS

V. GENE THERAPIES

- A. MICRO-RNA
- B. VIRAL VECTORS
- C. CRISPR

MICRO-RNA DUPLEX



ADENO ASSOCIATED VIRUS

VI. CONCLUSIONS

VII. EXPERT OPINION

Box1.

Review Article Outline.

Table 1. Summary of Completed and Ongoing Clinical Trials Targeting Specific Molecular Contributors to Cerebral Edema.

Trial Completed	Reference	Target	Drug and Dose	Phase	Sample Size	Population	Key Findings
NCT03000283	Corry et al., Clin Drug Invest, 2020	AVP	Conivaptan 20 mg twice daily X 2 days	1	7	Intracerebral hemorrhage	No safety concerns ↑ in serum sodium (p =0.01) No rescue therapy for intracranial hypertension required
NCT00526214	Lee et al., Eur J Neurol, 2013	COX2	Celecoxib 400 mg twice daily X 14 days	NA	44	Intracerebral hemorrhage	No effect on blood pressure or heart rate ↑ in perihematomal edema (p =0.005) ↑ in hemorrhage progression (p =0.046)
NCT02002390	Fu et al., JAMA Neurol, 2014	SIP	Fingolimod 0.5 mg daily X 3 days	2	23	Intracerebral Hemorrhage	↑ in perihematomal edema (p =0.04, relative perihematomal edema p =0.003) ↑ neurologic deficits (p =0.001)
NCT01268683, (GAMES Pilot)	Fu et al., PNAS 2014	SURI-TRPM4	Intravenous glyburide (RP1127) 3 mg/day	1	10	Acute Ischemic Stroke	↑ circulating lymphocytes ↑ neurologic deficits (p =0.0001) ↑ vascular permeability (p =0.005)
NCT01794182 (GAMES RP)	Sheth et al., Stroke, 2014	SURI-TRPM4	Intravenous glyburide (RP1127) 0.13 mg bolus 0.16 mg/h X 6 h 0.11 mg/h X 66 h	2	86	Large Hemispheric Infarction	Well tolerated, no dose reductions required Secondary analysis- decreased vasogenic edema on MRI
NCT00943826	Chimot et al., NEJM, 2014	VEGF	Bevacizumab 10 mg/kg every 2 weeks (in conjunction with radiotherapy and temozolomide)	3	452	Newly Diagnosed Glioblastoma Multiforme	No change in 90 day mRS without decompressive craniectomy Trend towards improved survival (p =0.06) ↑ matrix metalloproteinase levels with treatment (p =0.006) ↑ midline shift (8.5 to 4.6 mm) with treatment (p =0.0006)
NCT00884741	Gilbert et al., NEJM, 2014	VEGF	Bevacizumab 10 mg/kg every 2 weeks (in conjunction with radiotherapy and temozolomide)	3	637	Newly Diagnosed Glioblastoma Multiforme	↑ in progression free survival (p =0.007) No change in overall survival (p =0.21)
No identifier	Friedman et al., J Clin Oncol, 2009	VEGF	Bevacizumab 10 mg/kg (in conjunction with irinotecan)	2	167	Recurrent Glioblastoma Multiforme	↑ in tumor area, radiographic markers of edema, steroid requirements.
Ongoing							
NCT03804476	NA	AQP4	AER-271	1	80 (planned)	Healthy Subjects	Recruiting Results to inform Phase-2 in ischemic stroke
NCT03338998	NA	SIP	Siponimod (BAF312)	2	60 (planned)	Intracerebral Hemorrhage	Recruitment Suspended (COVID19)
NCT02524379 (SCING)	NA	SURI-TRPM4	Oral glyburide	1-2	10 (planned)	Spinal Cord Injury	Recruiting

Trial Completed	Reference	Target	Drug and Dose	Phase	Sample Size	Population	Key Findings
NCT03284463 , SE-GRACE	NA	SURI-TRPM4	Oral glyburide	2–3	306 (planned)	Acute Ischemic Stroke	Recruiting
NCT02864953 (CHARM)	NA	SURI-TRPM4	Intravenous glyburide (BIIB093)	3	768 (planned)	Large hemispheric infarction	Recruiting
NCT03954041 (ASTRAL)	NA	SURI-TRPM4	Intravenous glyburide (BIIB093)	2	160 (planned)	Contusional traumatic brain injury	Recruiting