Cerebral neuroprotection for acute ischemic stroke (AIS) is defined as a therapy aimed at enhancing the brain’s resilience to ischemia to improve the clinical outcome of affected individuals. Although traditionally aimed at the salvage of neurons, this term may be equally applicable to all the cellular constituents of the brain, including cells of cerebral blood vessels, neurons, and glia. Pharmacological neuroprotection (hereafter referred to as neuroprotection) would be achieved by drugs targeting one or more critical components of the ischemic cascade that lead to ischemic damage. The feasibility of neuroprotection has a strong basis in animal experiments, but research for several decades has failed to translate neuroprotective treatments from animals to humans. The disappointing results of all controlled clinical neuroprotection trials for AIS have cast doubts as to whether neuroprotection in humans is biologically possible and, given the complexities of human stroke syndromes, whether it is a clinically practicable therapy for patients experiencing AIS in the community.

Is Neuroprotection in Humans Feasible?
The questions of feasibility and practicability cannot be resolved simultaneously. Feasibility is a question of biology: Whether it is possible to achieve tissue sparing after AIS in the high-order brains of humans? The gloomiest hypothesis is that tissue sparing and preservation of neurological function may be possible in low-order species, such as rats, but not in humans. Under this hypothesis, there might be as-yet-unresolved but fundamental anatomic, genetic, or biological differences between low-order species and humans that preclude neuroprotection in the high-order brain. However, recent studies with postsynaptic density-95 (PSD-95) protein inhibitors, a promising class of neuroprotectant that uncouple PSD-95 from neurotoxic signaling pathways in central neurons, repudiate this concern. They show that neuroprotection is unequivocally possible not only in the rodent brain, but also in the high-order brain of old-world primates and in humans. Among these studies were experiments conducted in cynomolgus macaques, which bear genetic, anatomic, and behavioral similarities to humans, and who were subjected in cynomolgus macaques, which bear genetic, anatomic, and behavioral similarities to humans, and who were subjected in cynomolgus macaques, which bear genetic, anatomic, and behavioral similarities to humans, to middle cerebral artery occlusion (MCAO) in various clinically relevant paradigms. In brief, treatment with the PSD-95 inhibitor, NA-1, reduced infarct volumes as gauged by MRI and histology, preserved the capacity of ischemic cells to maintain gene transcription in genome-wide screens of ischemic brain tissue, and significantly preserved neurologic function in neurobehavioral assays. These results demonstrated that neuroprotection with the goal of obtaining tissue salvage and improved neurological function is achievable in the high-order brain even in severe experimental strokes.

A subsequent study in macaques demonstrated that small embolic strokes, similar to those incurred by humans undergoing vascular neurointerventional procedures, could be treated with NA-1. This experiment served to bolster a novel clinical trial paradigm: a proof-of-concept (POC) study to explore whether administering NA-1 after stroke onset could reduce ischemic brain damage in patients who underwent an endovascular brain aneurysm repair. Such individuals have a high incidence of small, procedurally induced ischemic strokes. One hundred eighty-five such subjects were enrolled in a multicenter randomized, double-blinded trial to receive a single intravenous infusion of NA-1 or saline control at the termination of the endovascular procedure (the Evaluating Neuroprotection in Aneurysm Coiling Therapy [ENACT] trial; ClinicalTrials.gov; NCT00728182). Ischemic damage and clinical outcomes were assessed using MRI, neurological evaluations, and a cognitive battery throughout a 30-day study period. Subjects who received NA-1 sustained fewer ischemic infarcts as gauged by MRI. Among subjects with ruptured aneurysms, NA-1 treatment reduced the number and volume of strokes and improved neurological outcome at 30 days. Consequently, this phase 2 multicenter trial provided evidence that tissue neuroprotection in the ischemic human brain is feasible. Overall, the biological efficacy of PSD-95 inhibitors in rats, primates, and humans suggests that they might be useful tools with which to probe the second major as-yet- unanswered question: Whether or not neuroprotection in humans is practicable?

The question of practicability of neuroprotection in AIS, that is, whether a neuroprotectant can be tested in a paradigm that will demonstrate a clinical benefit of the treatment, is unresolved. To date, all trials of neuroprotectants have failed to provide a roadmap or a validated basis from which to build better trials. By contrast, the strategy of early recanalization of blocked arteries with intravenous thrombolitics...
is established and is shown to improve clinical outcome in AIS. However, it is presently unclear whether trial designs developed to evaluate recanalization strategies also apply to neuroprotectants. A novel approach to neuroprotection must, therefore, consider lessons learned from all sources to devise a plausible path to success.

Stroke Progression Impacts Future Trials of Neuroprotectants

Therapeutic benefit from recanalization strategies or from neuroprotection can only be obtained if treatment is administered before ischemic damage is complete. Each approach is aimed at rescuing the ischemic penumbra, that portion of the ischemic territory that is still potentially salvageable if appropriate and timely treatment is given. The ideal neuroprotectant is one that, when administered to a patient experiencing a stroke, would safely halt or slow stroke progression and improve clinical outcome. The time urgency of salvageable brain is illustrated in the Figure, which shows the temporal progression of ischemic damage produced by MCAO in cynomolgus macaques using diffusion weighted MRI (DWI). The penumbra, defined here as the difference between the volume of tissue at risk and of tissue exhibiting increased DWI signal, shrinks to ~10% of its original size by 3 hours post-MCAO. Presumably, even ideal neuroprotectants would have limited usefulness if given after the penumbra shrinks to a point where rescuing the remaining tissue is insufficient to provide clinical benefit. The rate at which stroke damage progresses is thus a major consideration for stroke trial design. The key questions are: What is the duration of time from stroke onset until irreversible brain damage precludes the demonstration of clinical use from neuroprotection? What proportion of all patients with AIS has salvageable brain in a given timeframe? The answers are suggested by preclinical animal studies and by human studies including controlled clinical trials.

Preclinical Neuroprotectants Are Generally Ineffective More Than 3 Hours From Stroke Onset

The main literature on the therapeutic window of neuroprotection focuses on experimental strokes induced by permanent or temporary MCAO (pMCAO or tMCAO). Given that early spontaneous recanalization is rare in human AIS (~15% of cases <6 hours), the pMCAO animal model is highly relevant. Imaging experiments of rats subjected to pMCAO suggest that, in the absence of intervention, stroke damage measured by 3 hours is similar to that observed at 24 hours. Cats are even more sensitive to ischemia such that, irrespective of the level of reperfusion, markers of tissue death 2 to 3 hours after pMCAO already predict the size of the final infarcts. In cynomolgus macaques, stroke damage in animals subjected to pMCAO also reaches a plateau by 3 to 4 hours (Figure). Perhaps, because of this rapid progression in most species, only a few animal studies have claimed effectiveness of neuroprotectants administered in an early timeframe are effective in models of pMCAO. The use of early treatment is also corroborated in a primate study in which cynomolgus macaques were subjected to small embolic strokes induced by intracarotid injections of polystyrene microemboli, effectively producing a permanent vessel occlusion. Administration of the neuroprotectant NA-1 at 1 hour after stroke onset reduced the number and volume of the resulting infarcts. These results paralleled the outcome of the human ENACT trial of NA-1 in which treatment was instituted within an average window of 2 hours post–ischemia onset and revealed efficacy of NA-1 in reducing stroke burden. Accordingly, preclinical data in rats and primates, and clinical data in humans, suggest that neuroprotection can reduce stroke burden even in permanent cerebral ischemia if treatment is instituted early.

The most robustly supported scenario in the literature is that of early therapy initiation in tMCAO. Numerous agents have been studied in mice and rats. For most, ischemia was of short duration (<2 hours), and therapies were initiated either during the ischemic period or after reperfusion. A study using NA-1 in primates showed that initiation of treatment 1 hour after the onset of a 90-minute tMCAO salvaged ~70% of the brain at risk, whereas treatment initiation 1 hour after the onset of a 4.5-hour tMCAO salvaged ~40% of brain at risk. This result suggests that earlier treatment, preceding early reperfusion, is most likely to be effective.

A number of published studies suggest that neuroprotection can be beneficial when initiated ≥3 hours after a tMCAO.
However, many did not report the use of randomization, sample size calculation, blinded treatment allocation, and blinded assessment of outcomes. Generally, longer therapeutic windows are described in studies using mice in which tMCAO is of brief duration (≤30 minutes), whereas most studies in rats used a 90- to 120-minute tMCAO. A study using NA-1 in primates suggests efficacy in a 3.5-hour tMCAO when the treatment was administered 3 hours after ischemia onset. However, this study, conducted in our laboratory, was in a tMCAO model in which a collateral circulation was purposely maintained to preserve a penumbra by 3 hours.

Overall, the preponderance of preclinical evidence supports the potential for a benefit of neuroprotection in multiple species, experimental stroke models, and laboratories when neuroprotection is initiated early after stroke onset. By contrast, evidence in support of the use of a delayed administration of neuroprotection is sparse.

The Window of Opportunity for Treating Human AIS is Short

Because there are no approved neuroprotectants from which to estimate the therapeutic window of neuroprotection in humans, a potentially relevant approach is to infer it from the therapeutic window of reperfusion therapies. These include tissue plasminogen activator (tPA), the only Food and Drug Administration–approved reperfusion agent for AIS, and endovascular stroke therapies.

Treatment with tPA is only approved for initiation <3 hours of stroke onset. The usefulness of initiating thrombolysis >3 hours has been examined in several trials, including the European Cooperative Acute Stroke Study (ECASS-3) and the Third International Stroke Trial (IST-3). Based on these, the AHA in its most recent recommendations suggested that “tPA should be administered to eligible patients who can be treated in the time period of 3 to 4.5 hours after stroke onset.” However, in a meta-analysis of all the evidence from randomized trials for tPA in AIS to date, including 7012 patients in ≤12 trials including ECASS-3 and IST-3, Wardlaw et al suggested that there was no demonstrable benefit of tPA use in patients treated between 3 and 6 hours after stroke (7 trials). In addition, on May 7, 2012, the Food and Drug Administration rejected an application to expand the tPA label to include treatment ≥4.5 hours, concluding that the ECASS data were not “sufficiently compelling to warrant a label extension.” These analyses reflect evidence from the most inclusive controlled clinical trials, ones that enrolled patients with AIS from the community primarily on the basis of the time elapsed from symptom onset. They suggest that on average the time during which treatment initiation is beneficial is short, in the order of 3 hours, and no longer than 4.5 hours.

Not all strokes progress similarly. Many factors, including the site of arterial occlusion and the presence of a collateral circulation, may cause some patients to exhibit an ischemic penumbra at a time when others have progressed to a completed stroke. Thus, there may exist a subset of patients in whom penumbra identification is more important for determining eligibility for intervention than the time elapsed from stroke onset. However, although the penumbra concept is central to AIS therapy, its definition as the target of therapy should not be confused as criterion for patient selection. In fact, attempts at targeting thrombolysis to patients who exhibit a penumbra >3 hours after stroke onset have been disappointing. These comprised studies in patients with ischemic penumbra identified using various imaging techniques. A meta-analysis of articles describing the Desmoteplase in Acute Ischemic Stroke (DIAS) trial, DIAS-2, Dose Escalation of Desmoteplase for Acute Ischemic Stroke (DEDAS), Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evolution (DEFUSE), and Echoplanar Imaging Thrombolytic Evaluation Trial (EPITHET) trials, giving a total of 502 patients thrombolysed ≥3 hours, concluded that delayed thrombolysis in these patients was not confirmed to improve clinical outcome.

The early administration of tPA results in recanalization rates of 40% to 50% ≤6 hours of stroke onset, ≤70% in 24 hours, and ≤90% if the occlusion is distal to the internal carotid artery. These relatively low rates of early recanalization have prompted efforts to develop endovascular therapies to achieve better early recanalization. The potential for effective early recanalization using endovascular devices remains attractive. Unfortunately, controlled trials of endovascular therapies with first-generation devices failed to demonstrate a benefit, even if such patients are selected with penumbra imaging. Whereas the role of future of endovascular therapies remains controversial, a strong conclusion is that there is no benefit from recanalization if it occurs too late.

Accordingly, analyses of human data to date indicate that the effectiveness of recanalization therapies diminishes greatly >3 hours from stroke onset. If conclusions from these data apply to neuroprotection for patients with AIS in the community, then there is a clear scientific and medical rationale for limiting the investigation and potential use of neuroprotectants only to patients who can be treated <3 hours from stroke symptom onset. Despite this, no controlled trial of a neuroprotectant completed to date has enrolled patients within this timeframe. In fact, among all phase 2 and 3 trials of neuroprotectants completed since 2000, all except the ENACT trial have enrolled subjects in a window averaging ≥24 hours (Table 1). Given the apparent rapidity of stroke damage progression in animals and in humans, one must consider whether or not neuroprotectants, similarly to thrombolitics, should be considered as emergency drugs and studied in earlier timeframes, before ischemic damage is complete.

Considerations for Future Trials of Neuroprotectants

Large trials of future neuroprotectants will need to be preceded by strong POC. POC would inform and enable a larger, definitive phase 3 trial. A POC trial might make use of knowledge gained from modern imaging and from preclinical studies to demonstrate clinical benefit of a neuroprotectant. However, research has not yet established which imaging criteria might be useful as surrogates for patient outcomes or for patient selection. Presently no surrogate markers, including stroke imaging, have been validated in clinical trials as being reasonably likely to predict clinical benefit. Consequently, although stroke is a serious and life-threatening condition, neuroprotection trials
Table 1. Controlled Clinical Trials of Neuroprotectants Completed Since 2000 (Phase 2 and Higher)

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Study Title</th>
<th>Phase</th>
<th>Completed</th>
<th>Early Termination</th>
<th>Number of Subjects</th>
<th>Age, y</th>
<th>Enrollment Window</th>
<th>Overall Neuro Result (90 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLASS-I</td>
<td>Clomethiazole Acute Stroke Study in Ischemic Stroke</td>
<td>2</td>
<td>2001</td>
<td>No</td>
<td>1198</td>
<td>18–90</td>
<td>12 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>ASTIN</td>
<td>Acute Stroke Therapy by Inhibition of Neutrophils</td>
<td>2</td>
<td>2002</td>
<td>Yes</td>
<td>966</td>
<td>&gt;50</td>
<td>6 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>ARTIST-MRI</td>
<td>Effects of YM872 on Stroke Lesion Volume in Acute Stroke Patients</td>
<td>2</td>
<td>2003</td>
<td>Yes</td>
<td>&gt;18</td>
<td></td>
<td>6 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>ARTIST+</td>
<td>Effects of YM872 on Stroke Lesion Volume in Acute Stroke Patients</td>
<td>2/3</td>
<td>2003</td>
<td>Yes</td>
<td>400</td>
<td>&gt;18</td>
<td>6 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>IMAGES</td>
<td>Intravenous Magnesium Efficacy in Stroke trial (randomized controlled trial)</td>
<td>3</td>
<td>2003</td>
<td>No</td>
<td>2589</td>
<td>&gt;18</td>
<td>12 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>IL-1-ra in AIS</td>
<td>Interleukin-1 Receptor Antagonist in Acute Stroke Patients</td>
<td>2</td>
<td>2004</td>
<td>No</td>
<td>34</td>
<td>&gt;18</td>
<td>6 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>Repinotan</td>
<td>Repinotan in Patients With Acute Ischemic Stroke</td>
<td>3</td>
<td>2004</td>
<td>No</td>
<td>681</td>
<td>&gt;18</td>
<td>4.5 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>Traxiprodil</td>
<td>A Double-Blind, Placebo-Controlled, Multi-Center Study to Evaluate the Efficacy and Safety of a 72-Hour Infusion of CP-101,606 in Subjects With Acute Ischemic Stroke</td>
<td>2</td>
<td>2005</td>
<td>Yes</td>
<td>300</td>
<td>40-90</td>
<td>6 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>SAINT-I</td>
<td>Safety and Effectiveness of NXY-059 for the Treatment of Patients Who Have Suffered From a Stroke</td>
<td>3</td>
<td>2005</td>
<td>No</td>
<td>1700</td>
<td>&gt;18</td>
<td>6 h</td>
<td>Positive*</td>
</tr>
<tr>
<td>SAINT-II</td>
<td>Safety and Effectiveness of NXY-059 for the Treatment of Patients Who Have Suffered From a Stroke</td>
<td>3</td>
<td>2006</td>
<td>No</td>
<td>3306</td>
<td>&gt;18</td>
<td>6 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>SUN N4057</td>
<td>Efficacy of SUN N4057 in Subjects With Acute Ischemic Stroke and Measurable Penumbra on MRI</td>
<td>2</td>
<td>2007</td>
<td>Yes</td>
<td>43</td>
<td>18–85</td>
<td>9 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>EAST</td>
<td>Edaravone and Argatroban Stroke Therapy Study for Acute Ischemic Stroke</td>
<td>2</td>
<td>2008</td>
<td>No</td>
<td>808</td>
<td>&gt;20</td>
<td>24 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>ESS</td>
<td>Multicenter Efficacy Study of Recombinant Human Erythropoietin in Acute Ischemic Stroke</td>
<td>3</td>
<td>2008</td>
<td>No</td>
<td>522</td>
<td>19–100</td>
<td>6 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>ONO-2506</td>
<td>Study of ONO-2506 in Patients With Acute Ischemic Stroke</td>
<td>3</td>
<td>2008</td>
<td>Yes</td>
<td>757</td>
<td>20–79</td>
<td>72 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>Ginsenoside-Rd</td>
<td>Ginsenoside-Rd for Acute Ischemic Stroke</td>
<td>2</td>
<td>2008</td>
<td>No</td>
<td>199</td>
<td>18–75</td>
<td>72 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>Ginsenoside-Rd</td>
<td>Efficacy and Safety of Ginsenoside-Rd for Acute Ischemic Stroke</td>
<td>3</td>
<td>2008</td>
<td>No</td>
<td>390</td>
<td>18–75</td>
<td>72 h</td>
<td>Not published</td>
</tr>
<tr>
<td>TEST</td>
<td>Tolerability of Enecadin (INN) in Acute Ischemic Stroke Trial</td>
<td>2</td>
<td>2009</td>
<td>Yes</td>
<td>24</td>
<td>18–85</td>
<td>9 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>CASTA</td>
<td>The Safety and Efficacy of Cerebrolysin in Patients With Acute Ischemic Stroke</td>
<td>4</td>
<td>2011</td>
<td>No</td>
<td>1070</td>
<td>18–85</td>
<td>12 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>TANDEM-1</td>
<td>Thrombolysis and Deferoxamine in Middle Cerebral Artery Occlusion</td>
<td>2</td>
<td>2011</td>
<td>No</td>
<td>62</td>
<td>18–80</td>
<td>3 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>AXIS-2</td>
<td>AX200 (GCSF) for the Treatment of Acute Ischemic Stroke</td>
<td>2</td>
<td>2011</td>
<td>Yes</td>
<td>328</td>
<td>18–85</td>
<td>9 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>ENACT</td>
<td>Evaluating Neuroprotection in Aneurysm Coiling Therapy (NA-1 vs Placebo)</td>
<td>2</td>
<td>2011</td>
<td>No</td>
<td>185</td>
<td>&gt;18</td>
<td>2.5 h</td>
<td>n/a†</td>
</tr>
<tr>
<td>ICTUS</td>
<td>International Citicoline Trial on Acute Stroke</td>
<td>3</td>
<td>2011</td>
<td>Yes</td>
<td>2078</td>
<td>&gt;18</td>
<td>24 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>MACSI</td>
<td>Efficacy and Safety Study of DP-b69 in Treating Acute Ischemic Stroke</td>
<td>3</td>
<td>2012</td>
<td>Yes</td>
<td>770</td>
<td>18–85</td>
<td>9 h</td>
<td>Neutral</td>
</tr>
<tr>
<td>ALIAS</td>
<td>Albumin in Acute Ischemic Stroke Trial</td>
<td>3</td>
<td>2012</td>
<td>Yes</td>
<td>1100</td>
<td>18–83</td>
<td>5 h</td>
<td>Neutral</td>
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<tr>
<td>Ongoing trials to be reported this year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAST-MAG</td>
<td>Field Administration of Stroke Therapy–Magnesium Trial</td>
<td>3</td>
<td>Ongoing</td>
<td>Ongoing</td>
<td>1700</td>
<td>40–95</td>
<td>2 h</td>
<td>Pending</td>
</tr>
<tr>
<td>URICO-ICTUS</td>
<td>A phase 3 study of combined treatment with uric acid and rPA administered intravenously in acute ischemic stroke patients within the first 4.5 h of onset of symptoms</td>
<td>3</td>
<td>Ongoing</td>
<td>Ongoing</td>
<td>420</td>
<td>&gt;18</td>
<td>4.5 h</td>
<td>Pending</td>
</tr>
</tbody>
</table>

*Positive result not substantiated by subsequent, larger, SAINT-II trial.
†Study had imaging rather than neurological function as outcome measure.
cannot rely on as-yet-unvalidated surrogates for POC. Thus, in the absence of validated surrogate measures, initial POC trials in AIS must be sufficiently powered to demonstrate a potential for producing a clinical benefit. Ideally, POC that precedes a phase 3 trial should also seek to validate surrogate measures demonstrating that the neuroprotectant is biologically efficacious and that it has the potential to provide benefit in clinically relevant scenarios, similar to those encountered outside of trials. A trial that shows efficacy in highly rigid or complex scenarios or in a small subset of carefully preselected patients may be uninformative about a drug’s potential in the broader stroke population. In addition, a POC trial should be achievable in a reasonable timeframe and at an acceptable cost.

Considerations for Trial Design
The possible approaches to AIS trials are summarized in Table 2, which considers the most likely combinations of interventions and investigations that could be conducted. However, the risks and benefits of these various approaches must be carefully considered.

In-Hospital Trials
Virtually all stroke trials completed to date were conducted in patients enrolled after hospital arrival. The obvious advantages of this approach are that trial validity can be maximized by careful selection of enrolling sites, relative ease of staff training, monitoring, and data quality assurance. Hospital infrastructures are already protocol-driven and include the capacity to conduct the necessary screening tests, including the potential for sophisticated medical imaging. Hospitals have trained staff, including research coordinators, nurses, and physicians, who are able to obtain informed consent and implement the screening process.

The simplest approach to testing a drug is a monotherapy trial in which the neuroprotectant is evaluated against a placebo without other confounding interventions. However, in developed countries, the advent of intravenous tPA as a standard of care within 3 to 4.5 hours of stroke onset has made it more difficult to conduct trials of novel therapies in the same time-window as that of tPA eligibility. Monotherapy would thus be relegated to patients who are not tPA candidates. About 50% of patients arriving at emergency departments within the tPA window do not receive thrombolysis on the basis of mild stroke severity,54,55 hemorrhagic stroke, medical and surgical history, or blood tests.54,56,57 However, this population would be an unlikely resource for a neuroprotectant trial because of the heterogeneity of this subpopulation in terms of diagnosis, stroke severity (very mild or very severe), and comorbidities. In addition, by the time ineligibility for tPA is determined, a neuroprotectant might be administered in a timeframe when it is least likely to be effective, and to a population that may not benefit.

A trial can be conducted in conjunction with thrombolysis or other recanalization therapies. One such ongoing trial is the Efficacy Study of Combined Treatment with Uric-Acid and rtPA in Acute Ischemic Stroke (Urigo-Ictus), a phase 3 trial aimed at determining whether the combined treatment with uric acid and tPA is superior to tPA alone in patients with AIS treated <4.5 hours of symptom onset.58 In this study, the test

Table 2. Potential Approaches to Clinical Trials of Novel Neuroprotectants

<table>
<thead>
<tr>
<th>Trial Design</th>
<th>Intervention</th>
<th>Imaging for Subject Selection</th>
<th>Imaging Surrogate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug or Placebo</td>
<td>tPA Device</td>
<td>Exclude ICH</td>
</tr>
<tr>
<td>In-hospital Monotherapy only</td>
<td>On enrollment</td>
<td>✓ x x</td>
<td>✓</td>
</tr>
<tr>
<td>tPA permitted &lt;4.5 h (but not required)</td>
<td>On enrollment</td>
<td>✓ ± x</td>
<td>✓</td>
</tr>
<tr>
<td>With tPA</td>
<td>Before tPA</td>
<td>✓ ✓ x</td>
<td>✓</td>
</tr>
<tr>
<td>tPA required &lt;4.5 h</td>
<td>After tPA</td>
<td>✓ ✓ x</td>
<td>✓</td>
</tr>
<tr>
<td>No tPA (after tPA window)</td>
<td>&gt;4.5 h</td>
<td>✓ x x</td>
<td>✓</td>
</tr>
<tr>
<td>With mechanical reperfusion</td>
<td>Before tPA pre-device</td>
<td>✓ ✓ ✓</td>
<td>✓</td>
</tr>
<tr>
<td>With tPA</td>
<td>After tPA pre-device</td>
<td>✓ ✓ ✓</td>
<td>✓</td>
</tr>
<tr>
<td>Pre-hospital Monotherapy only</td>
<td>On enrollment</td>
<td>✓ x x</td>
<td>x</td>
</tr>
<tr>
<td>With in-hospital tPA permitted (but not required)</td>
<td>On enrollment</td>
<td>✓ ± x</td>
<td>x</td>
</tr>
<tr>
<td>With in-hospital tPA and device permitted (but not required)</td>
<td>On enrollment</td>
<td>✓ ± ±</td>
<td>x</td>
</tr>
</tbody>
</table>

CTA indicates CT-angiogram; ICH, intracranial hemorrhage; MRA, MR-angiogram; tPA, tissue plasminogen activator; ✓, takes place; x, does not take place; and ±, may or may not take place.
agent (uric acid or placebo) is initiated as soon as possible after starting the infusion of tPA treatment. The strength of this trial is that it demonstrates the feasibility of conducting a large trial of a stroke neuroprotectant in the tPA era and of successfully targeting subjects who are candidates for reperfusion, a subgroup expected to have a good response to neuroprotection. However, on arrival, the main priority is to evaluate the patients’ eligibility for recanalization. These activities, geared to shorten the door-to-needle time for thrombolytic therapies, must not be delayed by research. A trial superimposing a neuroprotectant on a recanalization therapy requires physicians to first conduct the studies needed to confirm eligibility for, and to begin, recanalization. The physician is then free to obtain informed consent to randomize the patient and administer the study treatment. Fonarow et al have reported on 25,504 patients with AIS treated with tPA <3 hours of symptom onset at 1082 hospital sites. Of those, the mean door-to-needle time for intravenous tPA administration was 79.3±28.1 minutes and the median was 78 minutes (25th to 75th percentile; 60–98 minutes). There were 6790 (26.6%) patients with door-to-needle time ≤60 minutes and 18,714 (73.4%) with door-to-needle time >60 minutes. For patients with door-to-needle time ≤60 minutes, the median time from stroke onset to arrival was 60 minutes. Thus, approximately only a quarter of tPA candidates begin thrombolysis therapy <140 minutes of stroke onset. This is significantly worse than in the National Institute for Neurological Disorders and Stroke (NINDS) tPA trial, in which about half of enrolled patients received tPA <90 minutes. Therefore, in practice, enrollment into a neuroprotection trial could be delayed by other time pressures, causing the neuroprotectant to be administered at a time when it is least likely to be effective. Despite these caveats, the UROIC-ICTUS trial demonstrates that this trial design is implementable, and the results (due in late 2013) may illuminate whether in-hospital neuroprotection in conjunction with thrombolysis is achievable.

A trial could be considered to study neuroprotection in patients who are no longer candidates for tPA (ie, after the tPA window) but who would be exposed to delayed endovascular recanalization. Presumably, such a trial would only enroll patients based on imaging to exclude patients with completed strokes, as was done in the Mechanical Retrieval and Recanalization of Stroke Clots Using Embolectomy (MR RESCUE) trial. However, there are still ongoing controversies about the use of penumbra imaging as a selection criterion for reperfusion, and evidence from controlled trials in favor of a benefit of endovascular recanalization >3 hours is lacking. Therefore, a trial in which an unproven neuroprotectant is superimposed on an as-yet-unproven recanalization paradigm would be questionable. Also, such a trial would have the least support from preclinical animal studies; it would be testing the neuroprotectant in a highly restricted patient population; and it would not inform about safety or efficacy in a broader stroke population.

**Prehospital Trials**

A controlled clinical trial for a drug should be optimally designed to maximally discriminate the clinical benefit of the drug over placebo. A central tenet of neuroprotection is that time is brain, and even an ideal neuroprotectant, one that arrests stroke damage on administration, can be of benefit only if there is brain left to save. A concern with all in-hospital trial designs for testing neuroprotectants is that they are not easily amenable to prioritizing an early administration of the drug. Instead, hospital-based trials to date have sought to optimize enrollment by increasing enrollment windows or by using sophisticated screening to enroll homogeneous patient populations of presumed responders. No completed clinical trial to date has determined whether early enrollment trumps all other considerations. However, preclinical studies provide strong evidence that neuroprotection is feasible when the drugs are administered early, and such evidence exists in a large variety of preclinical stroke study designs, for numerous neuroprotectants, and in several animal species. By contrast, there is comparatively little preclinical evidence for efficacy of neuroprotectants when they are administered several hours after stroke onset in any circumstance.

One approach to achieving early treatment is a trial in which the neuroprotectant is administered before hospital arrival. Completed stroke trials to date have not yet reported on the results of this approach. However, prehospital therapy trials have been conducted for other neurological emergencies, including the Multicenter Trial of Early Hypothermia in Severe Brain Injury, Progesterone for Traumatic Brain Injury Experimental Clinical Treatment, and the Intramuscular Versus Intravenous Therapy for Prehospital Status Epilepticus. The Field Administration of Stroke Therapy–Magnesium (FAST-MAG) trial is adapting the prehospital therapy approach to stroke. FAST-MAG is a multicenter, randomized, double-blind phase 3 clinical trial, using intention-to-treat analysis, of magnesium sulfate versus placebo among ambulance-transported patients with acute stroke, with study agent initiated in all individuals <2 hours of stroke onset. It will serve 2 purposes, first as a pivotal test of the neuroprotective value of magnesium sulfate in acute stroke. Equally importantly, FAST-MAG provides POC for the practicability of conducting prehospital therapy trials for acute stroke.

A stroke diagnosis would be based on clinical criteria in the field. Enrollment criteria would need to differentiate between the diagnosis of stroke (ischemic or hemorrhagic), subarachnoid hemorrhage, intracranial mass lesions, and stroke mimics, such as patients with alcohol and drug intoxication, postictal hemiparesis, migraine, hypoglycemia or other metabolic encephalopathies, and other nonstroke causes of acute neurological deficits. The FAST-MAG trial team has validated tools to assist with the clinical recognition of stroke in the ambulance. Paramedics in FAST-MAG recognize stroke with an accuracy of 96% based on the Los Angeles Prehospital Stroke Screen (LAPSS). The LAPSS is validated to have a sensitivity of 91%, specificity of 97%, positive predictive value of 97%, and negative predictive value of 98%. Application of the LAPSS in the field has resulted in the enrollment of 1700 patients into the FAST-MAG trial by December 2012. On hospital arrival, enrolled patients would receive all further treatments in accordance with institutional standards of care.
A prehospital trial has the following benefit: Randomization and treatment with test drug can occur early. In FAST-MAG, the median time from stroke onset to drug administration in the first 1463 patients was 46 minutes. Seventy-four percent of patients received drug <1 hour from stroke onset. Administration before recanalization might magnify the effects of reperfusion therapy, a hypothesis consistent with the improved efficacy of neuroprotection in preclinical studies using tMCAO. The trial would be representative of a broader stroke population and could inform not only on patients with AIS, but also on patients with intracranial hemorrhage (ICH) and stroke mimics. The trial would provide a safety database for a broad range of patients with stroke. The prehospital approach ensures that enrollment and randomization do not interfere with needs to determine tPA eligibility on hospital arrival. However, the prehospital approach is limited to agents with an acceptable safety profile, especially in the face of hemorrhagic strokes. Importantly, enrolling patients with diagnoses other than AIS (ICH, stroke mimics) will dilute the likelihood of benefit and will require a larger sample size to detect a treatment effect, and such a trial removes the possibility for imaging selection of any kind.

Beyond the First Hours
Although the focus of most trial designs is on the initial hours leading to the test intervention, the clinical outcome is usually evaluated weeks or months after the intervention was administered. Many factors arise in this interval that affect stroke outcome and, therefore, could confound the outcome of a neuroprotection trial. One such factor is recurrent stroke. Symptomatic ICH <7 days after a stroke occurs most frequently in patients treated with tPA (7% to 8%), but can also arise in 1% to 2% of patients not exposed to thrombolysis because of hemorrhagic transformation of the infarct.34,35 Another 10% of patients may experience fatal or nonfatal deterioration because of swelling of the infarct.35 In addition, recurrent ischemic stroke may afflict 6% to 7% of stroke victims <3 months, especially if the original cause remains untreated.68 Regardless of the impact of the neuroprotectant, recurrent stroke may erase any therapeutic benefits gained at the time of the original intervention. Thus, a clinical trial design should contemplate the impact of recurrent strokes on sample size.

Another major factor is whether the patient is admitted to a stroke unit. A Cochrane Review of organized inpatient care for stroke concluded that care in a stroke unit was consistently associated with improved outcomes, reducing significantly the odds of death or institutionalized care (odds ratio [OR], 0.82; 95% CI, 0.73–0.92; P=0.0006). This was independent of patient age, sex, or stroke severity.69 The use of physical therapy is another postacute phase intervention that may impact long-term outcome. For example, Van Peppen et al67 provided an analysis of randomized trials in which strong evidence was found in favor of task-oriented training to restore balance and gait, and for strengthening the lower paretic limb. It is probable that such training would translate to improved clinical outcomes. Hospitals with organized stroke care could be more likely to have organized stroke triage mechanisms and uniform approaches to the initial and delayed care of stroke patients. It might be reasonable to restrict clinical trials of new neuroprotectants to such institutions.

Conclusions
A clinical trial aimed at determining the clinical benefit of a neuroprotectant needs to prioritize the neuroprotectant, rather than other aspects, such as maximizing treatment window or enrollment rates. At the same time, a trial must conform to practicalities of trial conduct that include achievable treatment windows and enrollments. A major unresolved issue is whether or not the value of early enrollment trumps other considerations, such as using imaging-based patient selection or restricting the trial to patients relegated to reperfusion therapies. Regardless, the trial design should be consistent with paradigms tested in preclinical studies, especially the possibility that the impact of early neuroprotection may be magnified by subsequent reperfusion. Because of the diminishing efficacy of neuroprotection with time, the design should permit the study drug to be given as early as possible, in a timeframe in which patients still exhibit a salvageable penumbra. Its conduct should not interfere with time-sensitive standard-of-care therapies, such as thrombolysis with tPA. It should take into account that subjects may be exposed to potentially confounding therapeutic interventions, such as the use of tPA or of endovascular devices. Results should be generalizable to the target population of a phase 3 study. The design must be adaptable to the larger sample sizes and to enrolling sites needed in phase 3 to provide safety and efficacy data in the target population. Given the more exploratory nature of a phase 2 trial, results should ideally inform about a broader patient population than that targeted by a phase 3 trial, especially if the drug has a mechanism of action that might apply to broader indications (eg, hemorrhagic strokes). Overall, a trial that enables early treatment with a neuroprotectant and for which preclinical studies suggest a high potential for efficacy has the greatest chance of success. Time is brain, and neuroprotectants are emergency drugs that should be tested as such.

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References
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