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Toward Wisdom From Failure
Lessons From Neuroprotective Stroke Trials and New Therapeutic Directions

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Background—Neuroprotective drugs for acute stroke have appeared to work in animals, only to fail when tested in humans. With the failure of so many clinical trials, the future of neuroprotective drug development is in jeopardy. Current hypotheses and methodologies must continue to be reevaluated, and new strategies need to be explored.

Summary of Review—In part 1, we review key challenges and complexities in translational stroke research by focusing on the "disconnect" in the way that neuroprotective agents have traditionally been assessed in clinical trials compared with animal models. In preclinical studies, determination of neuroprotection has relied heavily on assessment of infarct volume measurements (instead of functional outcomes), short-term (instead of long-term) end points, transient (instead of permanent) ischemia models, short (instead of extended) time windows for drug administration, and protection of cerebral gray matter (instead of both gray and white matter). Clinical trials have often been limited by inappropriately long time windows, insufficient statistical power, insensitive outcome measures, inclusion of protocol violators, failure to target specific stroke subtypes, and failure to target the ischemic penumbra. In part 2, we explore new concepts in ischemic pathophysiology that should encourage us also to think beyond the hyperacute phase of ischemia and consider the design of trials that use multiagent therapy and exploit the capacity of the brain for neuroplasticity and repair.

Conclusions—By recognizing the strengths and limitations of animal models of stroke and the shortcomings of previous clinical trials, we hope to move translational research forward for the development of new therapies for the acute and subacute stages after stroke. (*Stroke*. 2002;33:2123-2136.)

Key Words: cerebral ischemia ∎ clinical trials ∎ models, animal ∎ neuroprotection ∎ rehabilitation ∎ stroke ∎ translations

Neuroprotection for Stroke: A Fantasy Invented by Basic Scientists?

The quest for effective stroke treatments remains an urgent priority. A stroke occurs every 53 seconds in North America,[1] and by 2020, cerebrovascular disease is projected to become the fourth-leading burden of disease worldwide, after heart disease, depression, and motor vehicle collisions.[2] According to the summary of historical trends in clinical stroke trials by Kidwell et al,[3] the 20th century saw the publication of 178 controlled trials of acute stroke therapies in the English-language literature, yet only a few produced "positive" results: the Neurological Institute of Neurological Disorders and Stroke (NINDS) rt-PA trial,[4] Prolyse in Acute Cerebral Thromboembolism (PROACT II),[5] a low-molecular-weight heparin trial,[6] and most recently a trial of ancrod.[7] The successful translation of these "vascular approaches" from the animal laboratory to the hospital emergency room has demonstrated that stroke is a treatable disorder in the hyperacute stage and has provided optimism that additional therapies to improve stroke outcome will be possible in the future.

In contrast, neuroprotective drugs that aim to salvage ischemic tissue, limit infarct size, prolong the time window for reperfusion therapy, or minimize postischemic reperfusion injury or inflammation have shown great promise in preclinical testing but disappointment in clinical trials.[8–10] Of >49 neuroprotective agents studied in >114 stroke trials, none has proven successful clinically.[3] Similarly, neuroprotective therapy has been unsuccessful in clinical trials of head trauma.[11–14] With the failure of so many trials, some clinicians may ask, "Is neuroprotective stroke therapy just a fantasy invented by basic scientists?" Will it ever play a clinical role? The answer is unclear. It must be acknowledged that neuroprotection may never be effective for promoting functional recovery after brain injury in humans. However, a lack of evidence of efficacy does not necessarily mean a lack

of efficacy. Because of problems in basic science experiments and in clinical trial design, the evidence against neuroprotection is not conclusive, as is discussed below.

The purpose of this article is to provide clinicians and investigators with an up-to-date summary of the complex issues involved in translating stroke-related research from bench to bedside and to argue that experience from recent trials can provide important lessons that can be translated from the bedside back to the bench. Part 1 reviews pitfalls that have arisen in the development of neuroprotective therapies and reinforces recent recommendations regarding preclinical and clinical evaluation of new drugs.[15,16] Part 2 highlights emerging concepts in ischemic pathophysiology that should encourage us to think beyond the hyperacute phase of ischemia and consider the design of trials that use multiagent therapy and exploit the capacity of the brain for neuroplasticity and repair.

Part 1: Reconciling the Results of Negative Stroke Trials

Reasons for the failure of so many neuroprotective agents in clinical trials, despite their apparent benefit in animal (mostly rodent) models, have been the subject of intense discussion recently.[15–31] It is becoming clear that existing animal models of focal cerebral ischemia are an imperfect representation of human stroke and may be relevant only to a minority of human stroke types.[32] Neff[33] reminds us that the lissencephalic brain of a rat is about the size of a lacunar infarct in humans, and some humans have infarcts the size of an entire rat. In addition, despite significant similarities between the rodent and human genomes, the differences that do exist are sufficient to remind us that conclusions reached regarding genomic and proteomic characteristics in rodent studies may not apply to human stroke.

Neuroanatomical, pathophysiological, pharmacokinetic, and genetic differences between rodents and humans notwithstanding, there has been a fundamental "disconnect" in the way that the efficacy of putative neuroprotective agents has been assessed in animal studies compared with clinical trials. The dramatic rise in the number of stroke trials over the past several decades has been accompanied by an improving, yet still highly variable, quality in study design.[3,34] Some trials could not have been expected to succeed because of conceptual or methodological flaws. Perhaps we have abandoned efficacious treatments prematurely on the basis of results of flawed trials. Other trials have been criticized, in retrospect, for proceeding on the basis of insufficient evidence of efficacy in preclinical studies (eg, only 50% of published animal studies were in favor of nimodipine).[35] Other studies, still, have raised safety concerns because of drug toxicity, a danger of accelerating research by combining phase II and phase III trials.[36–38]

In this section, we highlight 9 pitfalls that have arisen in trying to extrapolate from animals to humans in the investigation of neuroprotective therapy. By understanding how animal models may be made more relevant to human stroke and how the design of clinical trials may be improved, we can move forward translational research for the development of stroke therapies.

Pitfall 1: Preclinical Studies Have Used Very Short Time Windows for Drug Administration, Whereas Clinical Trials Allow Longer Time Windows

Most neuroprotective studies in animals have relied on drug administration either before the ischemic insult or very soon after the onset of ischemia.[39,40] In contrast, time windows for entry in acute stroke neuroprotective trials have been longer and highly variable; in studies published between 1995 and 1999, the median time to entry was 12 hours (range, 4 hours to 12 days), with a median time to treatment of 14 hours.[3] None of the published neuroprotective trials has used a 3-hour window.[3,16] Treatment within 3 hours would be expected to have a greater chance of efficacy because more patients would be expected to have potentially reversible ischemic tissue.[40–42] The effects of neuroprotective agents in the laboratory are even more time dependent than thrombolytics, leading Jonas et al[19] to summarize the failure of neuroprotective trials as a matter of "too little, too late." Certain "failed" drugs could potentially have clinical value if given at earlier time periods (within 2 hours after ischemia or prophylactically).[19]

On the other hand, Baron et al[43,44] and Fisher et al[45] emphasize that we need not stipulate a fixed time limit for neuroprotective therapy because the duration of the ischemic penumbra is highly individualized. For example, PET studies suggest that the window of opportunity may be extended in some patients[46]; in 1 study, about one third of patients still had evidence of penumbra when assessed at 5 to 18 hours (mean, 10 hours) after stroke onset.[44] The PROACT II results further support the fact that salvageable tissue is present up to 6 hours after onset in some patients.[5] The rate of progression of the penumbra from reversible to irreversible ischemic injury is dependent on many factors and may be accelerated in the presence of poor collateral circulation, hyperglycemia, and other exacerbating factors.[45]

Putative neuroprotective drugs should not be advanced into clinical stroke trials until preclinical studies have investigated their effects when administered many hours, not minutes, after ischemia. Clinical trialists must aim for the shortest possible door-to-needle times, particularly given the tendency that physicians have of "waiting until the last minute" of the time window to treat, regardless of when the patient arrives at hospital.[47] The NINDS rt-PA study showed that enrollment within 3 hours can be achieved, although 17 324 patients were screened to recruit the 624 subjects eligible for the study, with most excluded because of the time window.[4] With increasing public awareness of this issue and improvements in regional organization of stroke services and stroke teams, response times are improving, and at some centers, >25% of patients are reaching hospital within 3 hours.[48] Clinical trial protocols should enforce benchmarks for door-to-needle times and stratify patients by time of treatment with appropriate power calculations. If the long time windows are a major reason for the lack of efficacy of neuroprotective therapy in human trials, then the investigation of agents given prophylactically (eg, before surgical procedures with an increased risk of ischemic cerebrovascular events)[49–52] or by paramedics in the field (a phase I trial is already underway) may provide the necessary "proof-of-principle" data that are

much needed as long as a strict target population can be defined by preplanned posthoc analysis.

Pitfall 2: Preclinical Studies Target the Ischemic Penumbra, Whereas Clinical Trials Do Not

As Fisher,[45] Baron,[53] and others[54] have emphasized, the target of current neuroprotective therapy is the penumbra, ischemic tissue that is functionally impaired but whose damage is potentially reversible.[55,56] If reversible ischemic tissue is not present at the time of treatment, then neuroprotective therapy cannot be expected to work. Perhaps we have discarded some agents prematurely because clinical trials have not been selective enough in targeting patients with evidence of penumbra. Future trials may need to use stricter entry criteria to target not just cortical strokes but specifically those with a sufficient volume of penumbra.[16,45]

Patients with potentially salvageable penumbra tissue may be identified by functional neuroimaging.[44,45,53,57–59] According to PET studies by Heiss et al[57] performed in patients within 3 hours of acute stroke, the penumbra made up 18% (range, 8% to 34%) of the final infarct volume; 70% (range, 51% to 92%) was already critically hypoperfused, and 12% (2 to 25%) was sufficiently perfused. Although such observations imply that on average neuroprotective therapy may be able to salvage only a relatively small fraction of an infarct (supporting the rationale for combination reperfusion-neuroprotective studies), some patients can be identified with larger volumes of penumbra.[46,60] A recent PET study suggested that 45% of the final infarct (and in some patients, up to 85%) remained viable for up to 12 hours.[60,61]

With MRI, a perfusion-diffusion mismatch (perfusion abnormality greater than diffusion abnormality) can be identified in ≈70% of patients studied within 6 hours of stroke onset and may indicate salvageable tissue.[62,63] Some trials are now using MRI criteria to improve patient selection[64] (eg, a trial of sipatrigine currently underway requires a perfusion-diffusion mismatch at baseline of at least 30%[65]). Dynamic CT perfusion imaging also provides promise as a method of acute stroke imaging that may allow rapid identification of tissue compartments perfused within predetermined blood flow thresholds.[66]

In the absence of perfusion imaging, a mismatch between the clinical deficit and imaging findings has been suggested as a way to optimize patient selection (ie, severe clinical deficit with limited early lesion on diffusion-weighted MRI or CT).[54] A prediction formula that incorporates the time elapsed, National Institutes of Health Stroke Scale (NIHSS) score, and diffusion-weighted MRI lesion has recently been validated.[67] The Alberta Stroke Program Early CT Score, a 10-point score quantifying the signs of early infarction, also aims to improve patient selection.[68,69]

Therefore, in future trials, there should be a major effort to improve patient selection through the use of imaging criteria, in combination with other descriptors, (1) to select candidates who are expected to benefit from treatment, ie, those who have perfusion abnormality greater than diffusion abnormality, and (2) to exclude inappropriate patients, ie, those with lacunes or large infarcts with no perfusion-diffusion mismatch.[44,54,70,71] If imaging analysis cannot be performed

online in the acute stage in time for decision making, the scans should be analyzed by prespecified criteria as soon as possible to select the target population; patients with no evidence of penumbra posthoc should then be excluded from an efficacy analysis.

Pitfall 3: Preclinical Studies Have Demonstrated Protection of Gray Matter, Whereas Clinical Trials Frequently Enroll Patients Without Specifying Location of Damage

A particular concern is that preclinical neuroprotective studies have concentrated almost exclusively on the protection of cerebral gray matter from ischemic injury; the effects of neuroprotective therapy on cerebral white matter tracts are largely unknown.[23,72] The human brain contains a greater proportion of white matter compared with the rat brain,[72] and the failure of some neuroprotective trials may be due to an inability of certain agents to protect against axonal damage.[23,32,72] Approximately one third of human strokes are small-vessel lacunes, yet adequate animal models of lacunar stroke are lacking.[32,72] Until such data are available, it may not be reasonable to expect lacunes or subcortical white matter infarcts to respond to neuroprotective therapy. The pathophysiology of ischemic injury in white matter is different than in gray matter, and treatment targets likely differ as well.[23,72,73] *N*-methyl-D-aspartate (NMDA) receptors, for example, are preferentially located at synapses rather than along axons.[74,75] Blockade of sodium channels, calcium, or alpha-amino-3-hydroxy-5-methyl-4-isoxasole-propionic acid (AMPA) receptors (rather than blockade of glutamate-mediated excitotoxicity) has been hypothesized to be more important for white-matter protection.[73,74] The NMDA antagonist MK-801 reduced cortical gray-matter injury but not the amount of axonal damage after middle cerebral artery occlusion in cats.[76] Interestingly, however, a recent study showed that axonal and myelin damage could be reduced in rats with the NMDA antagonist CNS 1102 (Cerestat).[74] More studies like this that take into account both gray-matter and white-matter pathophysiology are needed if we are to achieve "total brain protection."[72] For now, only cortically based strokes should be enrolled in neuroprotective trials, unless the agent being tested is specifically designed to protect white matter also.[16] Diffusion-weighted MRI is beginning to be used in some trials to select patients with cortical involvement and exclude those with lacunar infarction.

Pitfall 4: Optimal Duration of Neuroprotectant Administration Is Unknown

In recent trials, drug administration has varied from a single injection to continuous infusions to multiple doses lasting up to 3 months after stroke.[77] Because acute treatment may only delay but not prevent cell death, Dyker and Lees[77] advocate continuing neuroprotective therapy for at least the first 72 hours, if not longer. Prolonged elevation of excitatory amino acids after stroke in some patients[78,79] and MR spectroscopy evidence suggesting ongoing neuronal loss over many days after stroke[80] support the concept of extended treatment. In rats, longer-lasting neuroprotection was achieved when a

glutamate antagonist was given for 1 week compared with administration in only the acute phase.[31]

However, certain drugs may exert different or even opposite actions, depending on the timing of administration. NMDA antagonists, benzodiazepines, or barbiturates may be beneficial if given early after ischemia but may have detrimental effects if given at later times.[81–84] For example, GABA-ergic agonists may be neuroprotective when given acutely (a trial of diazepam within the first 3 days after stroke is currently underway[85]), yet may impair recovery if administered at later stages after stroke.[86] Additionally, neurite outgrowth requires both NMDA and voltage-sensitive calcium channel activation.[87] Because neurite outgrowth is likely an essential process during recovery, patients maintained on NMDA antagonists and voltage-sensitive calcium channel blockers may suffer impaired recovery. The time point at which the therapeutic transition from neuroprotection to repair occurs merits further study.

Pitfall 5: Preclinical Studies Have Relied on Infarct Size to Judge Therapeutic Efficacy, Whereas Clinical Trials Rely on Behavioral Outcomes

Traditionally, animal studies have relied on reduction in infarct size within the first few hours after stroke as the primary measure of therapeutic efficacy. In contrast, clinical trials judge efficacy by using neurological and/or functional outcomes, not infarct volume, most often at 3 months after stroke.[3,34] As an example, preclinical studies of the antineutrophil adhesion agent Hu23F2G (LeukArrest) and the glycine antagonist GV150526 (gabestinel; Glycine Antagonist in Neuroprotection study)[88] assessed infarct volume within hours of occlusion, while patients were evaluated on day 28 or 90, respectively, for neurological and functional outcomes. Such discrepancies suggest that reliance on infarct size measurement alone in animals can be misleading as an indicator of therapeutic efficacy.[89,90] Histological end points cannot tell whether surviving neurons are functional or dysfunctional or will go on to die in a delayed fashion, and they are less predictive of long-term histology than early behavioral assessments.[90] Moreover, some compounds [eg, basic fibroblast growth factor (bFGF), osteogenic protein-1] have been associated with functional improvement without affecting infarct size in animals, suggesting that they act by other mechanisms, eg, enhancement of neural repair, rather than by neuroprotection.[91,92]

Therefore, assessment of therapeutic efficacy in preclinical studies should require, in addition to infarct size, demonstration of benefit on functional measures of motor, sensory, or cognitive deficits.[89,93] Examples include tests of limb placing, beam walking, grid walking, Rotorod performance, grip strength, balance beam inclined plane performance, prehensile traction, and cognition (eg, Morris water maze, radial maze, 1-trial passive avoidance, T-maze retention test).[89,90,94] These measurements are not equivalent in their reliability or predictive value. For this reason, it is better to use a battery of appropriate tests rather than a single measure. The staircase test has been recommended because of its greater sensitivity in detecting persisting deficits in forepaw dexterity months after ischemia, unlike simpler sensorimotor tasks on which

animals can recover quickly.[89,90] The bilateral sticky tape test may be a useful indicator of poststroke neglect.[94] Further development, refinement, and standardization of reliable functional assessments will continue to be a priority.[89,90,94]

Pitfall 6: Preclinical Studies Have Relied on Early Outcomes, Whereas Clinical Trials Rely on Late Assessments

Preclinical studies of neuroprotective drugs have rarely shown that early therapeutic benefit, when it is achieved, has a lasting impact. That is, of studies that have explored the long-term results when a drug showed early favorable influence on histological outcome, most have concluded that the early reduction in infarct volume does not persist if one continues to observe the animal; ie, most therapeutic attempts delay but do not arrest cell death.[26,90,95] For example, the NMDA antagonist MK-801 appeared neuroprotective at day 3, but at 4 weeks, there was no significant difference in infarct size.[95] Similarly, the cyclin-dependant kinase inhibitor flavopiridol, AMPA antagonist NBQX, and N-type calcium channel antagonist SNX-111 all appeared neuroprotective histologically when assessed at 1 week, but this was not sustained at 4 weeks.[96,97]

Such findings demonstrate that reliance on early end points is not sufficient and can be misleading; assessments at extended time points after ischemia are necessary to determine whether there is evidence of sustained neuroprotection.[15,26,95] Indeed, histopathological studies in animals show that infarcts evolve over time and may take many days to months to acquire their final appearance.[95,98,99] Late consequences of ischemia (eg, inflammation) or slow death mechanisms that are unleashed (eg, apoptosis) may in part explain such findings. Thus, if single-dose neuroprotective treatment only postpones the evolution of an infarct, perhaps multidose, extended treatments or combination therapy will be required for optimal neuroprotection (see below).[77,100]

Pitfall 7: Experimental Stroke Models Are Homogeneous, Whereas Human Stroke Is Heterogeneous

Another problem confounding the evaluation of neuroprotective therapy is the tremendous variability of human stroke types, recovery patterns, and associated clinical factors. Preclinical studies usually involve middle cerebral artery occlusion in young, healthy animals under anesthesia with tightly controlled temperature, blood pressure, oxygenation, and glucose levels ("interventional homeostasis"[54]).[101] In contrast, clinical trials often permit entry of multiple stroke types (cortical, mixed cortical-subcortical, pure subcortical white-matter strokes, and in some neuroprotective trials, both ischemic and hemorrhagic stroke), and there is a lack of standardized control over physiological parameters.[54] Unlike the animal model, stroke patients typically have a multitude of associated variables that may affect prognosis,[102–104] including old age, comorbidities, polypharmacy, recurrent ischemia, poor collateral circulation, or prior strokes. Hyperglycemia and other metabolic prognostic markers[105] may be particularly important variables to control or adjust for in future trials.[106–108]

TABLE 1. Influence of Outcome Measures on the Perception of Recovery*

Outcome Measure	Percent of Patients Considered Recovered,† %
Rankin 0–2	54
Rankin 0 or 1	24
Barthel >90	57
Barthel ≥75	40–50‡
Barthel ≥60	49–70‡
NIHSS 0 or 1	45
Fugl-Meyer >90	37
SF-36–PFI >75	28

SF-36–PFI indicates Short Form 36, physical functioning index.
*Data from the Kansas City Stroke Study (n=459), Duncan et al.[113]
†At 6 months after stroke.
‡From Duncan et al.[34]

Instead of viewing stroke as a single disease entity, future trials should more appropriately be directed only to specific homogeneous stroke subtypes. Currently, of 178 published acute stroke trials, only 2% specified a target stroke mechanism, and 35% specified a specific stroke territory.[3] Although a lack of specificity regarding stroke types in trial entry criteria may be appropriate for thrombolysis (where the pathophysiologic target, ie, clot, is similar regardless of stroke location, perhaps with the exception of lacunes), this may not be appropriate in neuroprotective trials in which efficacy in some patients (eg, cortical stroke), if present, could be diluted by the inclusion of other stroke types (eg, subcortical strokes). Indeed, posthoc analysis of some neuroprotective trials has suggested that a benefit may exist for certain subgroups, eg, patients with large cortical stroke [total anterior circulation stroke in the Clomethiazole Acute Stroke Study (CLASS)].[109,110] A follow-up study specifically targeting these patients failed to validate this hypothesis, however, but it set a good example for how we should approach future studies.[111]

Pitfall 8: Choice of Outcome Measures Can Determine the Success of a Clinical Trial More Than Actual Drug Efficacy

The choice of outcome measures in clinical trials is critical to the success or failure of a putative therapeutic intervention.[16,34,54] However, there is a lack of agreement about the most appropriate measures that should be used and about what constitutes "recovery" or "favorable outcome."[34,112–114] For example, on the Barthel Index, cutoff points anywhere between 50 to 95 of 100 have been used to define recovery.[115] Kidwell et al[3] showed that less than half of published acute stroke trials used a validated outcome measure and that only 17% had a prespecified primary end point. In the review of 51 phase II and III acute stroke trials by Duncan et al,[34] there were 14 different impairment level measures, 11 different activity (disability) measures, 8 miscellaneous scales, and only 1 quality-of-life measure. If disability scales are used (eg, Barthel Index, modified Rankin Scale), more patients will be considered recovered; if impairment scales are used (eg, NIHSS) fewer patients will be considered recovered.[34]

Table 1 shows how one's impression of recovery is directly dependent on the type of outcome measure chosen.

This variability in outcome assessment has made the stroke literature appear confusing and at times conflicting. Indeed, much of the controversy regarding the thrombolytic trials has resulted from inconsistency in the definition of recovery and differences in end points used among the various trials. In the NINDS study,[4] the benefit of tPA at 24 hours did not reach statistical significance on the prespecified NIHSS end point. However, posthoc analysis showed that if recovery is instead defined as an NIHSS score of 0 to 2, a striking difference is found: 24% of tPA-treated patients versus 5% of placebo-treated patients are recovered at 24 hours.[116] Moreover, the 24-hour outcome results would have been statistically significant on the predefined primary end point if the recently published modified version of the NIHSS had been used instead.[117] Furthermore, in the European Cooperative Acute Stroke Study (ECASS II), which used "favorable outcome" as defined in the NINDS study (modified Rankin scale score of 0 or 1), the result was statistically negative.[118] However, when a different dichotomization that classifies outcome in terms of self-care independence (Rankin score of 0 to 2) was used, the study was positive.[118]

Thus, greater consensus and standardization in outcome measures for acute stroke studies are needed, and this would facilitate meta-analysis. It is recommended that future efficacy trials incorporate outcome assessments that span the spectrum of stroke recovery, ie, impairment, activity limitations (disability), and participation restrictions (handicap).[34] As suggested by Duncan et al,[34] a single scale likely is inadequate to capture recovery, and dichotomized outcomes should be avoided; inclusion of extended/instrumental activities of daily living assessments, advanced mobility measures, and quality-of-life assessments is recommended.[34] Although it may be appropriate in 3-hour thrombolysis trials to aim for neurological cures (eg, NIHSS score 0 or 1) or functional recovery (eg, modified Rankin score of 0 or 1), in trials of neuroprotective agents or longer time windows, our expectations should be different. Grotta[40] reminds us that we should be aiming for neuronal protection, not neuronal "reincarnation." Therapeutic efficacy will likely be of a smaller magnitude, one that may be captured only by using less stringent criteria for recovery (eg, NIHSS <7 or modified Rankin score of 0 to 2), or by measuring shifts in disability states.[118a] A newer end point, neurological deterioration in hospital, has been proposed for traumatic brain injury trials.[119]

Different measures of recovery may be necessary, depending on the severity of the stroke population under investigation (ie, mild, moderate, or severe), the particular type of treatment being studied, or the specific neurological function targeted by the intervention. For example, in a recent trial of stem cell implantation for hemiparetic stroke patients,[120] the global neurologic deficit scales used (NIHSS and European Stroke Scale) would not be expected to capture meaningful change in motor impairment; instead, a motor-specific impairment scale such as the Fugl-Meyer Stroke Assessment[121,122] might be more revealing given the location of the target stroke in the subcortical basal ganglia region. As we learn the limitations of existing scales, newer stroke-specific

indexes incorporating quality-of-life measurements are being developed; eg, the Stroke Impact Scale[123] and SS-QOL[124] are intended to provide more comprehensive and more meaningful outcomes from the patient's perspective.[124] Inclusion of specific scales for aphasia, neglect, or apraxia may reveal benefits in subgroups of patients that are not apparent on global deficit rating scales. The concept of separate "motor-Rankin" and "cognitive-Rankin" scales has been advocated.[125] Stratification of patients in clinical trials by initial stroke severity is important. The Orpington prognostic scale, recently shown to have excellent predictive value for stroke recovery, may have value for stratifying patients in treatment trials.[126] The optimal time for outcome assessment is debatable, 3 months after stroke (or earlier) according to some[16] and 6 months according to others.[34] In some studies, even if a therapeutic effect on final outcome (ie, at 3 or 6 months) cannot be demonstrated, it may be desirable to detect whether the intervention was able to accelerate the rate of recovery. Comparison of change scores (the difference between baseline and final scores for each subject) may have the advantage of minimizing interindividual variability in stroke severity and recovery if reliable outcome measures are used.

Pitfall 9: Small Trials Are Trying to Answer Questions That Only Large Trials Can Answer

Have some stroke trials been negative because of a lack of efficacy or because of a lack of statistical power? To detect efficacy of neuroprotective compounds, which are likely to have small rather than large treatment effects, we need large trials (thousands of patients, according to some experts) to prevent type 2 statistical error.[54,127] The mean sample size of neuroprotective trials has been 186 (median, 69).[3] Only 2% of acute stroke efficacy trials have had sufficient statistical power to demonstrate a 5% absolute clinical benefit, and only 7% of trials have been powered to detect a 10% benefit.[3] The use of "adaptive randomization" in future trials may reduce sample size requirements.[128] This Bayesian statistical technique, being used in a neuroprotective trial currently underway,[129] aims to maximize the number of patients assigned to the dose(s) that appear most efficacious; outcome data from each patient provides feedback to the randomization computer as the trial proceeds to optimize the chance that the correct drug dose will be studied.[54]

Part 2: New Therapeutic Frontiers

Most trials to date have attempted to modulate the early metabolic events in ischemia, particularly those involving glutamate activation of the calcium cascade. With a growing understanding of the pathophysiology of ischemic brain injury in the acute phase of stroke, as well as progress in understanding the mechanisms that underlie functional recovery in the subacute stages, newer therapeutic strategies are emerging.[81,130–134] The concept of a single narrow time window for intervention is being replaced by the potential for multiple overlapping therapeutic windows and the possibility of multiagent chemotherapy "cocktails" administered at selected time periods after stroke (Table 2).[130] In this section, we discuss a few selected examples of strategies that may

TABLE 2. Possible Stroke Treatment Options for the Future: Evolving Time Windows and Combination Therapy Approaches

Prestroke
  Prophylactic neuroprotection for high-risk patients?

Minutes to hours after stroke
  Acute reperfusion therapies
    Intravenous thrombolysis
    Intra-arterial thrombolysis
    Combined intravenous/intra-arterial thrombolysis
    Mechanical reperfusion techniques

Minutes, hours, or days after stroke
  Neuroprotective therapy: chemotherapy cocktail of agents targeting different aspects of the ischemic cascade, perhaps administered sequentially at various time points after stroke
    Antinecrotic agents
    Antiadhesion/anti-inflammatory agents
    Antiapoptotic agents
  Combined thrombolysis and neuroprotective therapy
  Tight control of glucose, perhaps insulin administration
  Tight control of temperature, perhaps antipyretic administration, perhaps hypothermia

Days, weeks, or months after stroke
  Restorative treatments targeting specific deficits, eg, gait retraining, arm function
  Pharmacotherapy coupled closely with rehabilitation (rehabilitation pharmacology)?
  Growth factors coupled with rehabilitation?
  Stem cell therapy coupled with rehabilitation?
  Gene therapy?

Ongoing
  Avoidance of "detrimental" drugs
  Secondary stroke prevention therapies, including combination antithrombotic agents

form the basis for future trials, either alone or in combination with traditional neuroprotective approaches.

Delayed Neuronal Death After Ischemia: A Role for Antiapoptotic Therapy?

Observations of delayed neuronal death after ischemia have suggested the possibility of an "apoptosis-necrosis" continuum. Depending on the degree and duration of ischemia, brain cells may die by an ionic cascade, rapidly (necrotic cell death), or by a molecular cascade, slowly (apoptotic cell death).[131,132,135–137] At one end of the spectrum, severe focal ischemia produces infarction through excitotoxic necrosis usually evident in rats by 6 hours and maximal at 24 hours. At the other end of the spectrum, when ischemia is mild and short lasting, the resulting cell death may be apoptotic; ie, neurons appear to be spared initially but later go on to die slowly.[138] For example, after transient focal ischemia in rats Du et al[138] found no infarction at 24 hours; however, by 3 days, a small infarct had developed, and remarkably by 14 days, the infarct had progressed to the same volume as that induced by severe ischemia. Although excitotoxic necrosis is considered the predominant mechanism of ischemic cell

death in most cases, apoptosis may occur in penumbral neurons that escape excitotoxic death.[132] Importantly, if necrosis is attenuated by therapy (ie, by reperfusion or antiexcitotoxic agents), then apoptosis may be unmasked or even promoted.[132,136,138,139]

For these reasons, it is short-sighted to continue only with antinecrosis therapies without taking into account the role that apoptosis may play in ischemic cell death. Antiapoptotic agents (eg, cycloheximide, caspase inhibitors) have shown neuroprotective effects in animals in terms of both infarct volume reduction and functional improvement. Furthermore, the neuroprotection appears long lasting.[140,141] The therapeutic window for antiapoptotic neuroprotection is longer than that for most other neuroprotective agents (eg, 9 hours for the caspase inhibitor ZVAD-fmk and zDEVD-fmk after brief ischemia and up to 12 hours if combined with an NMDA antagonist).[142] The role of apoptosis in human stroke, however, and the clinical relevance of antiapoptotic therapy are not yet known and await further investigation. If further studies confirm the occurrence of apoptosis in the penumbra region, then cerebral blood flow measurement may become a clinically accessible surrogate marker for this outcome.

The Need for Polytherapy

On the basis of the complexity of events in cerebral ischemia and the disappointing results from single-agent trials, it may not be realistic to expect that 1 neuroprotective drug will have lasting benefit. Rather, effective neuroprotection may require "rational" polytherapy that combines drugs with different mechanisms of action, perhaps administered at different poststroke intervals, to maximize efficacy and/or extend the window for reperfusion, minimize reperfusion injury or hemorrhage, or inhibit delayed cell death.[16,130,143,144] Furthermore, because the failure of several neuroprotective trials has been attributed to dose-limiting toxicity,[145] combination therapy may permit lower doses of each agent and minimize adverse effects. Combinations with nonpharmacological (physiological) neuroprotective strategies such as hypothermia, insulin, and blood pressure control should also be subjected to clinical trials.[103,146]

Combined thrombolysis-neuroprotective approaches have shown promise in animal studies and are beginning to be investigated in clinical trials. For example, synergistic effects have been demonstrated in animals when thrombolysis is combined with citicoline,[147] an AMPA antagonist,[148] an NMDA antagonist,[149] or other agents.[150,151] Administration of antileukocytic adhesion antibodies has been shown to extend the therapeutic window for thrombolysis.[152,153] Recently, 2 trials have demonstrated the feasibility and safety of intravenous tPA treatment followed by neuroprotectant administration: the CLASS-T trial of clomethiazole[154] and a study of lubeluzole.[155]

In animals, synergy has been demonstrated by the combination of 2 neuroprotective agents with different actions; some examples include the NMDA antagonist MK-801 in combination with a GABA agonist,[156–158] a free radical scavenger,[159] a calcium antagonist,[160] citicoline,[161] or bFGF.[162] Similarly, synergy has been observed with the combination of the antioxidant tirilazad and magne-

sium,[163,164] the combination of 2 different antioxidants,[165] and citicoline combined with bFGF.[166]

Synergistic effects on infarct size and therapeutic window have also been found in animals through the use of antiexcitotoxic agents in combination with antiapoptotic agents, eg, the NMDA antagonist dextrorphan plus the protein synthesis inhibitor cyclohexamide,[167] or MK 801 with the caspase inhibitor ZVAD-fmk.[168] Caspase inhibitors given with bFGF extended the therapeutic window and lowered the required doses for neuroprotection.[169]

Beyond Neuroprotection: Exploiting the Repair Mechanisms of the Brain

The poststroke recovery period (days, weeks, and months after stroke) represents another target for more active therapeutic development. Our preoccupation with hyperacute and acute intervention, while critically important, should not lead us to neglect the testing of "restorative" interventions that might enhance recovery in the subacute and chronic stages after stroke.[81,170] Unlike neuroprotective therapy that targets ischemic tissue and aims to limit infarct size, "recovery-enhancing drugs" and other novel interventions for stroke aim to promote functional recovery after a completed stroke by stimulating repair mechanisms.

The Changing Milieu of the Brain After Stroke

Within minutes of ischemia, a loss of dendritic spines can be observed at excitatory synapses; reestablishment of dendritic spine synapses in surviving neurons can occur rapidly and represents a potential substrate for functional recovery.[171] Ischemic cortical injury induces the expression of growth factors in peri-infarct regions, and behavioral recovery is accompanied by increased dendritic branching and synaptogenesis that peaks 2 to 4 weeks after stroke in the rat.[172,173] Mechanisms of neuroplasticity postulated to underlie recovery include unmasking of latent connections, redundancy that allows recruitment of alternate parallel pathways to take over lost functions, axonal sprouting from surviving neurons with formation of new synapses, and possibly even neurogenesis.[134,174–176a]

Intracisternal administration of bFGF beginning 24 hours after stroke or osteogenic protein-1 beginning 3 days after stroke promoted recovery in animals without affecting infarct size and stimulated new neuronal sprouting and synapse formation.[91,92,177,178] Clinical trials of bFGF administered within 6 hours have been underway, but 1 trial was terminated early because of safety concerns.[179,180]

Proteins that are normally expressed in the developing brain and suppressed in the adult brain become upregulated in response to ischemia; stroke "recapitulates ontogeny," as summarized by Cramer and Chopp.[181] Cell-cycle genes are re-expressed in response to ischemia; ie, neurons are activated to "divide," but they are hard wired not to. Activation of the cell cycle cascade results in cell death, and interrupting this cascade results in cell survival.[182] This may be another fruitful therapeutic avenue in ischemia. Stem cell therapy is being investigated for enhancement of stroke recovery. There are suggestions in the literature that bone marrow stromal cells or umbilical cord blood cells injected intravenously or

intra-arterially in rats can migrate toward the infarct, stimulate growth factors, and promote functional recovery.[183–185] Recently, human experimentation has begun with a phase I trial of transplantation of cultured neuronal cells into basal ganglia infarcts in stroke patients.[120] A great deal more animal work is needed to provide a firmer basis for intervention.

Remapping and Rehabilitation

Cortical reorganization after stroke is promoted by rehabilitation and an enriched environment.[134,186,187] Nudo et al[188,189] showed that in monkeys who do not receive rehabilitative training after a small focal injury in the hand motor cortical region, the surrounding intact cortical representation undergoes shrinkage; however, with repetitive training in the form of motor skill acquisition, behavioral recovery is promoted, and the cortical hand representation is maintained or expanded. Thus, physical therapy may derive its effectiveness from "teaching" the brain to learn. Improved recovery in humans may be achieved with increased intensity of rehabilitation (more hours and greater frequency of therapy).[190] Such dose-response effects suggest the concept that "more is better" applies to stroke recovery. One intensive physical therapy regimen that is gaining popularity is "forced use" or constraint-induced movement therapy, which can be applied if the weak arm has enough strength to at least move against gravity.[191] In this protocol, the unaffected arm is constrained in a sling, forcing the patient to use the affected arm as much as possible in meaningful daily activities. The aim is to minimize the development of learned helplessness resulting from overreliance on the "good arm." Based on reported success in chronic stroke patients, this therapy is now under investigation in multicenter trials as a rehabilitation intervention in the earlier stages after stroke. Bilateral arm use assisted by passive movement may enhance activation in the peri-infarct region of a stroke[192] and may have a role in early rehabilitation. Body weight–supported treadmill training is a promising technique being investigated to promote recovery of ambulation after stroke.[193]

Pharmacological Manipulation After Stroke

Recovery after stroke may also be modulated pharmacologically.[81,178,194–196] Accumulating evidence suggests that the recovery process is dynamic and vulnerable to neurotransmitter modulation.[82] For example, pharmacological studies in animals have emphasized the importance of central noradrenergic transmission in mediating some forms of recovery after focal cortical injury. Drugs augmenting noradrenergic activity (eg, dextroamphetamine) enhance functional recovery when coupled with symptom-relevant experience, whereas drugs decreasing noradrenergic activity impair recovery and can reinstate deficits after recovery has taken place.[197–200] (For a review of additional studies, see Reference 170.) Similar findings have been demonstrated for other classes of drugs and may relate to their ability to facilitate (or impair) long-term potentiation.[82,201] Histologically, dextroamphetamine administration after cortical infarction in rats has been associated with upregulation of neural sprouting and synaptogenesis in the peri-infarct cortex and contralesional cortex, correlating with behavioral recovery.[202,203] In animal models,

chronic amphetamine administration, coupled with another stimulus, has been shown to increase cortical responsiveness possibly through upregulation of CREB, a protein transcription factor, and induction of genes mediating other molecular changes.[204] Experiments from Leonardo Cohen's laboratory investigating the effects of various drugs on cortical plasticity have shown that dextroamphetamine administration facilitates the induction, magnitude, and retention of use-dependent plasticity in humans during performance of a motor training task.[205]

The concept of rehabilitation pharmacology, which dates back >20 years,[206] proposes that conventional physical, occupational, or speech/language therapy might be augmented if coupled with pharmacotherapy to enhance activity-dependent plasticity.[207] On the basis of small clinical studies showing some promise,[208–210] we are conducting a clinical trial in Canada to investigate the effects on motor recovery of dextroamphetamine versus placebo coupled with physiotherapy after hemiparetic stroke,[211] and in the United States, a multicenter trial of amphetamine-facilitated recovery is underway. A recently published study, however, showed no benefit of amphetamine on motor recovery in a randomized, controlled trial of 36 patients treated with intermittent doses of drug coupled with physical therapy beginning 5 to 10 days after stroke.[212] However, racemic amphetamine 10 mg was used instead of dextroamphetamine as in previous studies, and their patients were older than in previous studies (average age, 80 years). Stroke type and neuroimaging characteristics that might influence recovery or treatment response (eg, lesion size, location) were not reported, and patients with moderate hemiparesis were not analyzed separately in comparison to those with severe hemiparesis. The effects of dextroamphetamine coupled with speech/language therapy are also being investigated in ongoing studies by Walker-Batson et al[213–215] and have shown some promise for enhancing aphasia recovery. Trials of other noradrenergic agonists (methylphenidate, L-DOPS), levodopa, and fluoxetine have been conducted recently and provide further proof of concept for the strategy of poststroke rehabilitation pharmacotherapy.[216–219]

Goldstein et al[195,220,221] have drawn attention to the observation that several drugs shown to be detrimental in the laboratory are commonly prescribed to hospitalized patients after stroke and head trauma and may have similar detrimental effects on recovery in humans. These include the antihypertensives clonidine and prazosin (α-noradrenergic antagonists), haloperidol and other dopamine antagonists, benzodiazepines, phenytoin, and phenobarbital.[194,195] Retrospective reports suggest that exposure to such drugs is associated with poorer motor recovery, independent of the severity of initial deficit or comorbid conditions.[222,223] Aphasia recovery may also be impaired by certain drugs.[206,224] In a prospective study of chronic stroke patients, administration of increasing doses of a benzodiazepine reinstated previously recovered focal deficits (hemiparesis, aphasia, neglect).[86]

Clinical rehabilitation trials are promising for many reasons and may avoid some of the methodological obstacles encountered in many acute stroke trials.[125] (Table 3) Preclinical studies of restorative therapies necessarily rely more on behavioral outcomes and extended follow-up periods (weeks

More homogeneous patient population
Tighter inclusion criteria (more accurate diagnosis of stroke type before entry through imaging criteria)
Reduced variability because patients with early spontaneous improvement can be excluded (to concentrate on those patients with persisting deficits in need of rehabilitation and avoid a placebo responder effect)
Extended time windows
Less pressure of time to enroll patients
More detailed baseline and outcome assessments can be carried out
Wider applicability than acute intervention
More patients are eligible for rehabilitation interventions

*Modified from Finklestein.[125]

after stroke), making these animal models potentially more relevant to the human condition than models of acute neuroprotective therapy. Without the constraints of a narrow time window, effective restorative therapies may be able to reach many more patients than would qualify for acute treatment. The challenge remains, however, to design these trials well, select appropriate outcome measures, determine the clinically important difference, and power the samples accordingly.

Conclusions

Researchers and clinicians must become more cognizant of the pitfalls and paradoxes that have arisen in attempting to translate the results of animal studies into clinical trials of neuroprotective stroke therapy. Much needed recommendations to improve the quality of preclinical and clinical drug development have been published recently by the Stroke Therapy Academic Industry Roundtable (STAIR)[15,16] and others[20] and should be followed. Preclinical evaluation of therapeutic efficacy based solely on measuring infarct volume in the early phase is no longer adequate. Clinical trials should be based on preclinical evidence demonstrating improved functional outcomes at long-term end points measured on standardized batteries of validated behavioral tests. Assessment of neuroprotection should rely more on delayed time windows for drug administration, longer durations of ischemia, and models that take into account the protection of cerebral white matter in addition to gray matter.[15,16,72] Ideally, there should be converging evidence from >1 laboratory, different stroke models, and >1 animal species.[15]

Previous neuroprotective trials have not been selective enough in targeting homogeneous patients with cortical stroke and evidence for the presence of ischemic penumbra. Future trials need to use stricter entry criteria to target not just cortical strokes but specifically those with a sufficient volume of penumbra as determined by imaging. Although functional imaging is currently restricted to specialized centers, we urgently need proof of principle that neuroprotectives can work in humans if administered to the appropriate patient populations. Perfusion imaging with CT is worthy of further investigation because of the availability and practicality of CT for acute stroke imaging. With improved methods of patient selection, it is anticipated that future treatments will more appropriately be customized according to an individu-

al's "penumbra window" rather than a rigid time window. Patient selection should be "penumbra-specific" for acute treatment interventions and "deficit-specific" for restorative/rehabilitative interventions. Greater consensus on outcome measures is needed because the choice of outcome measure remains highly variable and can determine the success or failure of a putative therapeutic agent.

If current neuroprotective approaches only delay rather than arrest cell death, future strategies may require "rational" polytherapy that combines drugs with different mechanisms of action, perhaps administered at different poststroke intervals and in combination with reperfusion strategies. The concept of the therapeutic window is evolving from a single rigid and narrow time period to multiple potential overlapping and sequential windows, spanning minutes, hours, days, weeks, and months after stroke. Although most trials have concentrated on excitotoxicity, newer targets, including inhibition of inflammatory reactions and apoptosis, are being explored. The time point at which the therapeutic transition from neuroprotection to repair occurs merits further study.

Stroke is a chronic condition, and we must not ignore opportunities for therapeutic intervention in the subacute and chronic stages. With a growing understanding of the mechanisms that underlie recovery, research directed at enhancement of neural repair and rehabilitation should be a high priority.

Acknowledgments

References

1. American Heart Association. *2001 Heart and Stroke Statistical Update*. Dallas, Tex: American Heart Association; 2001.
2. Michaud CM, Murray CJ, Bloom BR. Burden of disease: implications for future research. *JAMA*. 2001;285:535–539.
3. Kidwell CS, Liebeskind DS, Starkman S, Saver JL. Trends in acute ischemic stroke trials through the 20th century. *Stroke*. 2001;32:1349–1359.
4. National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med*. 1995;333:1581–1587.
5. Furlan A, Higashida R, Wechsler L, Gent M, Rowley H, Kase C, Pessin M, Ahuja A, Callahan F, Clark WM, Silver F, Rivera F. Intra-arterial prourokinase for acute ischemic stroke: the PROACT II study: a randomized controlled trial: Prolyse in Acute Cerebral Thromboembolism. *JAMA*. 1999;282:2003–2011.
6. Kay R, Wong KS, Yu YL, Chan YW, Tsoi TH, Ahuja AT, Chan FL, Fong KY, Law CB, Wong A. Low-molecular-weight heparin for the treatment of acute ischemic stroke. *N Engl J Med*. 1995;333:1588–1593.
7. Sherman DG, Atkinson RP, Chippendale T, Levin KA, Ng K, Futrell N, Hsu CY, Levy DE. Intravenous ancrod for treatment of acute ischemic stroke: the STAT study: a randomized controlled trial: Stroke Treatment With Ancrod Trial. *JAMA*. 2000;283:2395–2403.
8. Martinez-Vila E, Sieira PI. Current status and perspectives of neuroprotection in ischemic stroke treatment. *Cerebrovasc Dis*. 2001;11(suppl 1):60–70.
9. Fisher M, Schaebitz W. An overview of acute stroke therapy: past, present, and future. *Arch Intern Med*. 2000;160:3196–3206.

10. Fisher M. Neuroprotection of acute ischemic stroke: where are we? *Neuroscientist*. 1999;5:392–401.

11. Faden AI. Neuroprotection and traumatic brain injury. *Arch Neurol*. 2001;58:1553–1555.

12. Maas AI, Steyerberg EW, Murray GD, Bullock R, Baethmann A, Marshall LF, Teasdale GM. Why have recent trials of neuroprotective agents in head injury failed to show convincing efficacy? A pragmatic analysis and theoretical considerations. *Neurosurgery*. 1999;44:1286–1298.

13. Bullock MR, Lyeth BG, Muizelaar JP. Current status of neuroprotection trials for traumatic brain injury: lessons from animal models and clinical studies. *Neurosurgery*. 1999;45:207–217.

14. Teasdale GM, Maas A, Iannotti F, Ohman J, Unterberg A. Challenges in translating the efficacy of neuroprotective agents in experimental models into knowledge of clinical benefits in head injured patients. *Acta Neurochir*. 1999;73:111–116.

15. Stroke Therapy Academic Industry Roundtable (STAIR). Recommendations for standards regarding preclinical neuroprotective and restorative drug development. *Stroke*. 1999;30:2752–2758.

16. Stroke Therapy Academic Industry Roundtable II. Recommendations for clinical trial evaluation of acute stroke therapies. *Stroke*. 2001;32:1598–1606.

17. Grotta J. Neuroprotection is unlikely to be effective in humans using current trial designs. *Stroke*. 2001;33:306–307.

18. Lees KR. Neuroprotection is unlikely to be effective in humans using current trial designs: an opposing view. *Stroke*. 2001;33:308–309.

19. Jonas S, Aiyagari V, Vieira D, Figueroa M. The failure of neuronal protective agents versus the success of thrombolysis for the treatment of ischemic stroke. The predictive value of animal models. *Ann N Y Acad Sci*. 2001;939:257–267.

20. Liebeskind DS, Kasner SE. Neuroprotection for ischaemic stroke: an unattainable goal?. *CNS Drugs*. 2001;15:165–174.

21. DeGraba TJ, Pettigrew LC. Why do neuroprotective drugs work in animals but not humans? *Neurol Clin*. 2000;18:475–493.

22. Lees KR. Neuroprotection. *Br Med Bull*. 2000;56:401–412.

23. Muir KW, Grosset DG. Neuroprotection for acute stroke: making clinical trials work. *Stroke*. 1999;30:180–182.

24. Grotta J. Why do all drugs work in animals but none in stroke patients? 2. Neuroprotective therapy. *J Intern Med*. 1995;237:89–94.

25. Gorelick PB. Neuroprotection in acute ischaemic stroke: a tale of for whom the bell tolls? *Lancet*. 2000;355:1925–1926.

26. Drummond JC, Piyash PM, Kimbro JR. Neuroprotection failure in stroke. *Lancet*. 2000;356:1032–1033.

27. Wardlaw JM, Warlow CP, Sandercock PA, Dennis MS, Lindley RI. Neuroprotection disappointment yet aGAIN. *Lancet*. 2000;356:597.

28. Lutsep HL, Clark WM. Neuroprotection in acute ischaemic stroke: current status and future potential. *Drugs R D*. 1999;1:3–8.

29. Hunter AJ, Green AR, Cross DT. Animal models of acute ischemic stroke: can they predict clinically successful neuroprotective drugs? *Trends Pharmacol Sci*. 1995;16:123–128.

30. Morgenstern LB. What have we learned from clinical neuroprotective trials? *Neurology*. 2001;57:S45–S47.

31. Slikker W, Saran J, Auer RN, Palmer GC, Narahashi T, Youdim MBH, Maynard KI, Carbone KM, Trembly B. Neuroprotection: past successes and future challenges. *Ann N Y Acad Sci*. 2001;939:465–477.

32. Small DL, Buchan AM. Animal models. *Br Med Bull*. 2000;56:307–317.

33. Neff SR. Rodent models of stroke. *Arch Neurol*. 1997;54:350–351.

34. Duncan PW, Jorgensen HS, Wade DT. Outcome measures in acute stroke trials: a systematic review and some recommendations to improve practice. *Stroke*. 2000;31:1429–1438.

35. Horn J, de Haan RJ, Vermeulen M, Luiten PGM, Limburg M. Nimodipine in animal model experiments of focal cerebral ischemia: a systematic review. *Stroke*. 2001;32:2433–2438.

36. Becker KJ, Tirschwell DL. Ensuring patient safety in clinical trials for treatment of acute stroke. *JAMA*. 2001;286:2718–2719.

37. Albers GW, Goldstein LB, Hall D, Lesko LM, for the Aptiganel Acute Stroke Investigators. Aptiganel hydrochloride in acute ischemic stroke: a randomized controlled trial. *JAMA*. 2001;286:2673–2682.

38. Use of anti-ICAM-1 therapy in ischemic stroke: results of the Enlimomab Acute Stroke Trial. *Neurology*. 2001;57:1428–1434.

39. Jonas S, Tran AQ, Eisenberg E, Azam M, Viera D, Grumet S. Does effect of a neuroprotective agent on volume of experimental animal cerebral infarct predict effect of the agent on clinical outcome in human stroke? *Ann N Y Acad Sci*. 1997;825:281–287.

40. Grotta JC. Acute stroke therapy at the millennium: consummating the marriage between the laboratory and bedside: the Feinberg lecture. *Stroke*. 1999;30:1722–1728.

41. Zivin JA. Factors determining the therapeutic window for stroke. *Neurology*. 1998;50:599–603.

42. Marler JR, Tilley BC, Lu M, Brott TG, Lyden PC, Grotta JC, Broderick. JP, Levine SR, Frankel MP, Horowitz SH, Haley EC Jr, Lewandowski CA, Kwiatkowski TP. Early stroke treatment associated with better outcome: the NINDS rt-PA stroke study. *Neurology*. 2000;55:1649–1655.

43. Baron JC, von Kummer R, del Zoppo GJ. Treatment of acute ischemic stroke. challenging the concept of a rigid and universal time window. *Stroke*. 1995;26:2219–2221.

44. Baron J. Mapping the ischaemic penumbra with PET: implications for acute stroke treatment. *Cerebrovasc Dis*. 1999;9:193–201.

45. Fisher M. Characterizing the target of acute stroke therapy. *Stroke*. 1997;28:866–872.

46. Marchal G, Beaudouin V, Rioux P, de la Sayette V, Le Doze F, Viader F, Derlon JM, Baron JC. Prolonged persistence of substantial volumes of potentially viable brain tissue after stroke: a correlative PET-CT study with voxel-based data analysis. *Stroke*. 1996;27:599–606.

47. Albers GW, Bates VE, Clark WM, Bell R, Verro P, Hamilton SA. Intravenous tissue-type plasminogen activator for treatment of acute stroke: the Standard Treatment With Alteplase to Reverse Stroke (STARS) study. *JAMA*. 2000;283:1145–1150.

48. Barber PA, Zhang J, Demchuk AM, Hill MD, Buchan AM. Why are stroke patients excluded from tPA therapy? An analysis of patient eligibility. *Neurology*. 2001;56:1015–1020.

49. Arrowsmith JE, Harrison MJG, Newman SP, Stygall J, Timberlake N, Pugsley WB. Neuroprotection of the brain during cardiopulmonary bypass. *Stroke*. 1998;29:2357–2362.

50. Fisher M, Jonas S, Sacco RL. Prophylactic neuroprotection for cerebral ischemia. *Stroke*. 1994;25:1075–1080.

51. Jonas S. Prophylactic pharmacologic neuroprotection against focal cerebral ischemia. *Ann N Y Acad Sci*. 1995;765:21–25.

52. Grieco G, d'Hollosy M, Culliford AT, Jonas S. Evaluating neuroprotective agents for clinical anti-ischemic benefit using neurological and neuropsychological changes after cardiac surgery under cardiopulmonary bypass: methodological strategies and results of a double-blind, placebo-controlled trial of GM1 ganglioside. *Stroke*. 1996;27:858–874.

53. Baron JC. Perfusion thresholds in human cerebral ischemia: historical perspective and therapeutic implications. *Cerebrovasc Dis*. 2001;11(suppl 1):2–8.

54. Lees KR. Advances in neuroprotection trials. *Eur Neurol*. 2001;45:6–10.

55. Hakim AM. The cerebral ischemic penumbra. *Can J Neurol Sci*. 1987;14:557–559.

56. Hakim AM. Ischemic penumbra: the therapeutic window. *Neurology*. 1998;51:S44–S46.

57. Heiss WD, Thiel A, Grond M, Graf R. Which targets are relevant for therapy of acute ischemic stroke? *Stroke*. 1999;30:1486–1489.

58. Schlaug G, Benfield A, Baird AE, Siewert B, Lovblad KO, Parker RA, Edelman RR, Warach S. The ischemic penumbra: operationally defined by diffusion and perfusion MRI. *Neurology*. 1999;53:1528–1537.

59. Heiss WD. Ischemic penumbra: evidence from functional imaging in man. *J Cereb Blood Flow Metab*. 2000;20:1276–1293.

60. Baron JC. Mapping the ischaemic penumbra with PET: a new approach. *Brain*. 2001;124:2–4.

61. Heiss WD, Kracht LW, Thiel A, Grond M, Pawlik G. Penumbral probability thresholds of cortical flumazenil binding and blood flow predicting tissue outcome in patients with cerebral ischaemia. *Brain*. 2001;124:20–29.

62. Baird AE, Warach S. Magnetic resonance imaging of acute stroke. *J Cereb Blood Flow Metab*. 1998;18:583–609.

63. Barber PA, Davis SM, Darby DG, Desmond PM, Gerraty RP, Yang Q, Jolley D, Donnan GA, Tress BM. Absent middle cerebral artery flow predicts the presence and evolution of the ischemic penumbra. *Neurology*. 1999;52:1125–1132.

64. Warach S. Use of diffusion and perfusion magnetic resonance imaging as a tool in acute stroke clinical trials. *Curr Control Trials Cardiovasc Med*. 2001;2:38–44.

65. Orgogozo JM. Sipatrigine in Stroke Trial: a European multicenter study to establish the maximum tolerated dose of sipatrigine and to assess the efficacy of that dose by measuring the change in volume of ischaemic lesion by magnetic resonance imaging. *Stroke*. 2001;32:1455. Abstract.

66. Nabavi DG, Cenic A, Henderson S, Gelb AW, Lee TY. Perfusion mapping using computed tomography allows accurate prediction of cerebral infarction in experimental brain ischemia. *Stroke*. 2001;32: 175–183.

67. Baird AE, Dambrosia J, Janket S, Eichbaum Q, Chaves C, Silver B, Barber PA, Parsons M, Darby D, Davis S, Caplan LR, Edelman RE, Warach S. A three-item scale for the early prediction of stroke recovery. *Lancet*. 2001;357:2095–2099.

68. Pexman JH, Barber PA, Hill MD, Sevick RJ, Demchuk AM, Hudon ME, Hu WY, Buchan AM. Use of the Alberta Stroke Program Early CT Score (ASPECTS) for assessing CT scans in patients with acute stroke. *Am J Neuroradiol*. 2001;22:1534–1542.

69. Barber PA, Demchuk AM, Zhang J, Buchan AM. Validity and reliability of a quantitative computed tomography score in predicting outcome of hyperacute stroke before thrombolytic therapy: ASPECTS Study Group: Alberta Stroke Programme Early CT Score. *Lancet*. 2000;355: 1670–1674.

70. Warach S. New imaging strategies for patient selection for thrombolytic and neuroprotective therapies. *Neurology*. 2001;57:S48–S52.

71. Baird AE, Warach S. Using pathophysiology in acute stroke trials. *Stroke*. 1999;30:1293. Letter.

72. Dewar D, Yam P, McCulloch J. Drug development for stroke: importance of protecting cerebral white matter. *Eur J Pharmacol*. 1999; 375:41–50.

73. Stys PK. Anoxic and ischemic injury of myelinated axons in CNS white matter: from mechanistic concepts to therapeutics. *J Cereb Blood Flow Metab*. 1998;18:2–25.

74. Schabitz WR, Li F, Fisher M. The *N*-methyl-D-aspartate antagonist CNS 1102 protects cerebral gray and white matter from ischemic injury following temporary focal ischemia in rats. *Stroke*. 2000;31:1709–1714.

75. Jones KA, Baughman RW. Both NMDA and non-NMDA subtypes of glutamate receptors are concentrated at synapses on cerebral cortical neurons in culture. *Neuron*. 1991;7:593–603.

76. Yam PS, Dunn LT, Graham DI, Dewar D, McCulloch J. NMDA receptor blockade fails to alter axonal injury in focal cerebral ischemia. *J Cereb Blood Flow Metab*. 2000;20:772–779.

77. Dyker AG, Lees KR. Duration of neuroprotective treatment for ischemic stroke. *Stroke*. 1998;29:535–542.

78. Castillo J, Davalos A, Noya M. Progression of ischaemic stroke and excitotoxic amino acids. *Lancet*. 1997;349:79–83.

79. Bullock R, Zauner A, Woodward J, Young HF. Massive persistent release of excitatory amino acids following human occlusive stroke. *Stroke*. 1995;26:2187–2189.

80. Saunders DE, Howe FA, van den Boogaart A, McLean MA, Griffiths JR, Brown MM. Continuing ischemic damage after acute middle cerebral artery infarction in humans demonstrated by short-echo proton spectroscopy. *Stroke*. 1995;26:1007–1013.

81. Goldstein LB. *Restorative Neurology: Advances in Pharmacotherapy for Recovery After Stroke*. New York, NY: Futura Publishing; 1998.

82. Goldstein LB. Pharmacology of recovery after stroke. *Stroke*. 1990;21: 139–142.

83. Lodder J. Stroke recovery. *Neurology*. 1996;46:1187. Letter.

84. Goldstein LB. Stroke recovery. *Neurology*. 1996;46:1187–1188. Letter.

85. Lodder J. Early GABA-Ergic Activation Study in Stroke (EGASIS). *Stroke*. 2001;32:2450. Abstract.

86. Lazar RM, Fitzsimmons BF, Marshall RS, Berman MF, Bustillo MA, Young WL, Mohr JP, Shah J, Robinson JV. Reemergence of stroke deficits with midazolam challenge. *Stroke*. 2002;33:283–285.

87. Maletic-Savatic M, Malinow R, Svoboda K. Rapid dendritic morphogenesis in CA1 hippocampal dndrites induced by synaptic activity. *Science*. 1999;283:1923–1927.

88. Sacco RL, DeRosa JT, Haley ECJ, Levin B, Ordronneau P, Phillips SJ, Rundek T, Snipes RG, Thompson JL. The Glycine Antagonist in Neuroprotection Americas Investigators: glycine antagonist in neuroprotection for patients with acute stroke: GAIN Americas: a randomized controlled trial. *JAMA*. 2001;285:1719–1728.

89. Hunter AJ, Mackay KB, Rogers DC. To what extent have functional studies of ischaemia in animals been useful in the assessment of potential neuroprotective agents? *Trends Pharmacol Sci*. 1998;19: 59–66.

90. Corbett D, Nurse S. The problem of assessing effective neuroprotection in experimental cerebral ischemia. *Prog Neurobiol*. 1998;54:531–548.

91. Kawamata T, Alexis NE, Dietrich WD, Finklestein SP. Intracisternal basic fibroblast growth factor (bFGF) enhances behavioral recovery

following focal cerebral infarction in the rat. *J Cereb Blood Flow Metab*. 1996;16:542–547.

92. Kawamata T, Ren J, Chan TCK, Charette M, Finklestein SP. Intracisternal osteogenic protein-1 enhances functional recovery following focal stroke. *Neuroreport*. 1998;9:1441–1445.

93. Hudzik TJBA, Bialobok P, Widzowski D, Sydserff S, Howell A, Gendron P, Corbett D, Miller J, Palmer GC. Long-term functional end points following middle cerebral artery occlusion in the rat. *Pharmacol Biochem Behav*. 2000;65:553–562.

94. Hunter AJ, Hatcher J, Virley D, Nelson P, Irving E, Hadingham SJ, Parsons AA. Functional assessments in mice and rats after focal stroke. *Neuropharmacology*. 2000;39:806–816.

95. Valtysson J, Hillered L, Andine P, Hagberg H, Persson L. Neuropathological endpoints in experimental stroke pharmacotherapy: the importance of both early and late evaluation. *Acta Neurochir*. 1994;129: 58–63.

96. Colbourne F, Li H, Buchan AM, Clemens JA. Continuing postischemic neuronal death in CA1: influence of ischemia duration and cytoprotective doses of NBQX and SNX-111 in rats. *Stroke*. 1999;30:662–668.

97. Wang F, Corbett D, Osuga H, Osuga S, Ikeda JE, Slack RS, Hogan MJ, Hakim AM, Park DS. Inhibition of cyclin dependent kinases improves CA1 neuronal survival and behavioral performance following global ischemia in the rat. *J Cereb Blood Flow Metab*. 2002;22:171–182.

98. Garcia JH, Yoshida Y, Chen H, Li Y, Zhang ZG, Lian J, Chen S, Chopp M. Progression from ischemic injury to infarct following middle cerebral artery occlusion in the rat. *Am J Pathol*. 1993;142:623–635.

99. Garcia JH, Liu KF, Ho KL. Neuronal necrosis after middle cerebral artery occlusion in Wistar rats progresses at different time intervals in the caudoputamen and the cortex. *Stroke*. 1995;26:636–642.

100. Coimbra C, Drake M, Boris-Moller F, Wieloch T. Long-lasting neuroprotective effect of postischemic hypothermia and treatment with an anti-inflammatory/antipyretic drug: evidence for chronic encephalopathic processes following ischemia. *Stroke*. 1996;27:1578–1585.

101. Alonso de Lecinana M, Diez-Tejedor E, Carceller F, Roda JM. Cerebral ischemia: from animal studies to clinical practice: should the methods be reviewed?. *Cerebrovasc Dis*. 2001;11(suppl 1):20–30.

102. Demchuk AM, Buchan AM. Predictors of stroke outcome. *Neurolog Clin*. 2000;18:455–473.

103. Jorgensen HS, Reith J, Nakayama H, Kammersgaard LP, Houth JG, Raaschou HO, Olsen TS, Copland S, for the Copenhagen Stroke Study. Potentially reversible factors during the very acute phase of stroke and their impact on the prognosis: is there a large therapeutic potential to be explored? *Cerebrovasc Dis*. 2001;11:207–211.

104. Counsell C, Dennis M. Systematic review of prognostic models in patients with acute stroke. *Cerebrovasc Dis*. 2001;12:159–170.

105. Boysen G, Christensen H. Early stroke: a dynamic process. *Stroke*. 2001;32:2423–2425.

106. Kagansky N, Levy S, Knobler H. The role of hyperglycemia in acute stroke. *Arch Neurol*. 2001;58:1209–1212.

107. Demchuk AM, Morgenstern LB, Krieger DW, Linda Chi T, Hu W, Wein TH, Hardy RJ, Grotta JC, Buchan AM. Serum glucose level and diabetes predict tissue plasminogen activator-related intracerebral hemorrhage in acute ischemic stroke. *Stroke*. 1999;30:34–39.

108. Capes SE, Hunt D, Malmberg K, Pathak P, Gerstein HC. Stress hyperglycemia and prognosis of stroke in nondiabetic and diabetic patients: a systematic overview. *Stroke*. 2001;32:2426–2432.

109. Wahlgren NG, Ranasinha KW, Rosolacci T, Franke CL, van Erven PM, Ashwood T, Claesson L. Clomethiazole Acute Stroke Study (CLASS): results of a randomized, controlled trial of Clomethiazole versus placebo in 1360 acute stroke patients. *Stroke*. 1999;30:21–28.

110. Wahlgren NG, Bornhov S, Sharma A. The Clomethiazole Acute Stroke Study (CLASS): efficacy results in a subgroup of 545 patients with total anterior circulation syndrome. *J Stroke Cerebrovasc Dis*. 1999;8: 231–239.

111. Lyden P, Shuaib A, Ng K, Levin K, Atkinson RP, Rajput A, Wechsler L, Ashwood T, Claesson L, Odergren T, Salazar-Grueso E, for the CLASS-I/H/T Investigators. Clomethiazole Acute Stroke Study in Ischemic Stroke (CLASS-I): final results. *Stroke*. 2002;33:122–128.

112. Roberts L, Counsell C. Assessment of clinical outcomes in acute stroke trials. *Stroke*. 1998;29:986–991.

113. Duncan PW, Lai SM, Keighley J. Defining post-stroke recovery: implications for design and interpretation of drug trials. *Neuropharmacology*. 2000;39:835–841.

114. Uchino K, Billheimer D, Cramer SC. Entry criteria and baseline characteristics predict outcome in acute stroke trials. *Stroke*. 2001;32: 909–916.

115. Sulter G, Steen C, De Keyser J. Use of the Barthel Index and modified Rankin Scale in acute stroke trials. *Stroke*. 1999;30:1538–1541.

116. Broderick JP, Lu M, Kothari R, Levine SR, Lyden PD, Haley EC, Brott TG, Grotta J, Tilley BC, Marler JR, Frankel M. Finding the most powerful measures of the effectiveness of tissue plasminogen activator in the NINDS tPA stroke trial. *Stroke*. 2000;31:2335–2341.

117. Lyden PD, Lu M, Levine SR, Brott TG, Broderick J, for the NINDS rtPA Stroke Study Group. A modified National Institutes of Health Stroke Scale for use in stroke clinical trials: preliminary reliability and validity. *Stroke*. 2001;32:1310–1317.

118. Hacke W, Kaste M, Fieschi C, von Kummer R, Davalos A, Meier D, Larrue V, Bluhmki E, Davis S, Donnan G, Schneider D, Diez-Tejedor E, Trouillas P. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). *Lancet*. 1998;352:1245–1251.

118a.Lai SM, Duncan PW. Stroke recovery profile and the modified Rankin assessment. *Neuroepidemiology*. 2001;20:26–30.

119. Morris GF, Juul N, Marshall SB, Benedict B, Marshall LF. Neurological deterioration as a potential alternative endpoint in human clinical trials of experimental pharmacological agents for treatment of severe traumatic brain injuries: Executive Committee of the International Selfotel Trial. *Neurosurgery*. 1998;43:1369–1372.

120. Kondziolka D, Wechsler L, Goldstein S, Meltzer C, Thulborn KR, Gebel J, Jannetta P, DeCesare S, Elder EM, McGrogan M, Reitman MA, Bynum L. Transplantation of cultured human neuronal cells for patients with stroke. *Neurology*. 2000;55:565–569.

121. Fugl-Meyer AR, Jaasko L, Leyman I, Olsson S, Steglind S. The post-stroke hemiplegic patient. *Scand J Rehabil Med*. 1975;7:13–31.

122. Gladstone DJ, Danells CJ, Black SE. The Fugl-Meyer Assessment of motor recovery after stroke: a critical review of its measurement properties. *Neurorehabil Neural Repair*. (In Press).

123. Duncan PW, Wallace D, Lai SM, Johnson D, Embretson S, Laster LJ. The stroke impact scale version 2.0. Evaluation of reliability, validity, and sensitivity to change. *Stroke*. 1999;30:2131–2140.

124. Williams LS, Weinberger M, Harris LE, Clark DO, Biller J. Development of a stroke-specific quality of life scale. *Stroke*. 1999;30: 1362–1369.

125. Finklestein S. Molecular and cellular therapies for stroke recovery. Presented at Neuroplasticity: The Key to Stroke Recovery; March 2000; Kananaskis, Alberta, Canada.

126. Lai SM, Duncan PW, Keighley J. Prediction of functional outcome after stroke: comparison of the Orpington Prognostic Scale and the NIH Stroke Scale. *Stroke*. 1998;29:1838–1842.

127. Sandercock P, Hennerici MG, Orgogozo JM, Davis SM, Gorelick PB. Mega trials versus small trials in stroke. In: Fisher M, Bogousslavsky J, eds. *Current Review of Cerebrovascular Disease*. Philadelphia, Pa: Current Medicine, Inc; 2001:241–246.

128. Malakoff D. Bayes offers a "new" way to make sense of numbers. *Science*. 1999;286:1460–1464.

129. Stroke Trials Directory. Stroke Trial - UK-279,276. Available at: http://www.strokecenter.org/trials/list/trialPage188.htm. Accessed February 2002.

130. Lindsberg PJ, Roine RO, Tatlisumak T, Sairanen T, Kaste M. The future of stroke treatment. *Neurol Clin*. 2000;18:495–510.

131. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci*. 1999;22:391–397.

132. Lee J, Zipfel GJ, Choi DW. The changing landscape of ischaemic brain injury mechanisms. *Nature*. 1999;399:A7–A14.

133. Choi D. Stroke. *Neurobiol Dis*. 2000;7:552–558.

134. Johansson BB. Brain plasticity and stroke rehabilitation: the Willis Lecture. *Stroke*. 2000;31:223–230.

135. Kuschinsky W, Gillardon F. Apoptosis and cerebral ischemia. *Cerebrovasc Dis*. 2000;10:165–169.

136. Schulz JB, Weller M, Moskowitz MA. Caspases as treatment targets in stroke and neurodegenerative diseases. *Ann Neurol*. 1999;45:421–429.

137. MacManus JP, Buchan AM. Apoptosis after experimental stroke: fact or fashion? *J Neurotrauma*. 2000;17:899–914.

138. Du C, Hu R, Csernansky CA, Hsu CY, Choi DW. Very delayed infarction after mild focal cerebral ischemia: a role for apoptosis? *J Cereb Blood Flow Metab*. 1996;16:195–201.

139. Gwag BJ, Lobner D, Koh JY, Wie MB, Choi DW. Blockade of glutamate receptors unmasks neuronal apoptosis after oxygen-glucose deprivation in vitro. *Neuroscience*. 1995;68:615–619.

140. Hara H, Friedlander RM, Gagliardini V, Ayata C, Fink K, Huang Z, Shimizu-Sasamata M, Yuan J, Moskowitz MA. Inhibition of interleukin 1beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. *Proc Natl Acad Sci U S A*. 1997;94: 2007–2012.

141. Fink K, Zhu J, Namura S, Shimizu-Sasamata M, Endres M, Ma J, Dalkara T, Yuan J, Moskowitz MA. Prolonged therapeutic window for ischemic brain damage caused by delayed caspase activation. *J Cereb Blood Flow Metab*. 1998;18:1071–1076.

142. Schulz JB, Weller M, Matthews RT, Heneka MT, Groscurth P, Martinou JC, Lommatzsch J, von Coelln R, Wullner U, Loschmann P, Beal MF, Dichgans J, Klockgether T. Extended therapeutic window for caspase inhibition and synergy with MK-801 in the treatment of cerebral histotoxic hypoxia. *Cell Death Differ*. 1998;5:847–857.

143. Kaste M. Thrombolysis in ischaemic stroke: present and future: role of combined therapy. *Cerebrovasc Dis*. 2001;11(suppl 1):55–59.

144. Sacchetti ML, Toni D, Fiorelli M, Argentino C, Fieschi C. The concept of combination therapy in acute ischemic stroke. *Neurology*. 1997;49: S70–S74.

145. Dawson DA, Wadsworth G, Palmer AM. A comparative assessment of the efficacy and side-effect liability of neuroprotective compounds in experimental stroke. *Brain Res*. 2001;892:344–350.

146. Auer RN. Non-pharmacologic (physiologic) neuroprotection in the treatment of brain ischemia. *Ann N Y Acad Sci*. 2001;939:271–282.

147. Andersen M, Overgaard K, Meden P, Boysen G, Choi SC. Effects of citicoline combined with thrombolytic therapy in a rat embolic stroke model. *Stroke*. 1999;30:1464–1471.

148. Meden P, Overgaard K, Sereghy T, Boysen G. Enhancing the efficacy of thrombolysis by AMPA receptor blockade with NBQX in a rat embolic stroke model. *J Neurol Sci*. 1993;119:209–216.

149. Zivin JA, Mazzarella V. Tissue plasminogen activator plus glutamate antagonist improves outcome after embolic stroke. *Arch Neurol*. 1991; 48:1235–1238.

150. Lekieffre D, Benavides J, Scatton B, Nowicki JP. Neuroprotection afforded by a combination of eliprodil and a thrombolytic agent, rt-PA, in a rat thromboembolic stroke model. *Brain Res*. 1997;776:88–95.

151. Sereghy T, Overgaard K, Boysen G. Neuroprotection by excitatory amino acid antagonist augments the benefit of thrombolysis in embolic stroke in rats. *Stroke*. 1993;24:1702–1708.

152. Bowes MP, Rothlein R, Fagan SC, Zivin JA. Monoclonal antibodies preventing leukocyte activation reduce experimental neurologic injury and enhance efficacy of thrombolytic therapy. *Neurology*. 1995;45: 815–819.

153. Zhang RL, Zhang ZG, Chopp M. Increased therapeutic efficacy with rt-PA and anti-CD18 antibody treatment of stroke in the rat. *Neurology*. 1999;52:273–279.

154. Lyden P, Jacoby M, Schim J, Albers G, Mazzeo P, Ashwood T, Norlund A, Odergren T, for the CLASS IHT Investigators. The Clomethiazole Acute Stroke Study in Tissue-Type Plasminogen Activator-Treated Stroke (CLASS-T): final results. *Neurology*. 2001;57:1199–1205.

155. Grotta J. Combination Therapy Stroke Trial: recombinant tissue-type plasminogen activator with/without lubeluzole. *Cerebrovasc Dis*. 2001; 12:258–263.

156. Lyden PD, Jackson-Friedman C, Shin C, Hassid S. Synergistic combinatorial stroke therapy: a quantal bioassay of a GABA agonist and a glutamate antagonist. *Exp Neurol*. 2000;163:477–489.

157. Lyden PD, Lonzo L. Combination therapy protects ischemic brain in rats. a glutamate antagonist plus a gamma-aminobutyric acid agonist. *Stroke*. 1994;25:189–196.

158. Lyden P, Lonzo L, Nunez S. Combination chemotherapy extends the therapeutic window to 60 minutes after stroke. *J Neurotrauma*. 1995; 12:223–230.

159. Barth A, Barth L, Newell DW. Combination therapy with MK-801 and alpha-phenyl-tert-butyl-nitrone enhances protection against ischemic neuronal damage in organotypic hippocampal slice cultures. *Exp Neurol*. 1996;141:330–336.

160. Uematsu D, Araki N, Greenberg JH, Sladky J, Reivich M. Combined therapy with MK-801 and nimodipine for protection of ischemic brain damage. *Neurology*. 1991;41:88–94.

161. Onal MZ, Li F, Tatlisumak T, Locke KW, Sandage BWJ, Fisher M. Synergistic effects of citicoline and MK-801 in temporary experimental focal ischemia in rats. *Stroke*. 1997;28:1060–1065.

162. Barth A, Barth L, Morrison RS, Newell DW. bFGF enhances the protective effects of MK-801 against ischemic neuronal injury in vitro. *Neuroreport*. 1996;7:1461–1464.

163. Schmid-Elsaesser R, Zausinger S, Hungerhuber E, Baethmann A, Reulen HJ. Neuroprotective effects of combination therapy with tirilazad and magnesium in rats subjected to reversible focal cerebral ischemia. *Neurosurgery*. 1999;44:163–171.

164. Schmid-Elsaesser R, Hungerhuber E, Zausinger S, Baethmann A, Reulen HJ. Combination drug therapy and mild hypothermia: a promising treatment strategy for reversible, focal cerebral ischemia. *Stroke*. 1999;30:1891–1899.

165. Schmid-Elsaesser R, Hungerhuber E, Zausinger S, Baethmann A, Reulen HJ. Neuroprotective efficacy of combination therapy with two different antioxidants in rats subjected to transient focal ischemia. *Brain Res*. 1999;816:471–479.

166. Schabitz WR, Li F, Irie K, Sandage BW Jr, Locke KW, Fisher M. Synergistic effects of a combination of low-dose bFGF and citicoline after temporary experimental focal ischemia. *Stroke*. 1999;30:427–432.

167. Du C, Hu R, Csernansky CA, Liu XZ, Hsu CY, Choi DW. Additive neuroprotective effects of dextrorphan and cycloheximide in rats subjected to transient focal cerebral ischemia. *Brain Res*. 1996;718: 233–236.

168. Ma J, Endres M, Moskowitz MA. Synergistic effects of caspase inhibitors and MK-801 in brain injury after transient focal cerebral ischaemia in mice. *Br J Pharmacol*. 1998;124:756–762.

169. Ma J, Qiu J, Hirt L, Dalkara T, Moskowitz MA. Synergistic protective effect of caspase inhibitors and bFGF against brain injury induced by transient focal ischaemia. *Br J Pharmacol*. 2001;133:345–350.

170. Gladstone DJ, Black SE. Enhancing recovery after stroke with noradrenergic pharmacotherapy: a new frontier? *Can J Neurol Sci*. 2000;27: 97–105.

171. Hasbani MJ, Schlief ML, Fisher DA, Goldberg MP. Dendritic spines lost during glutamate receptor activation reemerge at original sites of synaptic contact. *J Neurosci*. 2001;21:2393–2403.

172. Cramer SC, Chopp M. Recovery recapitulates ontogeny. *Trends Neurosci*. 2000;23:265–271.

173. Ay I, Ay H, Koroshetz WJ, Finklestein SP. Growth factors and cerebral ischemia. In: Fisher M, Bogousslavsky J, eds. *Current Review of Cerebrovascular Disease*. Philadelphia, Pa: Current Medicine, Inc; 2001: 25–33.

174. Seil FJ. Recovery and repair issues after stroke from the scientific perspective. *Curr Opin Neurol*. 1997;10:49–51.

175. Lee RG, van Donkelaar P. Mechanisms underlying functional recovery following stroke. *Can J Neurol Sci*. 1995;22:257–263.

176. Li Y, Chen J, Chopp M. Cell proliferation and differentiation from ependymal, subependymal and choroid plexus cells in response to stroke in rats. *J Neurol Sci*. 2002;193:137–146.

176a. Morshead CM, Reynolds BA, Craig CE, McBurney MW, Staines WA, Morassutti D, Weiss S, van der Kooy D. Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. *Neuron*. 1994;13:1071–1082.

177. Ren J, Kaplan PL, Charette MF, Speller H, Finklestein SP. Time window of intracisternal osteogenic protein-1 in enhancing functional recovery after stroke. *Neuropharmacology*. 2000;39:860–865.

178. Fisher M, Finklestein S. Pharmacological approaches to stroke recovery. *Cerebrovasc Dis*. 1999;9:29–32.

179. Clark W, Schim J. Trafermin in acute stroke: results of a phase II/III randomized efficacy study. *Neurology*. 2000;54:A88. Abstract.

180. Bogousslavsky J, Donnan G. Fibroblast (trafermin) in acute stroke: results of the European-Australian phase II/III safety and efficacy trial. *Cerebrovasc Dis*. 2000;10(suppl 2):106. Abstract.

181. Cramer SC, Chopp M. Recovery recapitulates ontogeny. *Trends Neurosci*. 2000;23:265–271.

182. Osuga H, Osuga S, Wang F, Fetni R, Hogan MJ, Slack RS, Hakim AM, Ikeda JE, Park DS. Cyclin-dependent kinases as a therapeutic target for stroke. *Proc Natl Acad Sci U S A*. 2000;97:10254–10259.

183. Li Y, Chen J, Wang L, Lu M, Chopp M. Treatment of stroke in rat with intracarotid administration of marrow stromal cells. *Neurology*. 2001; 56:1666–1672.

184. Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, Chopp M. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke*. 2001;32:1005–1011.

185. Chen J, Sanberg PR, Li Y, Wang L, Lu M, Willing AE, Sanchez-Ramos J, Chopp M. Intravenous administration of human umbilical cord blood

186. Nudo RJ. Recovery after damage to motor cortical areas. *Curr Opin Neurobiol*. 1999;9:740–747.

187. Nudo RJ, Plautz EJ, Frost SB. Role of adaptive plasticity in recovery of function after damage to motor cortex. *Muscle Nerve*. 2001;24: 1000–1019.

188. Nudo RJ, Wise BM, SiFuentes F, Milliken GW. Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. *Science*. 1996;272:1791–1794.

189. Nudo RJ, Milliken GW. Reorganization of movement representations in primary motor cortex following focal ischemic infarcts in adult squirrel monkeys. *J Neurophysiol*. 1996;75:2144–2149.

190. Kwakkel G, Wagenaar RC, Twisk JWR, Lankhorst GJ, Koetsier JC. Intensity of leg and arm training after primary middle-cerebral-artery stroke: a randomised trial. *Lancet*. 1999;354:191–196.

191. Kunkel A, Kopp B, Muller G, Villringer K, Villringer A, Taub E, Flor H. Constraint-induced movement therapy for motor recovery in chronic stroke patients. *Arch Phys Med Rehabil*. 1999;80:624–628.

192. Staines WR, McIlroy WE, Graham SJ, Black SE. Bilateral movement enhances ipsilesional cortical activity in acute stroke: a pilot fMRI Study. *Neurology*. 2001;56:401–404.

193. Hesse S, Werner C, Bardeleben A, Barbeau H. Body weight-supported treadmill training after stroke. *Curr Atheroscler Rep*. 2001;3:287–294.

194. Goldstein LB. Influence of common drugs and related factors on stroke outcome. *Curr Opin Neurol*. 1997;10:52–57.

195. Goldstein LB. Potential effects of common drugs on stroke recovery. *Arch Neurol*. 1999;55:454–456.

196. Dobkin BH. Neurorehabilitation: greater plasticity through chemicals and practice. *Neurol Network Commentary*. 1998;2:171–174.

197. Feeney DM, Gonzalez A, Law WA. Amphetamine, haloperidol, and experience interact to affect rate of recovery after motor cortex injury. *Science*. 1982;217:855–857.

198. Hovda DA, Fenney DM. Amphetamine with experience promotes recovery of locomotor function after unilateral frontal cortex injury in the cat. *Brain Res*. 1984;298:358–361.

199. Goldstein LB, Davis JN. Post-lesion practice and amphetamine-facilitated recovery of beam-walking in the rat. *Restorative Neurol Neurosci*. 1990;2:311–314.

200. Goldstein LB, Coviello A, Miller GD, Davis JN. Norepinephrine depletion impairs motor recovery following sensorimotor cortex injury in the rat. *Restorative Neurol Neurosci*. 1991;3:41–47.

201. Gold P, Delanoy R, Merrin J. Modulation of long-term potentiation by peripherally administered amphetamine and epinephrine. *Brain Res*. 1984;305:103–107.

202. Stroemer RP, Kent TA, Hulsebosch CE. Neocortical neural sprouting, synaptogenesis, and behavioral recovery after neocortical infarction in rats. *Stroke*. 1995;26:2135–2144.

203. Stroemer RP, Kent TA, Hulsebosch CE. Enhanced neocortical neural sprouting, synaptogenesis, and behavioral recovery with d-amphetamine therapy after neocortical infarction in rats. *Stroke*. 1998;29:2381–2395.

204. Turgeon SM, Pollack AE, Fink JS. Enhanced CREB phosphorylation and changes in cFOS and FRA expression in striatum accompany amphetamine-induced sensitization. *Brain Res*. 1997;749:120–126.

205. Butefisch CM, Davis BC, Sawaki L, Waldvogel D, Classen J, Kopylev L, Cohen LG. Modulation of use-dependent plasticity by d-amphetamine. *Ann Neurol*. 2002;51:59–68.

206. Feeney DM, Sutton RL. Pharmacotherapy for recovery of function after brain injury. *Crit Rev Neurobiol*. 1987;3:135–197.

207. Feeney DM. Rehabilitation pharmacology: noradrenergic enhancement of physical therapy. In: Ginsberg MD, Bogousslavsky J, eds. *Cerebrovascular Disease: Pathophysiology, Diagnosis and Management*. Oxford, UK: Blackwell Science; 1998:620–636.

208. Crisostomo EA, Duncan PW, Propst M, Dawson DV, Davis JN. Evidence that amphetamine with physical therapy promotes recovery of motor function in stroke patients. *Ann Neurol*. 1988;23:94–97.

209. Walker-Batson D, Smith P, Curtis S, Unwin H, Greenlee R. Amphetamine paired with physical therapy accelerates motor recovery after stroke. *Stroke*. 1995;26:2254–2259.

210. Volpe BT, Shelton F, Krebs HI, Hogan N, Diels C, Reding M. Dextroamphetamine paired with standard and robotic neurorehabilitation changes motor performance one day later. *Ann Neurol*. 2000;48:454. Abstract.

211. Gladstone DJ, Black SE, Danells CJ, Staines WR, McIlroy WE, Graham SJ, Herrmann N, Szalai JP. Enhancing recovery from hemiplegic stroke:

reduces behavioral deficits after stroke in rats. *Stroke*. 2001;32: 2682–2688.

design of a multicentre study of amphetamine-facilitated stroke rehabilitation. *Clin Invest Med*. 2001;24:219. Abstract.

212. Sonde L, Nordstrom M, Nilsson CG, Lokk J, Viitanen M. A double-blind placebo-controlled study of the effects of amphetamine and physiotherapy after stroke. *Cerebrovasc Dis*. 2001;12:253–257.

213. Walker-Batson D, Curtis S, Natarajan R, Ford J, Dronkers N, Salmeron E, Lai J, Unwin DH. A double-bind, placebo-controlled study of the use of amphetamine in the treatment of aphasia. *Stroke*. 2001;32:2093–2098.

214. Walker-Batson D. Use of pharmacotherapy in the treatment of aphasia. *Brain Lang*. 2000;71:252–254.

215. Walker-Batson D. Pharmacotherapy in the treatment of aphasia. In: Goldstein LB, ed. *Restorative Neurology: Advances in Pharmacotherapy for Recovery After Stroke*. New York, NY: Futura Publishing; 1998:257–270.

216. Grade C, Redford B, Chrostowski J, Toussaint L, Blackwell B. Methylphenidate in early poststroke recovery: a double-blind, placebo-controlled study. *Arch Phys Med Rehabil*. 1998;79:1047–1050.

217. Nishino K, Sasaki T, Takahashi K, Chiba M, Ito T. The norepinephrine precursor L-threo-3,4-dihydroxyphenylserine facilitates motor recovery in chronic stroke patients. *J Clin Neurosci*. 2001;8:547–550.

218. Scheidtmann K, Fries W, Muller F, Koenig E. Effect of levodopa in combination with physiotherapy on functional motor recovery after stroke: a prospective, randomised, double-blind study. *Lancet*. 2001; 358:787–790.

219. Dam M, Tonin P, De Boni A, Pizzolato G, Casson S, Ermani M, Freo U, Piron L, Battistin L. Effects of fluoxetine and maprotiline on functional recovery in poststroke hemiplegic patients undergoing rehabilitation therapy. *Stroke*. 1996;27:1211–1214.

220. Goldstein LB, Davis JN. Physician prescribing patterns following hospital admission for ischemic cerebrovascular disease. *Neurology*. 1988;38:1806–1809.

221. Goldstein LB. Prescribing of potentially harmful drugs to patients admitted to hospital after head injury. *J Neurol Neurosurg Psychiatry*. 1995;58:753–755.

222. Goldstein LB, for the Sygen in Acute Stroke Study Investigators. Common drugs may influence motor recovery after stroke. *Neurology*. 1995;45:865–871.

223. Graham GD, Ahmed W, Davis LE, Bryniarski E, Hansen MD, Woolson RF, Clark WR, Adams HP. Effects of commonly prescribed medications on stroke recovery: a TOAST study analysis. *Stroke*. 1999;30:236. Abstract.

224. Porch B, Wyckes J, Feeney DM. Haloperidol, thiazides and some antihypertensives slow recovery from aphasia. *Neurosci Abstracts*. 1985;11:52.