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Targeting Reperfusion Injury in the Age of Mechanical Thrombectomy

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Pharmacological recanalization with r-tPA (recombinant tissue-type plasminogen activator) has been the mainstay for acute ischemic stroke (IS) treatment.[1] Recent randomized controlled trials have additionally demonstrated the efficacy of mechanical thrombectomy (MT).[2–4] Although the restoration of blood flow is a major goal in acute treatment, if this occurs too late, worse damage can ensue, compared with no revascularization.[5] This worsening results because of the generation of excess reactive oxygen species (ROS) which leads to direct cellular damage and indirect damage through the triggering of inflammation. Inflammation causes the generation of damaging immune mediators, effector molecules, and more ROS.[6] ROS can also lead to apoptosis/necrosis via DNA/RNA damage and lipid peroxidation. This cycle is known as reperfusion injury (R/I; Figure). Experimental studies have shown that durations of >2- to 3-hours transient middle cerebral artery occlusion (tMCAO) lead to worsened injury compared with permanent MCAO.[7] At the clinical level, delayed revascularization can sometimes lead to worsened outcomes.[8] Hyperintense acute reperfusion marker seen on magnetic resonance imaging in some patients with stroke has been associated with hemorrhagic transformation (HTf) and clinical worsening, suggesting the existence of R/I in humans.[9] Hence, adjunctive treatments to recanalization to target R/I has the potential to improve current outcomes while reducing complications of r-tPA.

We will focus on the underlying mechanisms of R/I and laboratory studies that targeted these mechanisms in experimental revascularization models, such as tMCAO or thromboembolic stroke (Table 1).[10–18] We will also review past and present clinical trials that attempt to study these targets, some in the setting of combined use with revascularization treatments (Table 2).[19–30]

Anti-Inflammatory Approaches to R/I

Poststroke inflammation has largely been thought to exacerbate ischemic injury. Several immune molecules to contribute to this worsening, including inflammatory cytokines, chemokines, and immune cell–produced reactive species. Immune cell activation is thought to first occur in microglia after release of molecules elaborated by ischemic brain cells. Leukocyte activation and infiltration into the brain soon follows. Several laboratory studies showed that preventing leukocyte infiltration led to better outcomes in stroke models although its efficacy has not yet been shown clinically.[31]

Inflammation begins after stroke as ischemic brain cells elaborate molecules collectively known as damage-associated molecular patterns. These include high mobility group box-, peroxiredoxin, purines, nucleotides such as ATP and UDP, and nucleic acid fragments.[6,31] Damage-associated molecular patterns bind innate immune receptors, such as Toll-like and purinergic receptors, on microglia and leukocytes leading to their activation followed by activation of inflammatory transcription factors nuclear factor-κB and mitogen-activated protein kinase. Deficiency or pharmacological inhibition of these factors has largely been shown to protect against experimental stroke.[32,33] These factors give rise to cytokines, chemokines, adhesion molecules, matrix metalloproteinases-9 (MMP-9), inducible nitric oxide synthase, and NADPH oxidase (NOX), leading to exacerbation of ischemic injury. Proinflammatory cytokine interleukin (IL)-1β has moved the farthest forward in terms of translation. In a phase II clinical trial, human recombinant IL-1 receptor antagonist, IL-1 β's endogenous inhibitor, reduced infarct volume and improved neurological outcome at 3 months.[34] T-cell releasing proinflammatory cytokines (interferon-γ, IL-17, and IL-23) have recently emerged as therapeutic targets. IL-17 promotes tumor necrosis factor-α, IL-1β, and MMP-9 expression, whereas IL-23 induces the expression of IL-17.[35] Inhibiting these cytokines improves neurological outcome in experimental R/I. Tumor necrosis factor-α–inducible protein 8-like 2, expressed in microglia/macrophages after tMCAO, contributes to anti-inflammatory effects, and tumor necrosis factor-α–inducible protein 8-like 2–deficient mice subjected to tMCAO have exacerbated neurological and inflammatory outcomes.[36] IL-10, IL-4, and transforming growth factor-β1 are anti-inflammatory cytokines, and all seem associated with improved neurological outcomes in stroke models.[6,37] MMP-9, which is expressed by immune cells, contributes to inflammation by disrupting the blood–brain barrier (BBB). Furthermore, endogenous tPA activates plasmin, which activates MMP-9. Thus, administration of r-tPA may accelerate hemorrhage, and edema should it enter the brain, making MMP-9 a relevant target.[38]

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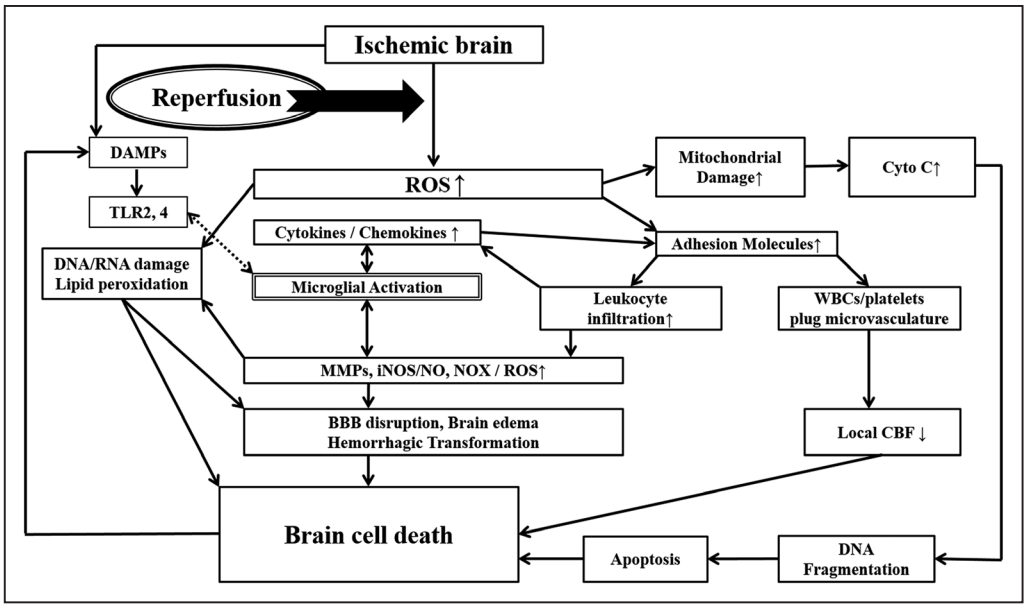

Figure. Reperfusion injury is thought to occur when a sudden influx of oxygenated blood introduces reactive oxygen species (ROS) into critically damaged ischemic brain. Ischemically damaged mitochondria become unable to efficiently neutralize ROS. Elevated ROS can directly damage DNA, RNA, and cause lipid peroxidation. ROS lead to immune cell activation, including brain resident microglia. Ischemic brain may also elaborate damage-associated molecular patterns (DAMPs) that act on Toll-like receptors (TLR) present on the surface of microglia. TLR activation triggers immune signaling with upregulation of cytokines and chemokines, which upregulate adhesion molecules involved in the recruitment and infiltration of circulating leukocytes. Once inside of the brain, leukocytes potentiate immune responses already established by microglia. Some leukocytes and platelets may remain in the intravascular space and form plugs that compromise local blood flow. Activated immune cells elaborate various toxic mediators, including matrix metalloproteinases (MMPs), which can disrupt the extracelluar matrix and blood–brain barrier (BBB) leading to brain edema and hemorrhage. Other immune molecules include inducible nitric oxide synthase (iNOS) and NOX2 (NADPH oxidase-2) that generate nitric oxide (NO) and superoxide, respectively. Mitochondria also release prodeath factors, such as cytochrome c (cyto c), which ultimately lead to DNA damage and apoptosis. CBF indicates cerebral blood flow; and WBC, white blood cell.

Several anti-inflammatory treatments have been studied in stroke models, especially in a combination therapy with r-tPA. Minocycline, with its pleiotropic effects against cell death, improved neurological outcome and decreased r-tPA–related HTf.[10] One mechanism of minocycline's protective effect may be its ability to suppress microglial activation by inhibiting p38-mitogen-activated protein kinase. Minocycline also improved BBB integrity via MMP inhibition.[39] BB-94, an MMP-9 inhibitor, reduced r-tPA–induced HTf in a rabbit stroke model.[11] However, MMPs are involved in neurovascular remodeling, and long-term inhibition may impede repair.[40] Epigallocatechin gallate, found in green tea, has gained interested for its antioxidant and neuroprotective properties. Epigallocatechin gallate downregulated MMP-2 and MMP-9 while upregulating plasminogen activator inhibitor-1 in stroke models.[12] Epigallocatechin gallate combined with r-tPA extended the therapeutic time window of r-tPA and reduced brain edema and BBB disruption.[12] Progranulin, a growth factor found in the brain, is thought to contribute anti-inflammatory and vasoprotective properties. It is particularly increased in microglia and endothelial cells after ischemia.[13] r-tPA plus progranulin in a stroke model was shown to improve neurological outcomes and reduce brain hemorrhage and edema. Granulocyte-colony stimulating factor may provide neuroprotection through anti-inflammatory effects.[41] In a model of tMCAO, r-tPA plus granulocyte-colony stimulating factor reduced HTf and improved of neurological function compared with r-tPA alone.[14]

Antioxidative/Nitrosative Approaches to R/I

Reperfusion after IS induces oxidative stress through the mitochondrial respiratory chain and NOX. Ischemic mitochondria, overwhelmed by ROS introduced by oxygenated blood, become unable to efficiently neutralize these species. Overexpressions of endogenous antioxidant enzymes superoxide dismutase, glutathione peroxidase, and catalase have been shown to improve outcome from experimental stroke. Transgenic mice overexpressing superoxide dismutase had significantly reduced infarct size in experimental tMCAO while superoxide dismutase–deficient mice had worsened outcomes.[42] Overexpressing other endogenous antioxidants, such as glutathione peroxidase and catalase, were similarly neuroprotective.[43] At the clinical level, ebselen, a glutathione peroxidase mimic, improved neurological outcomes in patients with IS treated within 6 hours of symptom onset.[44]

Superoxide generated by immune cells occurs via NOX, leading to more oxidative stress.[45] NOX inhibition has been shown to improve outcome in experimental stroke,[45] but NOX may also play an important role in hyperglycemia-induced stroke exacerbation. Glucose can be metabolized via the hexose monophosphate shunt to produce NADPH, thereby providing substrate to generate NOX. Apocynin, an NOX inhibitor, improved outcome from experimental hyperglycemic tMCAO[46] and reduced hyperglycemia-induced worsening of BBB disruption and HTf because of r-tPA use.[47]

Other related strategies have been investigated over the years. Free radical scavengers, such as tirilazad and

Table 1. Laboratory Studies of Antioxidant and Anti-Inflammatory Combination Therapies With r-tPA

Therapy (Author/y)	Animal Model	Therapeutic Target	Treatment	Outcomes
Minocycline (Fan et al[10] 2013)	Thromboembolic stroke, rat	p38 MAPK	r-tPA alone (1.5-h postembolization) vs r-tPA+minocycline 1-h postembolization)	Minocycline ↓ infarct volume, hemorrhage, and edema
BB-94 (Lapchak et al[11] 2000)	Large clot embolic stroke, rabbit	MMPs	r-tPA (1-h postembolization) vs r-tPA+BB-94 5 min after embolization	BB-94 ↓r-tPA–induced hemorrhage
EGCG (You et al[12] 2016)	Thromboembolic stroke, rat	MMPs, ROS	EGCG every 4 h after embolization treated+r-tPA	EGCG ↓r-tPA extended therapeutic window, ↓ infarct volume, edema, and BBB disruption
Progranulin (Kanazawa et al[13] 2015)	Thromboembolic stroke, rat	Inflammation	r-tPA (4-h post-MCAO) vs r-tPA+progranulin immediately before r-tPA	Progranulin ↓infarct size, cerebral edema, r-tPA–induced hemorrhagic transformation; improved motor outcome
G-CSF (dela Peña et al[14] 2015)	tMCAO×1 h, rat	IL-1β, iNOS, apoptosis	IV r-tPA vs r-tPA+G-CSF immediately before reperfusion	r-tPA+G-CSF ↓neurological deficit and hemorhhagic transformation vs r-tPA alone
NXY-059G (Lapchak et al[15] 2002)	Large clot embolic stroke rabbit	ROS	NXY-059G 5 min after embolization vs r-tPA 1 h after embolization+NXY-059G	NXY-059G+r-tPA ↓ r-tPA–induced hemorrhagic transformation and ↑behavioral function
Uric acid (Romanos et al[16] 2007)	Thromboembolic stroke, rat	ROS	r-tPA+uric acid 20 min after occlusion	Uric acid+r-tPA ↓infarct volume, ↑neurological function
Edaravone (Yagi et al[17] 2009)	tMCAO×3 h, rat	ROS and MMP-9	r-tPA alone vs r-tPA+edaravone immediately after reperfusion	Edaravone ↓ r-tPA–induced hemorrhagic transformation
Hypothermia (Tang et al[18] 2013)	tMCAO×1 or 3 h, rat	Multiple mechanisms	r-tPA 1 or 3 h after ischemia vs r-tPA plus cooling (33°C) before or concurrent with r-tPA	Hypothermia ↓infarct size, neurological deficits, brain hemorrhage, BBB disruption

BBB indicates blood–brain barrier; EGCG, epigallocatechin gallate; G-CSF, granulocyte-colony stimulating factor; IL, interleukin; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; ROS, reactive oxygen species; r-tPA, recombinant tissue-type plasminogen activator; and tMCAO, transient middle cerebral artery occlusion.

4-benzene-1,3-disulphonate N-oxide (NXY-059) plus r-tPA, showed efficacy in experimental stroke[15] but were negative when studied clinically.[48,49] PSD-95 protein (postsynaptic density-95) is associated with the *N*-methyl-D-aspartate receptor. It recruits neuronal nitric oxide synthase, responsible for generating neurotoxic nitric oxide.[50] The PSD-95 inhibitor, NA-1, improves outcome in experimental R/I. Insulin-like growth factor, a pleiotropic peptide involved in prosurvival signaling, may also inhibit oxidative and nitrosative stress.[51] Uric acid, through its antioxidant properties, improved outcomes in a thromboembolic stroke model.[16] ROS scavenger edaravone also improved neurological outcome in experimental models in combination with r-tPA[17] and is used clinically in Japan for acute IS.[19]

Antiapoptotic Approach to R/I

ROS-mediated damage leads to apoptosis and may be especially relevant to R/I as apoptosis requires the presence of cellular energy stores to drive cell death.[52] Mitochondrial initiation of apoptosis is referred to as the intrinsic pathway. This occurs when mitochondria release cytochrome c to the cytosol and forms a complex with apoptotic protease-activating factor 1 and procaspase-9 to form the apoptosome.[53] The apoptosome activates caspase-9 and activates effector caspase-3 which promotes DNA cleavage.[52] Superoxide dismutase–overexpressing mice showed reduced apoptosis and cytochrome c translocation in R/I,[53] as did blocking both second mitochondria-derived activator of caspase/direct IAP-binding protein (inhibitor of apoptosis protein) of low pI (Smac/DIABLO) and Omi stress-regulated endoprotease/high temperature requirement protein A2.[54]

Bcl-2 family molecules involved in apoptosis include BAX, BAD, and BID which trigger cytochrome c release, whereas Bcl-2 and Bcl-X_L prevent it.[52] Changing the balance of these molecules to favor antiapoptotic isoforms has been shown to improve outcome in experimental stroke. The extrinsic apoptotic pathway is activated when death receptors are bound by their ligands. The best studied of these receptor-ligand pairs is Fas/FasL. FasL ligates Fas and leads to caspase-8 activation, followed by eventual caspase-3 activation and DNA cleavage.[52] Mice with Fas mutations seem protected from R/I.[55]

Finally, estrogen is known to factor in IS. Female animals are known to have better outcomes after experimental stroke compared with male, and 17-β estradiol (E2)'s protective effect seems related to Bcl-2 upregulation and suppression of apoptosis.[56] Thus, neuroprotective effects for sex-specific steroids may be linked to the modulation of apoptosis.

Table 2. Clinical Studies of Antioxidant and Anti-Inflammatory Combination Therapies With Acute Revascularization

Therapy/Trial	Study Design	Patients Number/Inclusion Criteria	Intervention Time	Treatments	Outcomes
Edaravone 1. PROTECT4.5[19] 2. YAMATO[20] 3. RESCUE[21]	1, 3. Prospective observational study; 2. Multicenter, prospective, randomized, open-label study	1. n=11 384 2. n=165 (MCA stroke) 3. n=1442	<4.5 h	1. r-tPA+edaravone (30 mg twice/d×7 d) 2. Edaravone early group (before/during r-tPA) vs late group (after r-tPA) 3. Edaravone+IV r-tPA and MT	Edaravone+r-tPA safe; timing of edaravone did not affect recanalization; edaravone+r-tPA may be superior to MT
Uric acid URICO-ICTUS[22]	Randomized, double-blind, placebo-controlled, phase 2b/3	n=411 (NIHSS >6, ≤25, premorbid mRS ≤2	<4.5 h	1000 mg UA during r-tPA infusion	UA safe, did not affect outcomes from r-tPA treatment
Hypothermia 1. ReCCLAIM[23] 2. ICTus-2[24] 3. ReCCLAIM-II[25]	1. Prospective single-arm open-label clinical trial 2. Prospective, randomized, single-blind, multicenter phase 2/3study 3. Prospective, randomized, open-label study	1. n=20 (ASPECTS 5–7, NIHSS ≥13); 2. n=120 (NIHSS ≥7 and ≤20–24) 3. n=85 (ASPECTS 5–10, NIHSS 14–29, premorbid mRS <2)	1. <8 h 2. <3 h 3. <8 h	1. Immediate cooling to 33°C×12 h+MT/+r-tPA 2. Immediate cooling to 33°C for 24 h+r-tPA 3. Mild hypothermia to 33°C+r-tPA/MT	1, 2. Hypothermia safe after r-tPA and MT and may ↓HTf 3. stopped early
Verapamil SAVER-I[26]	Phase I	n=11 (MT with a TICI 2A or better)	<8 h	10 mg verapamil for 20 min into the previously occluded vessel	Combination is safe and feasible
Minocycline Kohler et al[27] 2013	Multicenter, prospective, randomized, open-label, blinded, pilot study	n=95	<24 h	r-tPA vs r-tPA +minocycline (100 mg daily×3d)	Minocycline safe but not efficacious
EGCG Wang et al[28] 2017	Randomized, double-blind, placebo-controlled	n=371 (clearly defined time of onset, measurable NIHSS deficit, no HTf)	<4.5 h	r-tPA vs r-tPA+EGCG (500 mg daily×7 d)	EGCG improved NIHSS; extended the time window for r-tPA
Fingolimod Zhu et al[29] 2015	Randomized, open-label, evaluator-blind, multicenter pilot study	n=47 (NIHSS ≥5)	4.5–72 h	r-tPA vs r-tPA+fingolimod (0.5 mg every 12 h×5 d)	Fingolimod+r-tPA well tolerated; improved outcomes
Simvastatin STARS07[30]	Multicentre, phase IV, prospective, randomized, double-blind, placebo-controlled	n=104 (NIHSS 4–22, premorbid mRS 0–1; 55 patients received r-tPA therapy)	<12 h	r-tPA vs r-tPA+simvastatin (40 mg once daily×90 d)	Simvastatin+r-tPA safe and ↓HTf

ASPECTS indicates Alberta Stroke Program Early CT Score; EGCG, epigallocatechin gallate; HTf, hemorrhagic transformation; MCA, middle cerebral artery; mRS, modified Rankin Scale; MT, mechanical thrombectomy; NIHSS, National Institutes of Health Stroke Scale; ReCCLAIM, Reperfusion and Cooling in Cerebral Acute Ischemia; r-tPA, recombinant tissue-type plasminogen activator; TICI, Thrombolysis in Cerebral Infarction; and UA, uric acid.

Clinical Studies of Combination Therapy With Revascularization

Although several drugs have been studied in combination with r-tPA in experimental stroke models to prevent R/I, clinical studies have also been performed in combination with r-tPA and MT. Although not specifically designed to target R/I, these studies may provide a framework to design future trials.

Combination treatment with r-tPA/MT plus edaravone is already being used clinically in Japan. Although lacking control groups, 2 observational studies were suggestive. PROTECT4.5 (Post-Marketing Registry on Treatment With Edaravone in Acute Cerebral Infarction by the Time Window of 4.5 Hours) evaluated edaravone plus r-tPA and suggested that this combination might increase the chances for better outcomes and reduce HTf.[19] YAMATO (Tissue-Type Plasminogen Activator and Edaravone Combination Therapy) indicated that favorable outcomes after r-tPA were not related to the timing of edaravone infusion.[20] Finally, a subanalysis of a stroke registry (RESCUE Japan Registry [Recovery by Endovascular Salvage for Cerebral Ultra-Acute Embolism]) indicated that edaravone was more effective in patients treated with r-tPA than MT.[21]

The URICO-ICTUS trial (Efficacy Study of Combined Treatment With Uric Acid and r-tPA in Acute Ischemic Stroke), which assessed the efficacy of uric acid plus r-tPA/MT in acute IS, confirmed safety in patients treated within 4.5 hours of symptom onset. This study also reported that uric acid was associated with reduced infarct growth and improved outcome in IS patients with early recanalization and hyperglycemia.[16] Efficacy was also demonstrated when uric acid was given in addition to MT.[22]

Therapeutic hypothermia, which is thought to target multiple R/I mechanisms, is already indicated to improve neurological outcomes after cardiac arrest and neonatal hypoxia-ischemia.[57] In combination with r-tPA in experimental tMCAO, therapeutic hypothermia led to reduced BBB disruption and HTf.[18] The ReCCLAIM (Reperfusion and Cooling in Cerebral Acute Ischemia)[23] and ICTuS-2 (Intravascular Cooling in the Treatment of Stroke 2)[24] trials examined this combination

in patients with stroke, and both showed that this approach was safe and feasible although RECCLAIM-II, which additionally examined MT, was stopped early for lack of funding.[25] Pilot studies of MT plus selective brain cooling via intra-arterial chilled saline infusion are ongoing and seem feasible and safe.[58]

The ACTION-I trial (Effect of Natalizumab on Infarct Volume in Acute Ischemic Stroke) studied safety and efficacy of natalizumab in acute IS. Natalizumab, an antibody to α4 integrin, is used in multiple sclerosis. Natalizumab is thought to reduce lymphocyte invasion and adhesion molecule upregulation. Although nataliizumab failed to show efficacy in experimental stroke,[59] ACTION-I included patients receiving r-tPA.[60] Infarct volume was not significantly different with the addition of natalizumab, but neurological outcomes were improved.

Other anti-inflammatory and antioxidant drugs, all of which are used clinically for other indications, have also been studied clinically in combination with r-tPA. The phase I trial, SAVER-I (Superselective Administration of VErapamil During Recanalization in Acute Ischemic Stroke) studied the therapeutic potential of verapamil with r-tPA and MT and found that this combination was safe.[26] Similarly, minocycline with r-tPA seems safe although efficacy is unclear.[27] Epigallocatechin gallate plus r-tPA seemed to extend the temporal therapeutic window of r-tPA and improve outcome.[28] Oral fingolimod in a small study indicated that it could be given safely with improved neurological recovery at 90 days[61] while a pilot study of fingolimod plus r-tPA demonstrated safety and trends toward favorable clinical outcomes and reduced HTf.[29] Recently, the STARS07 (Stroke Treatment with Acute Reperfusion and Simvastatin) trial, which was a phase IV trial to demonstrate the efficacy and safety of simvastatin treatment in acute stroke, showed that simvastatin plus r-tPA were safe and reduced HTf.[30]

There are currently several ongoing trials specifically evaluating the safety and efficacy of revascularization plus neuroprotection. Compared with earlier trials, these studies directly examined whether adjunctive treatments to r-tPA and MT improve outcome and may inadvertently study R/I. Activated protein C, which is thought to suppress inflammation and prevent BBB disruption, is being studied in combination with r-tPA/MT in The Safety Evaluation of 3K3A-activated protein C in Ischemic Stroke (RHAPSODY) trial (NCT02222714).[62] Another clinical trial of atorvastatin combined with MT, SEATIS (The Safety and Efficacy Study of High Dose Atorvastatin After Thrombolytic Treatment in Acute Ischemic Stroke) trial (NCT02452502), is also ongoing.[63] Combined therapy with NA-1 and MT in the Safety and Efficacy of NA-1 in Subjects Undergoing Endovascular Thrombectomy for Stroke (ESCAPE-NA1) trial (NCT02930018) plans to assess safety and efficacy. Although it did not limit enrollment to patients with IS receiving r-tPA/MT, ACTION II (NCT02730455) evaluates the safety and efficacy of intravenous natalizmab. Finally, FAMTAIS (Fingolimod with Alteplase bridging with Mechanical Thrombectomy in Acute Ischemic Stroke; NCT02956200), a phase II trial of bridging therapy (fingolimod plus r-tPA/MT), was recently started to assess safety and efficacy in large vessel occlusion.[64]

Conclusions

The broad field of R/I in acute stroke may identify potential treatment targets and lead to clinical translation. The phenomenon of R/I is well established in the laboratory. Although it is less clear clinically, recent advances in acute revascularization may make it possible to establish whether this occurs in humans. Regardless, pharmacological thrombolysis carries an increased risk of brain hemorrhage, and adjunctive therapies against the same targets that contribute to R/I in the laboratory could reduce HTf, lengthen the time window for intervention, and further improve outcomes. We propose that such approaches may also increase the numbers of patients with stroke eligible for treatment.

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Disclosures

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