Study Title: Fingolimod as a Treatment for Cerebral Edema after Intracerebral Hemorrhage

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Background/Rationale and Context:

1.1 Overview

Spontaneous intracerebral hemorrhage (ICH) is a catastrophic illness representing a global public healthcare problem. Unfortunately, there currently exists no specific treatment outside of supportive medical care. Of the 15 million strokes reported worldwide per annum, ICH accounts for approximately 10-15% of all stroke cases in the US, Europe and Australia; and approximately 20-30% of strokes in Asia.[1] This is associated with a 40% 30-day mortality rate[2], and those who survive have major neurologic deficits, with only around 20% of patients reaching functional independence at 6 months.[3] Although there has been a significant reduction in overall age-adjusted incidence of stroke, the incidence of ICH has remained unchanged in the past 40 years.[2] In the US alone, the annual healthcare costs of ICH is estimated at $12.7 billion.[4]

Hospitalizations for ICH have increased by 18% in the last decade[5], most likely due to the aging population and the increasing use of anticoagulants, thrombolytics, and antiplatelet agents. The most important risk factor is age. With each advance in decade after the age of 50, there is a 2-fold increase in ICH incidence.[6] ICH due to hypertension (HTN) accounts for approximately 65% of all spontaneous ICH[7] and 50-70% of patients are hypertensive at the time of hemorrhage.[8] ICH in the setting of HTN commonly occurs in the deep cerebral locations (basal ganglia, thalamus), followed by cerebral lobes, cerebellum, and brainstem (predominantly pons).[6] ICH occurring in the setting of cerebral amyloid angiopathy almost always present in a lobar location. Other causes of ICH include neoplasms, aneurysms, arteriovenous malformations (AVM), cerebral cavernous malformations, arteriovenous fistulae (AVF), and venous thrombosis.[7]

1.2 Primary Brain Injury

Primary brain injury from ICH typically occurs within the first few hours of ictus and is due to hematoma formation, leading to mechanical damage of the adjacent tissues from dissection and compression. The subsequent brain injury that occurs after an ICH typically involves both grey and white matter.[7] Hemorrhagic volume is an important factor in determining ICH outcome, with hemorrhage volumes > 100mL associated with poor prognosis.[9] The mass of the hemorrhage can increase intracranial pressure, leading to further compression of brain structures, which can potentially impact cerebral blood flow and lead to brain herniation. Because of the consequences due to mass effect, recent clinical trials have focused on examining the effects of early clot removal. New approaches to limit surgical morbidity are underway with promising results.[10-14] Location of the ICH is important for outcome, and it should be noted that guidelines support the evacuation of cerebellar clots as potentially life and function saving, whereas thalamic hemorrhage has a notoriously poor outcome despite treatment.[15] Although evacuation of the hematoma addresses the issue of physical disruption and mass effect, it does not address the sequelae of secondary brain injury from the body/tissue response to the hematoma.

1.3. Secondary Brain Injury

Secondary injury plays a significant role in the neurologic decline of ICH patients.[16] This is triggered by the presence of intraparenchymal blood, which activates cytotoxic, excitotoxic, oxidative, and inflammatory pathways[17], and is characterized by the mobilization and activation of inflammatory cells. It is believed that the microglia and astrocytes are the earliest of the inflammatory cells to respond to extravasated blood.[18] The activation of microglia leads to infiltration of various circulating inflammatory cells, most notably leukocytes, macrophages, and T-cells. This subsequently leads to the release of inflammatory cytokines (e.g. IL-1β, TNF-α, INF-γ**)** chemokines, free radicals, various adhesion molecules, cell surface receptors, and inflammatory enzymes (e.g. iNOS, COX-2, PLA2) coordinated via the transcription factor nuclear factor kappa B (NF- κB).[19, 20] These chemicals, coupled with cell death products, further activate intrinsic and migrating lymphocytes, leading to increased infiltration of lymphocytes and a continued cycle of inflammatory response. There is increasing evidence that this inflammatory response contributes to the formation of edema around the hematoma, which exacerbates mass effect, augments the cell death process through secondary ischemia, and produces further inflammatory insults to the surrounding brain tissue.[21] This pro-inflammatory state begins immediately and augments breakdown of the hematoma, with phagocytosis and increased blood brain barrier permeability. This gives way to a more anti-inflammatory phenotype around day 3 to begin the healing and rebuilding process.

Table 1. Different components of inflammation

Pro-inflammatory components	Anti-inflammatory components
M1 microglia	M2 microglia
Th1 cells	Th2 cells
Th17 cells	Treg cells
IL-1β	IL-4
IL-6	IL-10
IFN-γ	TGF-β
TNF-α	

1.3a Microglia (Table 2)

Microglia are the resident macrophages of the central nervous system and play an important role in the inflammatory and recovery phases as a result of injury to the CNS. They are vital in the maintenance of the overall homeostasis of the CNS, including clearance of pathogens and pathologic neurons, and remodeling of the extracellular matrix and synapses.[22] They account for approximately 5-20% of the total CNS glial population.[23] Under normal conditions, microglia reside in their ramified state, performing surveillance of the CNS. They contain short and fine processes, allowing for constant monitoring for any potential changes to the local brain milieu or pathogen/injury.[24] Once activated, they become morphologically indistinguishable from the infiltrating macrophages, becoming more amoeboid in shape with stout processes to allow for the phagocytosis process.

Microglia/macrophages are activated very early following ICH, occurring as early as 1 hour after ictus. In the murine model, once microglia have been activated, IL-1β can be detected as early as 6 hours, and may persist up to 24 hours.[25] By 72 hours, microglia/macrophages have reached their peak, and by day 7, the numbers begin to decrease. By 21 days, the microglia/macrophages have returned to their baseline numbers.[26] Once tissue damage occurs, microglia can be activated within minutes via neurotoxic substances released into the extracellular space by necrotic neurons.[27]

Based on ischemic injury and TBI models, two microglia phenotypes have been identified. The M1 phenotype is considered pro-inflammatory/damage-inducing and is known to produce inflammatory cytokines (IL-1β, IL-12, IL-23, and TNF-α), chemokines, nitric oxide (NO), and reactive oxygen species

(ROS), which initiates the breakdown process and the opening of the blood brain barrier (BBB).[26, 28] The M2 phenotype is considered to be anti-inflammatory and is generally involved in phagocytosis, release of trophic factors, and the overall regeneration and resolution of inflammation.[29] The presence of anti-inflammatory cytokine IL-4 can prime the polarization to M2 phenotype, leading to the production of IL-10 and upregulation of M2 specific surface phenotype markers. [29]

In the setting of spontaneous ICH, murine models demonstrated the acute increase in M1 microglia within 6 hours of ictus that decreased over the course of 14 days, whereas the M2 phenotype was noted at day 1 and increased over the course of 14 days.[30, 31] Most models indicate that a M1 to M2 phenotype switch occurs within the first 7 days of hemorrhage, but the exact timing and driving force of the phenotype switch remain unclear.[29] With the various roles they play during CNS development, injury, aging and associated neurodegenerative diseases, the microglia are not static and have a great deal of plasticity, allowing alterations to either phenotype based on cues from the microenvironment.[32, 33]

Table 2. Microglia phenotype and functions

Phenotype	Molecule	Type	Role
M1	IFN-γ	Cytokine	Pro-inflammatory, induces M1 phenotype
	IL-1β, IL-6, IL-23, TNF-α	Cytokine	Pro-inflammatory
	IL-12	Cytokine	Involved in the differentiation to Th1 cells
	IL-17	Cytokine	Pro-inflammatory, modulating factor of cytokine production
	ROS, iNOS	Metabolic enzyme	Oxidative damage
	CD11b, CD16, CD32	Surface receptors	Phagocytosis, chemotaxis
	CD68	Surface receptor	Scavenger receptor
	CD86	Surface receptor	Surface receptor, otherwise called B5-2, interacts with CD28
	MHC-II	Surface receptor	Presentation of extracellular proteins
	NF-κB	Transcription factor	Participate in modifying the detrimental M1 phenotype
	STAT1, STAT3	Transcription factor	Elevate the expression of NF-κB/p65
M2	IL-4	Cytokine	Anti-inflammatory, induces M2 phenotype
	IL-10	Cytokine	Anti-inflammatory, human cytokine synthesis inhibitory factor
	TGF-β	Cytokine	Regulation of lymphocyte proliferation, differentiation, and survival; regeneration; upregulate Bcl-2 and Bcl-x1
	YM-1	Protein	Binds heparin/heparin sulfate, proposed to prevent degradation of extracellular components
	Arginase 1(Arg1)	Protein	Downregulation of nitric oxide synthesis. Induces fibrosis and tissue regeneration
	FIZZ1	Protein	Associated with reconstruction of extracellular matrix

	CD36	Surface receptor	Scavenger receptor, involved in hematoma resorption in setting of upregulation of PPAR-γ
	CD163	Surface receptor	Scavenger receptor for haptoglobin-hemoglobin complex
	CD206	Surface receptor	Antigen internalization and processing/mannose receptor type 1
	PPAR-γ	Transcription factor	Induce M2 phenotype
	STAT6	Transcription factor	Inhibit the activation of NF-κB
	SOCS3	Signaling modulator	Cytokine-inducible negative regulators of cytokine signaling

1.3b T lymphocytes (Table 3)

T lymphocyte functions have been well-characterized in ischemic stroke models[34], but their role in ICH is less defined. Much of the T lymphocyte descriptions in ICH are extrapolations from pre-clinical ischemic models. In the healthy brain, there are very low numbers of T cells functioning as CNS surveillance[35] and appear to enter through the cerebrospinal fluid either through choroid plexus veins or meningeal blood vessels to monitor the subarachnoid space, allowing for the ability to initiate a local immune response or return to secondary lymphoid organs.[36] Following a stroke, immunohistochemistry has identified different T cell subtypes invading the infarcted region. It is theorized that T-cells can cross the BBB once a T-cell response is activated by a CNS autoantigen without antigen specificity.[37] In the stroke model, $CD4^+$ and $CD8^+$ T cells have been reported to have a detrimental role in perpetuating ischemic tissue injury by contributing to the microvascular dysfunction caused by cerebral ischemia/reperfusion (I/R) injury.[38] This was confirmed by models of $CD4^+$ or $CD8^+$ deficiencies resulting in decreased stroke volume and improved neurologic performance.[17, 39] On the other hand, there has been an abundance of evidence that regulatory T cells have a neuroprotective effect in stroke and promote recovery via their immunomodulating properties.[40, 41]

It has been reported that there is an increase in $CD4^+$ T helper (Th) cells starting on day 1 and peaking around day 3-4 days after a hemorrhagic event.[41, 42] Guo *et al.* confirms the presence of Th cells in the perihematomal region in surgical specimens of patients with ICH.[43] Another study showed infiltrating lymphocytes peaking around day 3. This T cell population was noted to have an equal number of T- and B-lymphocytes, with a predominance of T-helper cells at that time point.[44] Regulatory T (Treg) cells have shown a similar pattern in timing, but the peak was sustained up to 7 days after ICH.[41] It has also been documented in pre-clinical stroke models that the T cells persist as late as 7 weeks post-stroke, but their function in this post-acute phase remains unclear.[45]

T helper cell differentiation and their role in CNS injury and neuroregeneration have undergone extensive research. In the 1980's, two subtypes of $CD4^+$ T cells were described based on their cytokine secretion patterns: Th1 and Th2 cells.[35] Th1 cells are programmed to fight intracellular pathogens via cell-mediated response. Th1 cell differentiation is initiated by IL-12 and INF-γ, known to perpetuate pro-inflammatory reactions. Although the presence of Th1 cells have been confirmed at the site of lesion (ischemic infarct or intracerebral hemorrhage), their role in the pathogenesis of secondary brain injury yet remains to be defined. A possible mechanism is that these cells may, in part, induce apoptosis of neurons via production of inflammatory cytokines. In the case of ischemic stroke, Th1 cells may contribute its pathogenesis by accumulating within the microcirculation and impairing perfusion, thereby aggravating the hypoxic strain.[37]

Alternatively, Th2 cells function to fight extracellular pathogens via the humoral response. Their differentiation is typically initiated by IL-4 and IL-2, and they secrete IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, which are involved in various anti-inflammatory functions. It is presumed that Th2 cells act by producing the above cytokines with downregulation, and in some cases, direct suppression of Th1 cells.[37] In an effort to better characterize the subsets and functions of Th1 and Th2 cells in ischemic stroke, a pre-clinical study found that Th1 deficiency dramatically inhibited infarct size, whereas Th2 deficiency worsened infarct size and neurologic outcomes.[46] There is further evidence to suggest that inhibiting the proliferation of Th1 improves neurologic outcomes, while the inhibition of Th2 worsens brain injury in the setting of cerebral ischemia.[47]

There is increased infiltration of regulatory T (Treg) cells in the setting of both ischemic infarct and intracerebral hemorrhage. These cells are known for their anti-inflammatory function by mediating immune tolerance to self-antigens. Treg cells have been shown to decrease ICH-induced inflammatory injury.[41] In a rat ICH model, Treg transfusion resulted in the inhibition of IL-1β, TNF-α, and MMP-2 in the perihematomal tissue and increased presence of anti-inflammatory cytokines IL-4, IL-10, and TGF-β.[48] Treg cells have also been known to decrease perihematomal edema and BBB permeability after ICH, leading to improved short-term and long-term neurologic function in the animal model.[49]

Table 3. T lymphocyte phenotype and functions

Phenotype	Molecule	Type	Role
Th1	IL-12, TNF-α	Cytokine	Activate macrophages and are responsible for cell-mediated immunity and phagocyte-dependent protective responses
	T-bet	Transcription factor	Induce Th1 phenotype, enhances production of IFN-γ and mediates repression of IL-4 expression
	STAT1	Transcription factor	Primarily regulates T-bet and mediates IL-4 repression to mediate Th1 cell commitment
Th2	IL-4, IL-5, IL-10, IL-13	Cytokines	Responsible for strong antibody production, eosinophil activation, and inhibition of several macrophage functions, providing phagocyte-independent protective responses
	GATA3	Transcription factor	Key for Th2 cytokine production; inhibition of Th1 responses
	STAT3	Transcription factor	Required by STAT6 for interaction with appropriate gene loci
	STAT5	Transcription factor	Independent of IL-4 signaling; coordinates with GATA3 to induce Th2 phenotype
	STAT6	Transcription factor	Upregulates expression of GATA3 and mediates Th2 cell phenotype
Th17	IL-17A/F, IL-21, IL22, IL-23, TGF-β	Cytokines	Creates inflammation and tissue injury in autoimmune diseases; unclear of function in ICH
	RORγt	Transcription factor	Master regulator to induce Th17 phenotype via STAT3 activation
	STAT3	Transcription factor	Activated by IL-6 and IL-23 to induce expression of RORγt

Treg	IL-2, IL-10, TGF-β	Cytokines	Essential for the maintenance of peripheral tolerance, prevention of autoimmune disease, and limiting chronic inflammatory disease
	Foxp3	Transcription factor	Major lineage-specific transcription factor involved in Treg differentiation
	STAT5	Transcription factor	Enhances Foxp3 expression to promote Treg development

1.4 Sphingosine 1-Phosphate Receptor Modulation

Fingolimod (FTY720) is an oral immunomodulatory agent approved by the Food and Drug administration in 2010 for the treatment of relapsing-remitting multiple sclerosis (MS). It has been shown to reduce the number of MS relapses and limits the loss of brain volume via several mechanisms.[50-52] FTY720 is a functional analogue of phosphorylated sphingosine, a bioactive sphingolipid. In nature, sphingosine is phosphorylated by sphingosine kinase 1/2 (Sphk) to sphingosine 1-phosphate (S1P), which then binds to G-protein coupled receptors called sphingosine 1-phosphate receptors (S1PRs). There are five known S1PR subtypes, $S1PR_{1\text{-}5}$. $S1PRs_{1\text{-}3}$ are broadly expressed in the immune and cardiovascular systems and the CNS. $S1PR_1$ is highly expressed on T and B lymphocytes. $S1PR_4$ is generally found in the hematopoietic and lymphoid tissues. $S1PR_5$ is predominantly found within the CNS white matter.[53] These receptors participate in a wide variety of biologic processes, including leukocyte recirculation, cellular proliferation and differentiation, morphologic changes, cellular motility, endothelial cell function, vascular regulation, and cardiovascular and nervous system development.[54] S1P and S1PRs play a key role in the maintenance of normal immune function. Once an appropriate activating antigen is encountered in the lymph nodes, naïve T cells become activated and enter the circulation to reach the corresponding site of inflammation.

FTY720 is a sphingosine 1-phosphate receptor (S1PR) modulator and functions to regulate various cellular responses, including proliferation, apoptosis, and inflammation.[53] FTY720 binds with a high affinity to the following S1PR subtypes: $S1PR_1$, $S1PR_3$, $S1PR_4$, and $S1PR_5$.[53] As noted above, $S1PR_1$ is present on lymphocytes to aid in the egress of T cells with the appropriate antigen stimulation. The classic mechanism of action of FTY720 is presumed to be hematopoietic. Although structurally similarly to S1P, FTY720 acts as a functional antagonist at $S1PR_1$, causing internalization and degradation of this receptor and removing the lymphatic S1P gradient necessary for lymphocyte egress. As a result, this inhibits the egress of lymphocytes from lymph nodes, thereby reducing the overall number of inflammatory cells migrating to the CNS.[55] However, based on the high expression of S1PRs in the CNS, a new aspect of FTY720 function is emerging, focusing on direct neurologic cellular modulation. FTY720 has been found to easily cross the blood-brain barrier in its unphosphorylated form, where it is then phosphorylated by endogenous CNS sphingosine kinase 2.[53] It is then presumed that FTY720 influences the biology of CNS cells such as astrocytes and oligodendrocytes by reducing astrogliosis and demyelination.[56]

The metabolism and pharmacokinetics of FTY720 have been studied both in animal studies and healthy volunteers. It has been found that FTY720 undergoes extensive metabolism, with biotransformation occurring mainly through three pathways. The first pathway is the reversible phosphorylation of fingolimod phosphate, the active metabolite, predominantly via sphingosine kinase 2, with a lesser contribution by sphingosine kinase 1. It is then dephosphorylated by lipid phosphate phosphohydrolases LPP1a and LPP3.[57] The second pathway of metabolism involves hydroxylation and oxidation via the hepatic cytochrome P450 (CYP) 4F subfamily of enzymes, which produces several inactive carboxylic acid metabolites that are then excreted in the urine.[58, 59] CYP4 enzymes are primarily known for their role in the metabolism of endogenous fatty acids, prostaglandins, and steroids, therefore the low potential for drug-drug interactions.[58] The third pathway involves the formation of inactive non-polar ceramides.[57] In single dose studies of both fingolimod and fingolimod phosphate, it has been found that FTY720 has high oral

bioavailability, with an absolute oral bioavailability of 93%.[60] After oral administration of a single dose, blood absolute maximum concentration is reached by 12-16 hours and found to have a long half-life of 6-9 days that is independent of drug dose.[61, 62] Additionally, after oral administration of a single dose of fingolimod, there is a decrease in the total lymphocyte count to approximately 60% of baseline within 4-6 hours of administration and begins to recover within a few days after stopping the drug.[60]

As mentioned previously, S1PRs are expressed essentially by all neural cell lines in the CNS, and level of receptor subtype expression can vary based on the type of cell, and in the case of microglia, based on their state of activation.[63] In the ramified state, microglia express $S1PR_1$, $S1PR_2$, $S1PR_3$, and $S1PR_5$, with $S1PR_1$ expression being the dominant receptor type.[53] It has been found that S1P and its subsequent pathway is essential in key neuron specific functions such as the regulation of transmitter release and the proliferation and survival of neurons and glia.[64] It has been reported that sphingosine kinase 1 plays a critical part in the inflammatory processes mediated by TNF-α in immune cells.[65] *In vitro*, once microglia have been activated, S1P has been noted to amplify the release of pro-inflammatory cytokines, and with the suppression of Sphk1, there was suppression of TNF-α, IL-1β, and iNOS gene expression.[66] This was confirmed *in vitro* with the binding of S1PRs with FTY720, which downregulated the production of pro-inflammatory cytokines and promoted the increased expression and release of neurotrophic factors. The effect on gene expression was also shown to be dose-dependent.[67, 68] In addition to gene expression regulation of inflammatory and chemoattractant molecules, functional antagonism of the S1PRs with FTY720 may also limit the inflammatory response of activated microglia by affecting the polarization of microglia. In ischemic white matter damage murine models, treatment with FTY720 enhanced the protein expression and phosphorylation level of STAT3, polarizing microglia to the M2 phenotype, which suggests that S1P could be involved in the activation of the JNK/STAT3 cascade that is typically associated with M2 polarization.[69] Alternatively, gene expression profiling has been performed to better understand the molecular effects of FTY720. It has been found that FTY720 inhibited the expression of important transcription factors, most importantly STAT1, thereby suppressing the activation of microglia (M1 phenotype).[70] The effect of FTY720 on microglial activation was confirmed *in vivo* on MS patients, where serial PET imaging was performed, showing decreased microglial activation at the sites of focal inflammatory lesions.[71]

Based on literature showing FTY720 and its protective effects from ischemia-reperfusion injury in heart, liver, and kidney[72, 73], several groups hypothesized the potential neuroprotective effect of FTY720 in the setting of cerebral ischemia. FTY720 showed a neuroprotective effect after middle cerebral artery occlusion in the rat model with significantly diminished infarct volume and improved overall neurologic scores at 24 and 72 hours[74] and fewer activated neutrophils and microglia/macrophages.[75] Based on this pre-clinical evidence, a small human pilot study was recently performed to evaluate the efficacy and safety of FTY720 in the treatment of acute ischemic stroke. Their clinical results mirrored the results seen in animal models, with reduced infarct size and improved clinical outcomes.[76]

Beyond ischemic modulation, FTY720 has also been shown to potentially reduce the incidence of reperfusion hemorrhage in the setting of delayed tPA administration via enhancement of the BBB integrity.[77] In light of this evidence, Lu *et al* studied the effects of FTY720 in the setting of ICH in the rat model. It was found that FTY720 significantly reduced perihematomal edema (PHE), apoptosis, and associated brain atrophy, but they did not find a difference in the number of inflammatory cells between the treated and control groups.[78] However, Rolland *et al* compared single dosing to 3-day dosing and found significant reduction in hematogenous and intracerebral lymphocytes compared to control rats for both dosing regimens.[79] They also found decreased expression of intercellular adhesion molecule-1 (ICAM-1), interferon-γ (INF-γ), and interleukin-17 (IL-17) at 72 hours. At 8 and 10 weeks post-ICH, treated rats were noted to have significantly reduced spatial and motor learning deficits, brain atrophy, and neuronal cell

loss.[79] Given the multiple subtypes of S1PRs that are present in the CNS and the multiple target receptors of FTY720, efforts have been made to elucidate specific receptors associated with the improved clinical outcomes after FTY720 administration. In experimental ICH models, a selective $S1PR_1$ immunomodulator was used as a treatment paradigm. In the presence of the $S1PR_1$ immunomodulation, there was significant attenuation of neurologic deficits and improved post-ICH survival. Overall lesion volume was reduced. There was decreased edema and midline shift noted on MRI, most likely due to the exhibited reduced counts of microglia and T cells with associated decreased production of pro-inflammatory cytokines IL-1β and TNF-α.[80]

A small clinical pilot study of 3-day dosing of fingolimod in ICH patients also exhibited radiographic evidence of smaller perihematomal edema, significant reduction in neurologic impairment and improvement in GCS by day 7. At 3 months, 63% of fingolimod-treated patients achieved full neurologic recovery compared to 0% in the control group. There were no differences in adverse event rates between the treated and control groups.[81]

1.5 Data Sharing

As a member of the International Stroke Genetic Consortium, Wake Forest collaborates to identify medical, environmental and genetic risk factors and causes of stroke to better care for our patients.

The Ethnic/Racial Variations of Intracerebral Hemorrhage (ERICH) study originally recruited over 3000 cases of spontaneous hemorrhage with equal power among white, black and Hispanic cases. ERICH-Gene will combine additional ICH cases from around the world to maximize the power of our study to identify novel genetic variants across ethnicities. The new goal is to perform centralized genotyping of 5,200 new ICH cases under the ERICH-Gene Study with case harmonization of advanced clinical and neuroimaging phenotypes. These data will be de-identified prior to sharing in accordance with NIH dbGap broad data sharing regulations. De-identified data may be shared for future studies according to NIH road data sharing requirements.

Given the devastating nature of ICH and the data provided, a randomized double blinded, placebo-controlled pilot trial of fingolimod plus best standard of care management versus placebo plus best standard of care management in the treatment of ICH is justified. First, given the long half-life of fingolimod, this study will assess the safety of single dosing of fingolimod to be given to critically ill patients, considering that a large portion of patients with ICH requires a prolonged ICU stay (the rate of nosocomial infections in the ICH patient population is approximately 23.1%[82], and the incidence of clinically significant bradycardia after the first dose has been reported in 0.5%[60]). Secondly, we will explore the use of T and B cell subsets as biomarkers of drug effect in this critically ill patient population. And finally, we will be evaluating for any differences in clinical outcomes during an extended 365 day period and perihematomal edema on radiographic imaging.

Objectives:

Specific Aim #1: Assess the safety of a single dose of fingolimod in patients who present with ICH.

Hypothesis #1: We hypothesize that there will be no difference in adverse events compared with placebo, as defined by a) drug-related adverse events; b) nosocomial infections; and c) neurologic decline.

Specific Aim #2: Evaluate the use of CD4+ T, CD8+ T, and CD19+ B cells as biomarkers of drug effect in a critically ill ICH population.

Hypothesis#1: We hypothesize that similar to the effect in multiple sclerosis patients, there will be a decrease in inflammatory cells within 24 hours of drug administration, which will persist over 30 days, as

measured by the following biomarkers: CBC with differential and flow cytometry to measure levels of CD4+ T cells, CD8+ T cells, and CD19+ B cells.

Specific Aim #3: Evaluate for differences in clinical outcomes, including a) mortality and disability at 90 and 180 days, and b) perihematomal edema on standard of care radiographic imaging.

Hypothesis#1: We hypothesize that clinical improvement, as measured by mortality and global disability measures established by the NINDS Common Data Elements for Stroke, will be more evident at 180 days than at 90 days, and outcomes will be no worse compared with placebo.
Hypothesis#2: We hypothesize that the biomarker levels of inflammatory cells will correlate with volumetric measurements of perihematomal edema calculated on standard of care neuroimaging modalities (MRI and CT).

Specific Aim #4: Perform multi-omics characterization regarding the immune response related to ICH. We will collect and store DNA from peripheral whole blood at all assessment time points, with additional samples from CSF, if applicable. Stored blood and CSF samples will be used for DNA methylation and metabolomics evaluated with mass spectrometry.

Methods and Measures:

Study Design: We propose a randomized, double-blinded, placebo-controlled pilot trial of fingolimod in patients with primary spontaneous intracerebral hemorrhage. Eligible participants will be allocated to study groups using fixed allocation randomization and a computer-based random number-generating allocation. Allocation will be equal. For those patients who meet all inclusion criteria without exclusion criteria subjects will receive oral or nasogastric tube (NGT) or Dobhoff feeding tube administration of fingolimod versus placebo. Participants will be monitored at time of enrollment and days 1, 3 5, 7, and 14 (discharge dependent) by 2 blinded assessors (neuroscience subspecialists) and will receive standard of care for the duration of the study. After discharge from the hospital, participants will enter a follow up phase of 12 months, with clinic visits at 30±14 days, 90±14 days, 180±14 days, and 365±14 days. They will receive a standard of care Neuroimaging at at these follow up time-points day visit and will be assessed with the pre-selected outcome assessments established by the NINDS Common Data Elements for Stroke.

Laboratory studies: We will perform standard of care CBC with differential and flow cytometry to follow numbers of T helper cells, cytotoxic T cells, B cells, and macrophages in peripheral blood to assess the viability of these levels as a biomarker of drug effect.

Cells isolated from the peripheral blood or CSF will be incubated with a panel of antibodies to identify the following immune populations: CD4+ T cells: CD4+CD3+ and Tbet (Th1), GATA-3 (Th2), ROR□T (Th17) FoxP3 (Treg); CD8+ T cells: CD8+CD3+T-bet; CD19+ B cells; M1:CD68+CD163-CD80+CD64+; M2:CD68+CD163+CD206+TGM2+

Additional markers of activation/differentiation tested include P2Y12, S1PR1, S1PR5, PD-1. Cells will be washed and analyzed using the BD Fortessa flow cytometric analysis instrument. In some cases, populations will be isolated for further analysis by fluorescence-based cell sorting.

With the use of commercially available assays, quantification of the top 5-10 cytokines that are present in the peripheral blood will also be performed.

We will store all CSF and blood samples to perform future Next-Gen multi-omic analysis of all CSF and blood samples including: mRNA-seq, SPLiT-seq, antibody fluorescent, DNA methylation, and mass spectroscopy. Future laboratory study procedures as detailed below.

Blood for RNA analysis will be collected in PAXgene Blood RNA tubes (Qiagen, Inc.) before shipping to Wake University. This method requires that a "discard" tube of blood be drawn prior to the PAXgene tube, and we will use this tube to perform a CBC (with differentials) that will be used for analysis (see below). RNA will be isolated using the PAXgene 96 Blood RNA Kit (Qiagen, Inc.), which is a 96-well plate-based system for increased efficiency and quality control. RNA concentration, purity and integrity of the purified RNA will be assessed using a 2100 Bioanalyzer (Agilent). Samples with an RNA integrity number (RIN) <8 will be excluded from analyses. RNA-seq will be performed in the Cancer Genomics Core, where Dr. Hawkins (Co-Investigator) is Co-Director. RNA-seq libraries will be constructed using the Illumina TruSeq Stranded Total RNA kit with Ribo-Zero. This kit allows total RNA (100 ng – 1 ug per sample) to be analyzed after undergoing ribosomal RNA depletion. Only RNA samples with RIN > 8.0 will be utilized for highest quality sequencing. The indexed libraries will be sequenced using an Illumina NextSeq 500 platform (high throughput mode) using 1x75 single end (SE) reads. The NextSeq 500 has a flexible throughput platform and we have the option of running paired-end (PE) sequencing and performing read lengths up to 150 bp (SE) or 150x150 (PE). Pooling 8-10 libraries per flow cell and assuming >80% >Q30 reads, we should be able to generate 30-40 million reads per sample in 12 hours. This sequencing depth is standard for analysis of differential gene expression.

DNA will be isolated from the blood and CSF using standard protocols currently performed in Dr. Timothy Howard's laboratory. DNA will be bisulfite-converted using the EZ DNA Methylation Gold kit (Zymo, Irvine, CA), as performed routinely in our laboratory. This kit is designed to work with a wide range of DNA amounts, ranging from 500 pg to 2 µg of DNA. Bisulfite treatment of DNA converts all non-methylated cytosines in the genomic DNA to uracil, whereas methylated cytosines remain unchanged. To determine the proportion of DNA methylation at each site, we will use the Infinium MethylationEPIC BeadChip (Illumina, Inc.), which targets approximately 850,000 CpG sites at single-nucleotide resolution, and the HiScan Reader (Illumina, Inc.). This microarray assays CpG sites from RefSeq genes, including the promoter, 5' UTR, first exon, gene body, and 3' UTR. The proportion of methylation for each site is based on the ratio of the fluorescence intensity of the methylated versus the combined methylated and unmethylated probes (referred to as the beta value), and will be determined with GenomeStudio (Illumina, Inc.). A ratio value of 0 equals no methylation of the locus and a ratio of 1 equals total (100%) methylation.

Setting: The study will occur at Wake Forest Baptist Medical Center during the patient's hospitalization through admission, operation, Neurosciences Intensive Care Unit stay, Neurology/Neurosurgery floor stay, and outpatient follow up in the neurosurgical clinic. The duration of the study for each participant will be approximately 12 months.

Subjects selection criteria:

All adult patients ages 18-80 who present to Wake Forest Baptist Medical Center with the diagnosis of spontaneous intracerebral hemorrhage.

- **Inclusion Criteria**
  - Has given written informed consent to participate in the study in accordance with required regulations; if a participant is not capable of providing informed consent, written consent must be obtained from the participant's legally authorized representative (LAR). When the LAR is not available for consent, Docusign for econsent may be obtained.

- Stated willingness to comply with all study procedures and availability for the duration of the study.
- Men and non-pregnant women ages 18-80 years old
- Has a confirmed diagnosis of spontaneous supratentorial ICH. The presence of cerebellar ICH is exclusionary. Presence of hydrocephalus due to mass effect and cerebral edema is not exclusionary. If the patient has hydrocephalus requiring CSF drainage, an external ventricular drain will be placed as standard of care and will not be exclusionary.
- Symptoms less than 24 hours prior to enrollment if all eligibility criteria are met. An unknown time of onset is exclusionary. Use the time the patient was last known to be well for patients that awaken from sleep with symptoms.
- Has a GCS score ≥ 5 on presentation.
- Has a National Institutes of Health Stroke Scale (NIHSS) score ≥ 4 on presentation.
- Maintenance of SBP < 200 mmHg at the time of enrollment and randomization.
- Historical Modified Rankin Scale score of 0-2.

- **Exclusion Criteria**
  - Men or women < 18 years old
  - Incarcerated patients
  - ICH known as a result of trauma.
  - Primary intraventricular hemorrhage without significant intraparenchymal component.
  - Ruptured aneurysm, arteriovenous malformation (AVM), vascular anomaly, Moyamoya disease, hemorrhagic conversion of an ischemic infarct, recurrence of recent (< 1 year) hemorrhage, neoplasms diagnosed with radiographic imaging.
  - Patients with unstable mass or evolving intracranial compartment syndrome.
  - Brainstem hemorrhage or irreversible impaired brain stem function (bilateral fixed, dilated pupils and extensor motor posturing), GCS ≤ 4.
  - Platelet count < 100,000; INR > 1.4.
  - Any irreversible coagulopathy or known clotting disorder.
  - .
  - Known history of Mobitz Type II second-degree or third-degree atrioventricular (AV) block or sick sinus syndrome.
  - Admission within the past 6 months for the following: myocardial infarction, unstable angina, stroke, decompensated heart failure requiring hospitalization, or Class III/IV heart failure.
  - Baseline QTc interval $\geq$500 ms.
  - Current treatment with Class Ia or Class III anti-arrhythmic drugs.
  - Implanted cardiac devices that are not compatible with the desired MRI sequences needed for the study (non-contrast T1, T2, SWI/GRE, and FLAIR sequences).
  - Abnormal liver function or liver failure.
  - Active acute infection that is deemed by the Principal Investigator to be clinically significant.
  - Chronic viral or fungal infection.
  - Active use of antineoplastic, immunosuppressive, or immunomodulating therapies.
  - Leukopenia with a WBC < 2.0×10^9/L.
  - Not expected to survive to the 365 day visit due to co-morbidities or is DNR/DNI status prior to randomization.
  - Active drug or alcohol use or dependence that, in the opinion of the investigator, would interfere with adherence to study requirements.

- Concomitant enrollment in another interventional study.
- Inability or unwillingness of participant or legal guardian/representative to give written informed consent.

- **Sample Size**
  - Because this is a pilot study to assess the safety and feasibility of single dosing of an immunomodulator in a critically ill population, we estimate that a sample size of 30 total subjects, is sufficient to provide safety data that will help inform feasibility and elements of design for a future trial. We will include 10 patients to be enrolled in an open-label arm to assess the feasibility of administering fingolimod through an NGT or Dobhoff tube. Once this is determined to be without complication and drug delivery via the NGT or Dobhoff tube is not inhibited, we will include subjects with NGT or Dobhoff tube in the randomized portion of the sample.
  -

Interventions and Interactions:

I. For all patients presenting with symptoms of stroke, including arm/leg weakness, speech difficulties, altered mental status or vision changes:

- Medical history:
  - Obtained at time of admission to include last known well time
  - Should be focused on inclusion/exclusion criteria, as noted previously
  - Review of medications for documentation of any active use of antineoplastic, immunosuppressive, or immunomodulating agents
  - Documentation of historical Modified Rankin Scale for inclusion/exclusion criteria
  - Intracerebral hemorrhage score (ICH) will be calculated at this time to document the severity of the disease.

- NIHSS
  - Obtained at time of admission
  - Code stroke, including formal neurology consult

- Vital signs:
  - Obtained at time of admission, important for inclusion/exclusion criteria

- Physical exam:
  - Obtained at time of admission
  - Documentation of initial GCS score at time of admission for inclusion/exclusion criteria

- EKG:
  - Obtained at time of admission as part of standard of care and important for exclusion criteria

- Blood samples:
  - Obtained at time of admission for standard of care baseline set of admission labs, to include CBC with differential with a baseline lymphocyte subset analysis, basic metabolic panel, liver function panel (LFTs) to rule out underlying hepatic dysfunction, and coagulation panel to rule out underlying coagulopathy.
  - If they meet all eligibility criteria, prior to the administration of study drug, 5 cc of blood will be set aside in storage for future multi-omic analysis.

- Pregnancy test:
    - For women of child-bearing age, obtained at time of admission as part of standard of care.

- Urinalysis:
    - As part of admission standard of care to rule out UTI as cause of alterations in mental status.

- Neuro imaging: Most appropriate clinically indicated standard of care
    - CT scan of the head to evaluate for ischemic infarct or intracerebral hemorrhage
    - CT angiogram of the head and neck to be obtained as part of standard of care work up to exclude vascular etiologies of hemorrhage. If a CTA is contraindicated due to renal impairment, an MRA will be performed instead.
    - MRI of the brain to be obtained as part of standard of care work up to exclude neoplasms or cavernous malformations as etiology of hemorrhage.

- Speech and swallow evaluation to assess for any dysphagia, important for exclusion criteria. This procedure occurs as part of standard of care. A bedside swallow evaluation is appropriate.

II. For participants who meet the inclusion criteria, without exclusion criteria, and are enrolled in the study:

- Each participant is to be admitted to the Neuroscience Intensive Care Unit for standard of care close neurologic monitoring, vital signs as part of standard of care, intracranial pressure and drain output monitoring if an EVD is in place.
- Those participants randomized to the fingolimod plus best standard of care group will receive a single dose of 0.5 mg oral fingolimod within 24 hours of symptom onset. Those participants randomized to the control group will receive a single dose placebo pill within 24 hours of symptom onset that will appear, smell, and taste identical to the fingolimod capsule.
- For participants who are eligible for the open-label arm, they will receive a single dose of 0.5 mg fingolimod through an NGT or Dobhoff tube within 24 hours of symptom onset.
- For participants with evidence of clinical or radiographic hydrocephalus, they will undergo standard of care placement of an external ventricular drain (EVD). Unless it is emergent and needs to be placed in the emergency room as a life-saving procedure, each participant will be admitted to the Neuroscience Intensive Care Unit for expeditious placement of the EVD. Once the head is secured to the bed, the hair on the right side of the head will be clipped. Kocher's point will be identified, and incision will be marked with a skin marker. The area will be prepped sterilely with Chloroprep. The procedure is then completed in the usual sterile manner for twist drill burr hole placement and the placement of the EVD. This will then be tunneled subcutaneously under the scalp away from the incision and secured. At this time, 5 cc of CSF will be collected and saved for future multi-omic analysis.
- All participants enrolled are to be placed on telemetry to monitor for any cardiac events.
- Each participant will receive neurologic assessments by 2 blinded neuroscience subspecialists on Day 1, 3, 5, 7, and 14 (discharge dependent).
- Each participant will have their vitals performed and collected as part of standard of care. Vitals will be specifically recorded at the time of their neurologic assessment by the blinded assessors.
- Each participant will be getting daily standard of care blood draws, approximately 10 cc worth of blood, until time of discharge. The standard of care studies will include CBC with differential, basic metabolic panel (BMP), and LFTs. On Day 1, 3, 5-7 (discharge dependent), these standard of care blood draws will include 5 cc of blood that will be collected for lymphocyte subset analysis for research purposes for biomarker testing. Drug response will be assessed by lymphocyte subset levels. Additionally, 10 cc of blood will also be drawn at the 30 day visit for standard of care studies

(CBC with differential and lymphocyte subset analysis, BMP), in addition to 5 cc set aside in storage for future multi-omic analysis. However, there will be no blood draws if the participant is experiencing severe anemia.

- If an EVD is present, the drain output will be collected daily as part of standard of care. On Day 1, 3, 5, 7, 10, and 14 after placement, 5 cc at each time point will be collected and 4 cc to be sent off for standard of care infectious surveillance and 1 cc to be saved for future multi-omic analysis. The EVD will be removed in a standard fashion based on the clinical and radiographic determination that the participant no longer has hydrocephalus. If a patient undergoes EVD removal if clinically indicated prior to the Day 10 or 14 collection time points, they will not undergo these final CSF draws.
- Each participant will undergo a standard of care Neuroimaging of the brain 24 hrs and 5-7 days, 90 days, 180 days and 365 days after drug administration to assess the stability and resolution of the hemorrhage, follow the perihematomal edema, evaluate for evidence of hydrocephalus, and the development of subsequent brain atrophy that occurs after ICH. There will be a standard of care Neuroimaging on day 14 if the participant is still hospitalized or clinically indicated.
- 72 hours after admission, each participant will undergo a standard of care non-contrast Neuroimaging of the brain to assess the extent of perihematomal edema after drug administration. There will also be a standard of care Neuroimaging at the 30 day follow up to follow the resolution of hematoma and perihematomal edema. All radiographic data will be used to calculate volumetric measurements via RAPID protocol to measure hematoma and perihematomal edema volume.
- Each participant will undergo standard of care speech, cognitive, physical, and occupational therapy to be initiated upon admission. At time of discharge, post-ICH reference outcome measures established by the NINDS Common Data Elements for Stroke are to be performed and recorded. These will include interviewer-administered modified Rankin Scale (mRS), patient-reported PROMIS 10, Montreal Cognitive Assessment (MoCA), and the Western Aphasia Battery-Revised. These outcome measures will assess for various aspects of recovery, including physical, emotional, and neurocognitive. These clinical assessments will also be performed and recorded at the time of discharge, and at the 30, 90, 180, and 365 day visits. We will also be collecting each participant's disposition at time of discharge, 30, 90, 180, and 365 days.

III. Adverse events:

- All adverse events to be monitored from admission until day of discharge and at all follow up visits. These will include a) clinically significant cardiac events, b) nosocomial infections, and c) neurologic decline.
    - Clinically significant cardiac events: All subjects will be placed on telemetry for 72 hours after administration of drug and changes in heart rate or rhythm will be recorded. Maximal decrease in heart rate occurs within 6 hours of drug administration and recovers, with only 0.5% of patients experiencing clinically symptomatic bradycardia.[60]
    - Nosocomial infections: All UTI, sepsis, and pneumonia events will be recorded and compared to published ICH nosocomial infection rates (23%).[82]
    - Neurologic decline: Throughout hospitalization, all subjects will be monitored for neurologic decline, which has been reported to occur in up to 33% of patients within the first 48-72 hours.[83] Each subject will be assigned a GCS score and NIHSS score at time of enrollment, Day 1, 3, 5, 7, and 14 (discharge dependent). As per ischemia stroke criteria, a change ≥ 4 in the NIHSS will be considered a neurologic change and will be followed over time.
    - Additionally, during hospitalization and at all follow up visits, we will monitor for all drug related and other adverse events, not limited to hepatic function, in-hospital mortality, 30-day mortality, 90-day mortality, and all-cause mortality.

IV. Outpatient follow-up:

Follow-Up Visit 1 (30 ± 14 days after admission)

Standard of care in-person follow up visit to assess neurologic status and functional recovery based on the outcome measures above. At this time, a standard of care Neuroimaging of the brain will be done to follow the resolution of perihematomal edema and to assess for delayed hydrocephalus. 10 cc of blood will also be drawn for standard of care studies (CBC with differential and lymphocyte subset analysis, CMP), in addition to 5 cc set aside in storage for future multi-omic analysis. Any potential long-term drug adverse effects are to be followed at this time.

Follow-Up Visit 2 (90 ± 14 days after admission)

Standard of care in-person follow up visit to assess neurologic status and functional recovery based on the outcome measures above. At this time, there will be a standard of care Neuroimaging of the brain to follow the resolution of both the hematoma and perihematomal edema. Any potential long-term drug adverse effects will be followed at this time.

Follow-Up Visit 3 (180 ± 14 days after admission)

In-person follow up visit to assess neurologic status and functional recovery based on the outcome measures above. Any potential long-term drug adverse effects will be followed at this time. At this time, there will be a Neuroimaging of the brain to follow the resolution of both the hematoma and perihematomal edema. Any potential long-term drug adverse effects will be followed at this time

Follow-Up Visit 4 (365 ± 14 days after admission)

In-person follow up visit to assess neurologic status and functional recovery based on the outcome measures above. Standard of care Neuroimaging will be performed to assess brain injury and atrophy.

	Enrollment	24 hrs post -	72 hrs post - ictus	5-7 days	10-14 days	Discharge	30±14 days	90±14 days	180±14 days	365±14 days

		ictus +								
Neuroimaging Diagnostic CT,CTA,MRA or MRI	X	X	X	X	X‡		X	X	X	X
Medical history	X									
ICH score	X									
EKG	X									
Speech and swallow evaluation	X									
Informed consent	X									
Single drug dose (fingolimod or placebo)	X									
CSF*	X*	X*	X*	X*	X*					
Peripheral whole blood (CBC, CMP, lymphocyte subsets)	X	X	X	X	X‡		X			
Telemetry	X	X	X							
Vital signs	X	X	X	X	X		X	X	X	X
Neuro exam	X	X	X	X	X		X	X	X	X
NIHSS	X	X	X	X†	X†	X	X	X	X	X
mRS	Historic			X†	X†	X	X	X	X	X
PROMIS 10				X†	X†	X	X	X	X	X
MoCA				X†	X†	X	X	X	X	X
WAB-R				X†	X†	X	X	X	X	X

* If the participant requires EVD placement.
†Or at time of discharge
‡ If still hospitalized or clinically indicated
+ At least 6 hours after medication given

Outcome Measures:
Primary outcome measure:

- Safety of a single-dose administration of fingolimod to critically ill patients. We will be assessing rates of the following adverse events:
    - Clinically significant cardiac events at 30 days
    - Nosocomial infections (UTI, sepsis, and pneumonia) at 90 days
    - Neurologic decline, considered a change ≥ 4 points of the NIHSS at 30 days

Secondary outcome measures:

- Feasibility of administering fingolimod through an NGT or Dobhoff tube.
- Changes seen in the lymphocyte subpopulations at 30 days. The lymphocyte subsets of CD4+ T, CD8+ T, and CD19+ B cells will be compared among the three groups and the trends will be followed over time in all participants.
- All radiographic imaging performed (CT and MRI) will undergo volumetric measurement calculations of hematoma and perihematomal volumes via RAPID protocol. These volumes will be correlated with biomarker levels of inflammatory cells and clinical outcome assessments.
- NIHSS at 365 days post-ictus.
- *Interviewer-administered modified Rankin Scale score at 365 days post-ictus.*
- Patient-Reported Outcomes Measurement Information System 10 at 365 days (patient report of physical and neurobehavioral function). As referenced in the ICF, all these outcomes measures will be done in the context of a standard of care visit. If any participant endorses symptoms consistent with depression or are at risk of causing harm to self and others, we will follow the institutional policy regarding making the appropriate referral to their primary physician to address these concerns or appropriate psychiatric referral.
- Montreal Cognitive Assessment (MoCA) at 365 days post-ictus.
- Western Aphasia Battery – Revised (WAB-R) at 365 days post-ictus.
- Mortality at 30 days
- Mortality at 90 days
- All cause mortality within the study period
- Patient disposition: number of home days over 365 days
- Multi-omic analysis of inflammatory phenotypes related to ICH

Analytical Plan:

Randomization:

Randomization of the study participants should occur after the participant has undergone eligibility screening and has been consented if all criteria have been met. Consented participants will be allocated to study groups using fixed allocation randomization and a computer-based random number-generating allocation. Allocation will be equal. The study pharmacist will be the only unblinded member of the study and will provide the appropriate study agent based on the statistician's randomization schema. Enrollment into the open-label arm of fingolimod will not be randomized.

Statistical Analysis:

- **Aim 1. Safety of a single dose of fingolimod in ICH patients.** The purpose of the primary analyses will be to measure safety and feasibility of the intervention (rates of drug-related adverse events, nosocomial infections, and neurologic decline), and to measure these in the comparison group to help inform feasibility and elements of design for a future trial. In general, we will estimate composite outcome rates (95% CIs) in our study groups, for example of UTI, sepsis, and hospital-acquired pneumonia events, and we will compare these rates to historical rates, when available. This will help ensure the rates of adverse events are not elevated, and are comparable or lower than those in previous reports. Baseline demographic and clinical characteristics will be described and

compared between medical management groups, and differences may be used to help explain heterogeneity in the safety outcomes.

- **Aim 2. Evaluate CD4+ T, CD8+ T, and CD19+ B cells as biomarkers of drug effect in a critically ill ICH population.** To provide initial assessments of 'clinical activity," we will compare inflammation-related biomarkers between the fingolimod and placebo groups over time. In general, we will calculate individual-level changes in the biomarkers from baseline to the later biomarker collections. For example, changes in CD4+ and CD8+ T cells and CD19+ B cells at 24 hr, 72 hr, 5-7 days, and 30 days will be useful to evaluate if levels of inflammation are lower with fingolimod. Prior to analysis, summary statistics and graphic approaches will be used to examine data distributions, both overall, and by study group. Changes will then be compared between the groups using the two-sample *t* test or Wilcoxon rank sum test and alpha level = 0.05 (two-sided). These initial assessments will be interpreted cautiously, given the small sample sizes and possible missing data.
- **Aim 3. Evaluate differences in mortality and disability between placebo and treatment groups.** Analyses will provide 1) initial percentages of patients who benefit from intervention, specifically focusing on clinical improvements as measured by items from the NINDS Common Data Elements for Stroke, and will provide 2) estimates of variability and correlations in our sample over time. Additional analyses will explore correlations between biomarkers, or their changes over time, including with the perihematomal edema volume. Though the sample sizes in this pilot trial are quite small, these outcome measures have been used extensively in other studies, and our estimates will be helpful to assess comparability in this context, thus informing sample size calculations for a future trial.
- **Aim 4. Perform future multi-omics characterization regarding the immune response related to ICH.** RNAseq Data Analysis: Alignment of reads will be performed using the STAR sequence aligner,[84] and gene counts determined using feature Counts software.[85] Differential gene expression (DEG) will be analyzed using DESeq2 software.[86] All experimental conditions will be prepared in the same experiment, so that the paired-sample analysis can be calculated to detect DEGs at each time point using DESeq2 as described.[87] Although the primary purpose is to estimate parameters for subsequent power analysis, current analysis will consider Benjamini-Hochberg false discovery rate (FDR) adjusted $p<0.05$ as significant.[88] DEGs will be analyzed for significant enrichment of biological pathways and signaling networks using Ingenuity Pathway Analysis (IPA), Causal Network Analysis and Upstream Regulator Tools[89] and the DAVID software as described.[90] A parallel analysis to the detection of DEG will be computed on the percent methylation (i.e., estimation of parameters for future power calculations and study design, FDR-adjusted p-values). Further statistical analyses will necessarily rely on data descriptions, and the formal statistical inference (hypothesis testing) will be limited due to the small sample size (n=10).

Human Subjects Protection:

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 Code of Federal Regulations [CFR] Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR 312, and/or the International Council on Harmonisation E6.

The appropriate IRB/EC must approve the protocol and informed consent documents, agree to monitor the conduct of the study, and agree to review the study progress periodically, at intervals of no more than 1 year.

The study provides an opportunity to "bank" tissue samples from willing participants for future innovative ICH research, namely Next-Gen multi-omic analysis of the inflammatory process surrounding ICH.

Therefore, we plan to collect additional tissue samples (CSF) to be stored and analyzed in the future. For participants meeting eligibility criteria, peripheral whole blood will be collected prior to the dose of drug, and at 24 hr, 72 hr, 7 days, and 30 days after drug administration. If the participant requires CSF diversion for hydrocephalus, prior to the administration of the study agent, we will also collect CSF that would otherwise be discarded as medical waste. If an external ventricular drain is present, CSF will also be collected at 24 hr, 72 hrs, and 7 days after drug administration. Participants' participation in this tissue banking repository will be optional; they do not have to participate in the repository sub-study in order to participate initially or to continue their participation in the main study. All samples will be stripped of identifiers and assigned a unique code number. The key linking code numbers and identifying information will be kept in a secure location accessible only to the PI of the study or his or her designee, on a password-protected network drive. All samples will be stored at -80^{o} F low temperature, locked, and frozen until the multi-omic analysis can be performed. The samples will be stored indefinitely or until no more remains for research that can be performed at future dates.

Subject Recruitment Methods:

Adult patients coming the Emergency Department or direct admitted to the Neurosciences Intensive Care Unit with the diagnosis of spontaneous ICH will be identified as potentially eligible participants and screened by interview.

For patients meeting inclusion criteria without exclusion criteria, the participant or the participant's health power of attorney will be approached for consent for participating in the study. The study will be discussed with the participant and their surrogate as soon as approachable and available during the participant's care in the hospital. If the participant or health care power of attorney does not want to be included in the study, there will be no examinations or data collected.

Equal access by women and minorities will be ensured as inclusion criteria will apply to all patients presenting to Wake Forest Baptist Hospital, regardless of gender or race.

Privacy will be protected by using secured computers for data gathering and storage, and by following strict HIPAA protocol.

Informed Consent:

Consent forms describing in detail the study agent, study procedures, and benefits/risks are given to the participant, and signed, IRB-approved informed consent must be obtained from each participant before starting intervention/administering study product. The ICF will conform to applicable regulations, including the FDA regulation in 21 CFR Part 50, and to institutional requirements for informed consent.

Consent may be obtained by one of the study investigators or research coordinators once it has been determined that the participant is an appropriate candidate for the study. The study team will attempt to obtain the informed consent in person either in the emergency department, the neurology/neurosurgery floor, or the neurosciences ICU setting.

When the LAR is not available in person, the consent options to be utilized will be:

1. The study team can communicate via phone or webex with a family member of legal representative and explain the study and answer questions. If the person is present at the medical center or will be coming in within the allowed window, then a wet signature could be obtained, and the patient enrolled on study.
2. If the legal representative is not available for a wet signature within the allowed window, then the institution has recently obtained a validated and compliant version of DocuSign that may be available in this situation. The study team would still communicate the consent process over the phone or webex, but

instead of obtaining a wet signature, they would send an email with a link to DocuSign and obtain an electronic signature.

The ICF will be reviewed with the prospective participant (or participant's LAR), by the investigator/qualified designee, and they will answer any questions. Once completed satisfactorily, the participant (or LAR) and the investigator/qualified designee will sign and date the ICF. The participant will receive a copy of the signed ICF and the original signed and dated ICF will be kept in the site's files. Documentation of the participant's informed consent for, and participation in, this trial will be noted in the participant's medical record.

If a prospective participant is not capable of providing informed consent because of neurologic injury, consent will be sought from the participant's LAR. Diminished capacity does not limit a participant's right to refuse participation. If the prospective participant refuses participation, the refusal stands. Furthermore, if an LAR consents on behalf of a participant and the participant regains cognitive ability, the participant's consent must be sought directly, and the participant has the right to withdraw consent given by an LAR. Periodic assessment by site study staff will be made to evaluate the ability of a participant to give direct consent. Additionally, the subject's assent must also be obtained if he or she is able to understand the nature, significance, and risks associated with the study. A portion of the consent will also describe the possibility of storing blood for potential multi-omic testing that will be performed, and it will be clarified that the information collected from the stored blood or CSF samples is for research purposes only and does not serve a therapeutic purpose.

If the participant or LAR is not capable of reading the ICF, an impartial witness is required to be present as the consent is read aloud and while questions are answered or any discussion of consent takes place. Afterward, the participant or LAR should be asked to sign and date the ICF for the verbal consent process. The impartial witness and the individual who read the consent document and answered questions should also sign and date the verbal consent document. In every case, the participant should receive a copy of the full text of the ICF.

The participant (or participant's LAR) will be informed as soon as possible if new information becomes available that may be relevant to the participant's willingness to continue participation in the trial. The communication of this information to the participant will be documented.

Confidentiality and Privacy:

Participant information collected in this study will comply with the standards for protection of privacy of individually identifiable health information as promulgated in HIPAA and as mandated in Title 45 CFR, Parts 160 and 164. All records will be kept confidential, and the participant's name will not be released at any time. Participant records will not be released to anyone other than responsible regulatory authorities, when requested. In all cases, caution will be exercised to assure the data are treated confidentially and that the participant's privacy is guaranteed.

An authorization for use and disclosure of protected health information under the HIPAA Privacy Rule will be obtained from every trial participant before, or at the time of, enrollment. It will be presented to, and signed by, the participant at the same time as the study consent. The investigator is responsible for obtaining participants' (or their legal representatives') authorizations and signature and for explaining the elements of the HIPAA authorization form if necessary.

Data and Safety Monitoring:

The principal investigator will be responsible for the overall monitoring of the data and safety of study participants. The principal investigator will be assisted by other members of the study staff. Routine safety data in the review include SAEs, AEs, discontinuation due to AEs, clinically significant post-drug worsening of neurologic status, EKGs, vital signs, and laboratory assessments.

Reporting of Unanticipated Problems, Adverse Events, or Deviations:
Any unanticipated problems, serious and unexpected adverse events, deviations, or protocol changes will be promptly reported by the principal investigator or designated member of the research team to the IRB and sponsor or appropriate government agency if appropriate.

Adverse event reporting:
All AEs occurring between the times informed consent is obtained and the last study-related procedure is completed as part of the day 365 follow up will be recorded on the appropriate eCRF form. Adverse events may be volunteered by subjects and or solicited at each assessment by the investigator or designee.

Recording should be done in a concise manner using standard, acceptable medical terms. The investigator should make every effort to establish a diagnosis of the event based on the presenting signs, symptoms, and/or clinical information. In such cases, the diagnosis should be documented as the AE/SAE.

Diseases, signs, symptoms, or laboratory abnormalities already existing at enrollment are not considered AEs unless they worsen (i.e. increase in intensity or frequency). Surgical procedures themselves are not AEs – they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE. Surgical procedures planned before enrollment and the conditions leading to these measures are not AEs.

If, in the investigator's judgment, a clinically significant worsening from baseline is observed in any laboratory or other testing parameter (e.g. EKG, CBC, BMP), physical examination finding, or vital sign, a corresponding clinical AE should be recorded on the AE pages of the eCRF. If the cause is not known, the abnormal test or finding should be recorded as an AE, using appropriate medical terminology. Actual numeric values of test results should not be reported.

Serious adverse event reporting:
All SAEs occurring between the times informed consent is obtained and the last study-related procedure is completed as part of the day 365 follow up, whether or not deemed drug-related or expected, must be reported by the investigator or qualified designee within 1 working day of first becoming aware of the event. The investigator/qualified designee will enter the required information regarding the SAE into the appropriate module of the eCRF, which will automatically result in distribution of the information to DCRI Safety Surveillance. If the eCRF system is temporarily unavailable, the event, including the investigator-determined causality to study drug, should be reported via a paper back-up SAE form to DCRI Safety Surveillance. Upon return of the availability of the EDF system, the SAE information must be entered into the eCRF.

The investigator is responsible for reporting AE/SAEs to their IRB/ethics committee (EDC) according to local guidelines and/or requirements.

All SAEs will be reviewed for expectedness per the Investigator. Events determined to be related to the investigational drug and unexpected per the investigator will be reported in the expedited manner to the regulatory authorities and participating sites. For SAEs resulting in death or considered life threatening,

reporting should occur no later than 7 days after the event; for unexpected, non-life threatening SAEs, reporting should occur no later than 15 days after the event.

Unanticipated problems reporting:
It is the site investigator's responsibility to report UPs to their IRB/EC and to the data coordinating center. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB/EC project number.
- Detail description of the vent, incident, experience, or outcome.
- Explanation of the basis for determining that the event, incident, experience, or outcomes represents a UP.
- Description of any deviations from protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported as per the instructions for SAE reporting outlined above.
- Any other UP will be reported to the IRB/EC and to the DCC as per the IRB/EC's reporting requirements, but no later than 30 days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures).

References:

1. Qureshi AI, Mendelow AD, Hanley DF. Intracerebral haemorrhage. Lancet. 2009;373(9675):1632-44.
2. van Asch CJ, Luitse MJ, Rinkel GJ, van der Tweel I, Algra A, Klijn CJ. Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis. The Lancet Neurology. 2010;9(2):167-76.
3. Fogelholm R, Murros K, Rissanen A, Avikainen S. Long term survival after primary intracerebral haemorrhage: a retrospective population based study. J Neurol Neurosurg Psychiatry. 2005;76(11):1534-8.
4. Lloyd J. Heart Disease and Stroke Statistics-2010 Update: A Report From the American Heart Association (vol 121, pg e46, 2010). Circulation. 2011;124(16):E425-E.
5. Qureshi AI, Suri MFK, Abu N, Kirmani JF, Ezzeddine MA, Divani AA, et al. Changes in cost and outcome among US patients with stroke hospitalized in 1990 to 1991 and those hospitalized in 2000 to 2001. Stroke. 2007;38(7):2180-4.
6. Adeoye O, Broderick JP. Advances in the management of intracerebral hemorrhage. Nat Rev Neurol. 2010;6(11):593-601.
7. Xi GH, Keep RF, Hoff JT. Mechanisms of brain injury after intracerebral haemorrhage. Lancet Neurol. 2006;5(1):53-63.
8. Ariesen MJ, Claus SP, Rinkel GJE, Algra A. Risk factors for intracerebral hemorrhage in the general population - A systematic review. Stroke. 2003;34(8):2060-5.
9. Broderick JP, Brott TG, Duldner JE, Tomsick T, Huster G. VOLUME OF INTRACEREBRAL HEMORRHAGE - A POWERFUL AND EASY-TO-USE PREDICTOR OF 30-DAY MORTALITY. Stroke. 1993;24(7):987-93.
10. Mould WA, Carhuapoma JR, Muschelli J, Lane K, Morgan TC, McBee NA, et al. Minimally Invasive Surgery Plus Recombinant Tissue-type Plasminogen Activator for Intracerebral Hemorrhage Evacuation Decreases Perihematomal Edema. Stroke. 2013;44(3):627-34.
11. Mendelow AD, Gregson BA, Rowan EN, Murray GD, Gholkar A, Mitchell PM, et al. Early surgery versus initial conservative treatment in patients with spontaneous supratentorial lobar intracerebral haematomas (STICH II): a randomised trial. Lancet. 2013;382(9890):397-408.
12. Minimally Invasive Surgery Plus Rt-PA for ICH Evacuation Phase III [Available from: https://ClinicalTrials.gov/show/NCT01827046.
13. MIND: Artemis in the Removal of Intracerebral Hemorrhage [Available from: https://ClinicalTrials.gov/show/NCT03342664.
14. ENRICH: Early MiNimally-invasive Removal of IntraCerebral Hemorrhage (ICH) [Available from: https://ClinicalTrials.gov/show/NCT02880878.
15. Morgenstern LB, Hemphill JC, Anderson C, Becker K, Broderick JP, Connolly ES, et al. Guidelines for the Management of Spontaneous Intracerebral Hemorrhage A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke. 2010;41(9):2108-29.
16. Babu R, Bagley JH, Di C, Friedm AH, Adamson C. Thrombin and hemin as central factors in the mechanisms of intracerebral hemorrhage-induced secondary brain injury and as potential targets for intervention. Neurosurgical Focus. 2012;32(4).
17. Zhou Y, Wang Y, Wang J, Stetler RA, Yang Q-W. Inflammation in intracerebral hemorrhage: From mechanisms to clinical translation. Progress in Neurobiology. 2014;115:25-44.
18. Wang J. Preclinical and clinical research on inflammation after intracerebral hemorrhage. Progress in Neurobiology. 2010;92(4):463-77.
19. Wang J, Dore S. Inflammation after intracerebral hemorrhage. Journal of Cerebral Blood Flow and Metabolism. 2007;27(5):894-908.

20. Aronowski J, Zhao X. Molecular Pathophysiology of Cerebral Hemorrhage Secondary Brain Injury. Stroke. 2011;42(6):1781-6.
21. Keep RF, Hua Y, Xi G. Intracerebral haemorrhage: mechanisms of injury and therapeutic targets. Lancet Neurol. 2012;11(8):720-31.
22. Boche D, Perry VH, Nicoll JAR. Review: Activation patterns of microglia and their identification in the human brain. Neuropathology and Applied Neurobiology. 2013;39(1):3-18.
23. Lawson LJ, Perry VH, Dri P, Gordon S. HETEROGENEITY IN THE DISTRIBUTION AND MORPHOLOGY OF MICROGLIA IN THE NORMAL ADULT-MOUSE BRAIN. Neuroscience. 1990;39(1):151-70.
24. Xiong XY, Liu L, Yang QW. Functions and mechanisms of microglia/macrophages in neuroinflammation and neurogenesis after stroke. Progress in Neurobiology. 2016;142:23-44.
25. Wasserman JK, Zhu X, Schlichter LC. Evolution of the inflammatory response in the brain following intracerebral hemorrhage and effects of delayed minocycline treatment. Brain Research. 2007;1180:140-54.
26. Taylor RA, Sansing LH. Microglial Responses after Ischemic Stroke and Intracerebral Hemorrhage. Clinical & Developmental Immunology. 2013.
27. Mracsko E, Veltkamp R. Neuroinflammation after intracerebral hemorrhage. Frontiers in Cellular Neuroscience. 2014;8.
28. Kanazawa M, Ninomiya I, Hatakeyama M, Takahashi T, Shimohata T. Microglia and Monocytes/Macrophages Polarization Reveal Novel Therapeutic Mechanism against Stroke. International Journal of Molecular Sciences. 2017;18(10).
29. Zhao H, Garton T, Keep RF, Hua Y, Xi G. Microglia/Macrophage Polarization After Experimental Intracerebral Hemorrhage. Translational Stroke Research. 2015;6(6):407-9.
30. Lan X, Han X, Li Q, Yang Q-W, Wang J. Modulators of microglial activation and polarization after intracerebral haemorrhage. Nat Rev Neurol. 2017;13(7):420-33.
31. Zhang Z, Zhang Z, Lu H, Yang Q, Wu H, Wang J. Microglial Polarization and Inflammatory Mediators After Intracerebral Hemorrhage. Molecular Neurobiology. 2017;54(3):1874-86.
32. Hu X, Leak RK, Shi Y, Suenaga J, Gao Y, Zheng P, et al. Microglial and macrophage polarization -new prospects for brain repair. Nat Rev Neurol. 2015;11(1):56-64.
33. Eggen BJL, Raj D, Hanisch UK, Boddeke HWGM. Microglial Phenotype and Adaptation. Journal of Neuroimmune Pharmacology. 2013;8(4):807-23.
34. Shichita T, Sakaguchi R, Suzuki M, Yoshimura A. Post-ischemic inflammation in the brain. Frontiers in Immunology. 2012;3.
35. Hendrix S, Nitsch R. The role of T helper cells in neuroprotection and regeneration. Journal of Neuroimmunology. 2007;184(1-2):100-12.
36. Kivisakk P, Mahad DJ, Callahan MK, Trebst C, Tucky B, Wei T, et al. Human cerebrospinal fluid central memory CD4+ T cells: evidence for trafficking through choroid plexus and meninges via P-selectin. Proc Natl Acad Sci U S A. 2003;100(14):8389-94.
37. Arumugam TV, Granger DN, Mattson MP. Stroke and T-cells. Neuromolecular Medicine. 2005;7(3):229-42.
38. Yilmaz G, Arumugam TV, Stokes KY, Granger DN. Role of T lymphocytes and interferon-gamma in ischemic stroke. Circulation. 2006;113(17):2105-12.
39. Hurn PD, Subramanian S, Parker SM, Afentoulis ME, Kaler LJ, Vandenbark AA, et al. T- and B-cell-deficient mice with experimental stroke have reduced lesion size and inflammation. Journal of Cerebral Blood Flow and Metabolism. 2007;27(11):1798-805.
40. Liesz A, Suri-Payer E, Veltkamp C, Doerr H, Sommer C, Rivest S, et al. Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. Nature Medicine. 2009;15(2):192-9.

41. Zhou K, Zhong Q, Wang Y-C, Xiong X-Y, Meng Z-Y, Zhao T, et al. Regulatory T cells ameliorate intracerebral hemorrhage-induced inflammatory injury by modulating microglia/macrophage polarization through the IL-10/GSK3 beta/PTEN axis. Journal of Cerebral Blood Flow and Metabolism. 2017;37(3):967-79.
42. Loftspring MC, McDole J, Lu A, Clark JF, Johnson AJ. Intracerebral hemorrhage leads to infiltration of several leukocyte populations with concomitant pathophysiological changes. Journal of Cerebral Blood Flow and Metabolism. 2009;29(1):137-43.
43. Guo F-q, Li X-j, Chen L-y, Yang H, Dai H-y, Wei Y-s, et al. Study of relationship between inflammatory response and apoptosis in perihematoma region in patients with intracerebral hemorrhage. Zhongguo wei zhong bing ji jiu yi xue = Chinese critical care medicine = Zhongguo weizhongbing jijiuyixue. 2006;18(5):290-3.
44. Gelderblom M, Leypoldt F, Steinbach K, Behrens D, Choe C-U, Siler DA, et al. Temporal and Spatial Dynamics of Cerebral Immune Cell Accumulation in Stroke. Stroke. 2009;40(5):1849-57.
45. Selvaraj UM, Stowe AM. Long-term T Cell Responses in the Brain After an Ischemic Stroke. Discovery Medicine. 2017;24(134):323-33.
46. Gu L, Xiong X, Zhang H, Xu B, Steinberg GK, Zhao H. Distinctive Effects of T Cell Subsets in Neuronal Injury Induced by Cocultured Splenocytes In Vitro and by In Vivo Stroke in Mice. Stroke. 2012;43(7):1941-6.
47. Theodorou GL, Marousi S, Ellul J, Mougiou A, Theodori E, Mouzaki A, et al. T helper 1 (Th1)/Th2 cytokine expression shift of peripheral blood CD4(+) and CD8(+) T cells in patients at the post-acute phase of stroke. Clinical and Experimental Immunology. 2008;152(3):456-63.
48. Gao L, Lu Q, Huang L-J, Ruan L-H, Yang J-J, Huang W-L, et al. Transplanted Neural Stem Cells Modulate Regulatory T, gamma delta T Cells and Corresponding Cytokines after Intracerebral Hemorrhage in Rats. International Journal of Molecular Sciences. 2014;15(3):4431-41.
49. Mao LL, Yuan H, Wang WW, Wang YJ, Yang MF, Sun BL, et al. Adoptive Regulatory T-cell Therapy Attenuates Perihematomal Inflammation in a Mouse Model of Experimental Intracerebral Hemorrhage. Cellular and Molecular Neurobiology. 2017;37(5):919-29.
50. Kappos L, Radue E-W, O'Connor P, Polman C, Hohlfeld R, Calabresi P, et al. A Placebo-Controlled Trial of Oral Fingolimod in Relapsing Multiple Sclerosis. New England Journal of Medicine. 2010;362(5):387-401.
51. Calabresi PA, Radue E-W, Goodin D, Jeffery D, Rammohan KW, Reder AT, et al. Safety and efficacy of fingolimod in patients with relapsing-remitting multiple sclerosis (FREEDOMS II): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Neurol. 2014;13(6):545-56.
52. Groves A, Kihara Y, Chun J. Fingolimod: Direct CNS effects of sphingosine 1-phosphate (S1P) receptor modulation and implications in multiple sclerosis therapy. Journal of the Neurological Sciences. 2013;328(1-2):9-18.
53. Cohen JA, Chun J. Mechanisms of Fingolimod's Efficacy and Adverse Effects in Multiple Sclerosis. Annals of Neurology. 2011;69(5):759-77.
54. Chun J, Hartung H-P. Mechanism of Action of Oral Fingolimod (FTY720) in Multiple Sclerosis. Clinical Neuropharmacology. 2010;33(2):91-101.
55. Chiba K. FTY720, a new class of immunomodulator, inhibits lymphocyte egress from secondary lymphoid tissues and thymus by agonistic activity at sphingosine 1-phosphate receptors. Pharmacology & Therapeutics. 2005;108(3):308-19.
56. Lee CW, Choi JW, Chun J. Neurological S1P Signaling as an Emerging Mechanism of Action of Oral FTY720 (Fingolimod) in Multiple Sclerosis. Archives of Pharmacal Research. 2010;33(10):1567-74.
57. David OJ, Kovarik JM, Schmouder RL. Clinical pharmacokinetics of fingolimod. Clinical pharmacokinetics. 2012;51(1):15-28.

58. Jin Y, Zollinger M, Borell H, Zimmerlin A, Patten CJ. CYP4F enzymes are responsible for the elimination of fingolimod (FTY720), a novel treatment of relapsing multiple sclerosis. Drug metabolism and disposition: the biological fate of chemicals. 2011;39(2):191-8.
59. Zollinger M, Gschwind HP, Jin Y, Sayer C, Zecri F, Hartmann S. Absorption and disposition of the sphingosine 1-phosphate receptor modulator fingolimod (FTY720) in healthy volunteers: a case of xenobiotic biotransformation following endogenous metabolic pathways. Drug metabolism and disposition: the biological fate of chemicals. 2011;39(2):199-207.
60. Gilenya (fingolimod hydrochloride tablets):USprescribing information [online]. Available from URL: www.accessdata.fda.gov/drugsatfda_docs/label/2011/022527s002lbl.pdf. 2012.
61. Kovarik JM, Schmouder R, Barilla D, Wang Y, Kraus G. Single-dose FTY720 pharmacokinetics, food effect, and pharmacological responses in healthy subjects. British journal of clinical pharmacology. 2004;57(5):586-91.
62. Budde K, Schmouder RL, Brunkhorst R, Nashan B, Lucker PW, Mayer T, et al. First human trial of FTY720, a novel immunomodulator, in stable renal transplant patients. Journal of the American Society of Nephrology : JASN. 2002;13(4):1073-83.
63. Tham CS, Lin FF, Rao TS, Yu N, Webb M. Microglial activation state and lysophospholipid acid receptor expression. International Journal of Developmental Neuroscience. 2003;21(8):431-43.
64. Okada T, Kajimoto T, Jahangeer S, Nakamura S-i. Sphingosine kinase/sphingosine 1-phosphate signalling in central nervous system. Cellular Signalling. 2009;21(1):7-13.
65. Melendez AJ. Sphingosine kinase signalling in immune cells: Potential as novel therapeutic targets. Biochimica Et Biophysica Acta-Proteins and Proteomics. 2008;1784(1):66-75.
66. Nayak D, Huo Y, Kwang WXT, Pushparaj PN, Kumar SD, Ling EA, et al. SPHINGOSINE KINASE 1 REGULATES THE EXPRESSION OF PROINFLAMMATORY CYTOKINES AND NITRIC OXIDE IN ACTIVATED MICROGLIA. Neuroscience. 2010;166(1):132-44.
67. Noda H, Takeuchi H, Mizuno T, Suzumura A. Fingolimod phosphate promotes the neuroprotective effects of microglia. Journal of Neuroimmunology. 2013;256(1-2):13-8.
68. Rothhammer V, Kenison JE, Tjon E, Takenaka MC, de Lima KA, Borucki DM, et al. Sphingosine 1-phosphate receptor modulation suppresses pathogenic astrocyte activation and chronic progressive CNS inflammation. Proceedings of the National Academy of Sciences of the United States of America. 2017;114(8):2012-7.
69. Qin C, Fan W-H, Liu Q, Shang K, Murugan M, Wu L-J, et al. Fingolimod Protects Against Ischemic White Matter Damage by Modulating Microglia Toward M2 Polarization via STAT3 Pathway. Stroke. 2017;48(12):3336-46.
70. Das A, Arifuzzaman S, Kim SH, Lee YS, Jung KH, Chai YG. FTY720 (fingolimod) regulates key target genes essential for inflammation in microglial cells as defined by high-resolution mRNA sequencing. Neuropharmacology. 2017;119:1-14.
71. Sucksdorff M, Rissanen E, Tuisku J, Nuutinen S, Paavilainen T, Rokka J, et al. Evaluation of the Effect of Fingolimod Treatment on Microglial Activation Using Serial PET Imaging in Multiple Sclerosis. Journal of Nuclear Medicine. 2017;58(10):1646-51.
72. Delbridge MS, Shrestha BM, Raftery AT, El Nahas AM, Haylor JL. Reduction of ischemia-reperfusion injury in the rat kidney by FTY720, a synthetic derivative of sphingosine. Transplantation. 2007;84(2):187-95.
73. Man K, Ng KT, Lee TK, Lo CM, Sun CK, Li XL, et al. FTY720 attenuates hepatic ischemia-reperfusion injury in normal and cirrhotic livers. American Journal of Transplantation. 2005;5(1):40-9.
74. Hasegawa Y, Suzuki H, Sozen T, Rolland W, Zhang JH. Activation of Sphingosine 1-Phosphate Receptor-1 by FTY720 Is Neuroprotective After Ischemic Stroke in Rats. Stroke. 2010;41(2):368-74.
75. Wei Y, Yemisci M, Kim H-H, Yung LM, Shin HK, Hwang S-K, et al. Fingolimod Provides Long-Term Protection in Rodent Models of Cerebral Ischemia. Annals of Neurology. 2011;69(1):119-29.

76. Fu Y, Zhang N, Ren L, Yan Y, Sun N, Li Y-J, et al. Impact of an immune modulator fingolimod on acute ischemic stroke. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(51):18315-20.
77. Zhu Z, Fu Y, Tian D, Sun N, Han W, Chang G, et al. Combination of the Immune Modulator Fingolimod With Alteplase in Acute Ischemic Stroke A Pilot Trial. Circulation. 2015;132(12):1104-12.
78. Lu L, Barfejani AH, Qin T, Dong Q, Ayata C, Waeber C. Fingolimod exerts neuroprotective effects in a mouse model of intracerebral hemorrhage. Brain Research. 2014;1555:89-96.
79. Rolland WB, Lekic T, Krafft PR, Hasegawa Y, Altay O, Hartman R, et al. Fingolimod reduces cerebral lymphocyte infiltration in experimental models of rodent intracerebral hemorrhage. Experimental Neurology. 2013;241:45-55.
80. Sun N, Shen Y, Han W, Shi KB, Wood K, Fu Y, et al. Selective Sphingosine-1-Phosphate Receptor 1 Modulation Attenuates Experimental Intracerebral Hemorrhage. Stroke. 2016;47(7):1899-906.
81. Fu Y, Hao J, Zhang N, Ren L, Sun N, Li Y-J, et al. Fingolimod for the Treatment of Intracerebral Hemorrhage A 2-Arm Proof-of-Concept Study. Jama Neurology. 2014;71(9):1092-101.
82. Murthy SB, Moradiya Y, Shah J, Merkler AE, Mangat HS, Iadacola C, et al. Nosocomial Infections and Outcomes after Intracerebral Hemorrhage: A Population-Based Study. 2016;25(2):178-84.
83. Lord AS, Gilmore E, Choi HA, Mayer SA, Collaboration V-I. Time course and predictors of neurological deterioration after intracerebral hemorrhage. Stroke. 2015;46(3):647-52.
84. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013;29(1):15-21.
85. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. 2014;30(7):923-30.
86. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology. 2014;15(12):550.
87. Ching T, Huang S, Garmire LX. Power analysis and sample size estimation for RNA-Seq differential expression. Rna. 2014;20(11):1684-96.
88. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B (Methodological). 1995;57(1):289-300.
89. Kramer A, Green J, Pollard J, Jr., Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics. 2014;30(4):523-30.
90. Huang DW, Sherman BT, Tan Q, Kir J, Liu D, Bryant D, et al. DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. Nucleic acids research. 2007;35(Web Server issue):W169-75.